

**Retrovirus Epidemiology Donor Study (REDS):
General Serum Repository (GSR) and General Leukocyte/Plasma Repository (GLPR)
Database**

This document has been prepared to provide background information and the methodology used to accrue specimens for the National Heart, Lung, and Blood Institute's REDS GSR and GLPR. Additionally, this document will provide specific information regarding the format of the dataset that accompanies this document and its contents.

Background

REDS completed the collection of two major repositories that are a valuable resource for the scientific community, the General Serum Repository (GSR) and the General Leukocyte/Plasma Repository (GLPR). The GSR and GLPR provide researchers with the opportunity to conduct viral marker studies from a large representative sample of blood donors. Rapid investigative response can be initiated at any time to determine the prevalence of new or suspected retroviruses or other pathogens in the donor blood supply. Because of the unique linkage to demographic data and donation test results collected in the REDS donation database, investigations can be targeted to particular subsets of the donor population.

Specimens in these repositories were obtained through the use of a carefully developed statistical sampling plan, designed to obtain donations from representative of all donor racial/ethnic groups. Specimens from these repositories are linked to donation records that contain demographic and test result data. The GSR, collected from 1991 to 1994, contains specimens from more than 500,000 donations. The GLPR, collected from 1994 to 1995, contains specimens from nearly 148,000 donations. These repositories have already been used for scientific studies, such as a study conducted to determine the validity of test methodologies and the prevalence and significance of human herpes virus 8 (HHV-8) and assessing assays used for detection of genetic alleles, such as the CCR-5 delta marker.¹⁻² Additionally, a focused study of *T. cruzi* antibody prevalence was performed using GSR samples collected from donors born in countries with endemic Chagas disease.³

REDS blood centers that contributed to both repositories include:

- American Red Cross - Greater Chesapeake and Potomac Region, Baltimore, MD;
- American Red Cross - Southeastern Michigan Region, Detroit, MI;
- American Red Cross - Southern California Region, Los Angeles, CA;
- Blood Centers of the Pacific - San Francisco, CA; and
- Oklahoma Blood Institute - Oklahoma City, OK.

The REDS coordinating center for was:

- Westat, Rockville, MD.

GSR

Overview

Between December 1990 and 1994, the GSR was formed. Specimens were obtained from all donors at collection sites on certain days from those donors who consented. Sites and days enrollment were sampled by the coordinating center based on collection site information and schedules that were provided by the blood centers, and the need to ensure representation of all demographic groups in a given area. Specimens for the repository were collected for all types of donations, including whole blood, apheresis, directed, and autologous. After processing and temporary storage at the blood centers, the samples were shipped to NHLBI's central repository.

A GSR Database System was used at the blood centers to accession and inventory all samples placed in the repository and to create an automated link between the barcode laboratory ID on the aliquot and the donation ID. Linking of this information ultimately allowed the study's coordinating center to provide demographic and viral marker test results on each sample placed in the repository.

Specimen Collection

After the blood center completed collection of the whole blood unit or apheresis product and the tubes required for routine blood center testing, they were directed to collect an additional 10 ml clot tube of whole blood. The additional 10 ml tube was labeled with the Whole Blood Number/Blood Unit ID (WBN/BUI) and stored separately at the collection site in appropriate racks at room temperature. Upon arrival at the blood center's processing lab, the 10 ml specimen tubes were placed overnight in the refrigerator at 4° C to assure the formation of a firm clot in each tube, to minimize fibrin strands in the serum, and to maximize the amount of serum collected for the repository.

Specimen Processing

At the start of the study, each blood center was equipped with freezer racks for -40° C walk-in freezers; accompanying 2" storage boxes with 81 cell dividers; rack, tube, and box labels with barcode and eye-readable ID numbers; and appropriate aliquot tubes for serum storage. A specially designed GSR Database System was also provided to each blood center by the coordinating center, which allowed for accessioning and tracking of specimen inventories as the blood center processed the samples. Each laboratory ID label that was assigned to an aliquot as it was processed was automatically linked in the database to the WBN/BUI of the donation. Of special note for this repository, two aliquots were placed in the repository, one was designated as an NHLBI aliquot and one was designated as a REDS aliquot. These aliquots distinguishing NHLBI and REDS were stored in separate boxes, although the lab ID assigned to the aliquots was identical. The minimum volume for storage in the repository was 3 ml, 2 ml for the NHLBI aliquot and 1 ml for REDS. Priority was always given to the NHLBI aliquot.

The following morning (no later than 48 hours after collection), the clot tubes were removed from the refrigerator and centrifuged. Following centrifugation, 2 ml of serum was pipetted into the NHLBI aliquot and 2 ml was pipetted into the REDS aliquot. Less than minimum volume specimens were stored in the repository. Following the sample processing, specimen boxes were stored in the -40° C walk-in freezer until transfer to NHLBI's central repository.

