WNV Study

Viral and Immune parameters of Dengue and WNV in donors
- Blood safety implications -

WNV Manual Of Procedures

Sponsored by:
The National Heart, Lung, and Blood Institute (NHLBI)
National Institutes of Health (NIH)

Blood Centers:
Blood Systems Inc. and registered facilities (UBS and CTS)

Coordinating Center/Central Laboratory:
Blood Systems Research Institute, San Francisco, CA

Central Repository:
BioLINCC - SeraCare BioServices

WNV MANUAL OF PROCEDURES

TABLE OF CONTENTS

1. INTRODUCTION

1.1 Study Overview:

The study entitled "Viral/Immune parameters of Dengue and WNV in donors: blood safety implications" (WNV/DENV) is a research program sponsored by the National Heart, Lung, and Blood Institute (NHLBI) to build two repositories of samples (plasma and PBMCs aliquots) collected from WNV+ and DENV+ blood donors enrolled in the acute viremia stages of WNV and DENV infections, and then followed through viral clearance and development of symptomatic infection. These repositories of plasma and PBMC specimens and databases with linked clinical and laboratory data will be transferred to the NHLBI Specimen Repository so that these unique samples are accessible to qualified scientists studying blood safety and the pathogenesis of WNV and DENV.

Several blood centers, a coordinating center, a central laboratory and a central repository will be involved in this project.

Figure 1A. Geographic location of participating sites

1.1.1. Blood Centers

- **For WNV+ donors enrollment**

UBS Scottsdale, AZ
UBS Billings, MT
UBS Cheyenne, WY
UBS Fargo, ND
UBS Rapid City, SD
UBS Fort Smith, AR
UBS Las Vegas, NV
UBS Lafayette, LA
UBS Meridian, MS
UBS Tupelo, MS
UBS Reno, NV
UBS San Luis Obispo, CA
UBS Rancho Mirage, CA
UBS Albuquerque, NM
UBS El Paso, TX
UBS McAllen, TX
UBS Lubbock, TX
UBS Tempe, AZ

- **For DENV+ donors enrollment**

American Red Cross Blood Services, San Juan, Puerto Rico

1.1.2. Coordinating Center

Blood Systems Research