GLPR

Overview

The GLPR was developed as a resource to conduct future studies of transfusion-transmitted viruses using new testing technologies such as nucleic acid amplification testing and probe techniques. It was hypothesized that a newly discovered retroviral agent would most probably incorporate its genome within the host leukocyte's nucleic acids, thus the decision to collect leukocytes. This repository includes aliquots of plasma and whole blood (with and without dimethyl sulfoxide (DMSO)), compatible with viral and cellular nucleic acid amplification analyses, flow cytometric analyses, and viral and cell proliferation studies.⁴⁻⁵

The GLPR was collected over a 15-month period between October 1994 and December 1995, which obviously precedes the initiation of unit leukoreduction. It was designed to be a representative sample of donations from the five participating REDS blood centers. Specimens were collected from all donors and types of donations (e.g., apheresis, autologous, directed, whole blood) at selected collection sites on all days specified by the study's coordinating center. Collection sites were randomly selected by the coordinating center using collection schedules that were provided by REDS blood centers. After processing and temporary storage at the blood centers, the samples were shipped to NHLBI's central repository, which at that time was Ogden Bioservices.

A GLPR Database System was used at the blood centers to accession and inventory all samples placed in the repository and to link the laboratory ID assigned to the aliquot to the donation ID. Linking of this information ultimately allowed the study's coordinating center to provide demographic and viral marker test results on each sample placed in the repository.

Specimen Collection

After the blood center completed collection of the whole blood unit or apheresis product and the tubes required for routine blood center testing, they were directed to collect an additional 10 ml of whole blood. The additional 10 ml tube was labeled with the Whole Blood Number/Blood Unit ID (WBN/BUI) and stored separately at the collection site in appropriate racks at room temperature. To ensure the highest possible yield of leukocytes, blood centers were directed to process the GLPR specimens as soon as the samples arrived at the blood center from the collection sites. All specimens were processed within 48 hours of collection.

Specimen Processing

At the start of the study, each blood center was equipped with freezer racks for -40° C walk-in freezers; accompanying 2" storage boxes with 81 cell dividers; rack, tube, and box labels with barcode and eye-readable ID numbers; appropriate cryovials for plasma and whole blood; and cryovial holders. A specially designed GLPR Database System was also provided to each blood center by the coordinating center, which allowed for accessioning and tracking of specimen inventories as the blood center processed the samples. Each laboratory ID label that was assigned to an aliquot as it was processed was linked in the database to the WBN/BUI of the donation.

The minimum total volume necessary for inclusion in the GLPR was 6 ml. If the total volume was less than 6 ml, the sample was not processed. For each donation, six aliquots were prepared, four whole blood and two plasma. Whole blood, whole blood with DMSO, and plasma

aliquots were stored in separate boxes after processing. The minimum final GLPR volume per aliquot after processing was:

- 0.5 ml whole blood in each of two tubes
- 0.5 ml whole blood with DMSO in each of two tubes
- 1.5 ml plasma in each of two tubes.

Whole blood processing

The 10 ml tube collected off of the donation was vortexed to resuspend the blood cells evenly. The required volume of whole blood was then pipetted into each of the 4 whole blood aliquots referenced above. After capping off two of these aliquots, the remaining two were diluted with 50 μ l each of DMSO. The diluted samples were then capped and vortexed briefly to assure mixing with the samples. Samples were frozen within 30 minutes of dilution to prevent cell toxicity by the DMSO.

Plasma processing

The remaining blood left in the 10 ml donation tube was re-capped and centrifuged at 2,500 rpm for 20 minutes to permit removal of the plasma layer. The aliquots were then prepared by pipetting 1.5 ml of plasma from the donation tube into each of the two plasma aliquots. Volume corrections were made in the GLPR Database System in cases where the volume was not 1.5 ml.

Dataset Formation:

A weighted SAS dataset has been developed for the GLPR and GSR. Based on the design of the repositories to be random at the level of the donation, post-stratification weights were derived for age, sex, and race/ethnicity. The post-stratification procedure extrapolated back to the entire REDS donation dataset population for the time period that the repositories were formed, thus allowing estimates of prevalence or other statistics to be representative of the population of blood donations at the five participating REDS blood centers referred to on page 1 of this document.

Donation Classifications

Donations were classified by the following criteria:

- **Race/ethnicity** (see below);
 - Asian
 - Black
 - Hispanic
 - White
 - Other
 - Not Ascertained
 - Don't Know

- **Sex;** and
Male
Female
- **Age group.**
<25
25 to <35
35 to <45
45 to <55
55 to <65
≥65

Weights

Within these groups, the post-stratified weight was calculated as the overall count of donations divided by the count of donations in the repository:

$$\text{Post-stratified weight} = \frac{\text{Total donations}}{\text{Donations in repository}} .$$

The means of the post-stratified weights are 8.8 for the GSR and 8.5 for the GLPR. The design effect, which provides a measure of the impact of the sampling methods on standard errors and sample size, is 1.12 for the GSR, indicating a sample must be 12% larger to offset increased standard errors because of disproportionate representation of the GSR sample. The design effect for the GLPR is 1.02, indicating that the sample size need not be increased to offset disproportionate representation.

References:

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3. Williams A. Prevalence of *T. cruzi* antibodies in US blood donors born in Chagas' endemic areas. Presented at the 23rd International Meeting of Basic Research in Chagas' Disease; November 4-8, 1996; Caxambu, Brazil.

4. Fiebig EW, Johnson DK, Hirschorn DF, Knape CC, Webster HK, Lowder J, Busch MP for the Viral Activation Transfusion Study. Quantitative lymphocyte subset analysis on frozen whole blood. *Cytometry*. 1997; 29: 340-350.
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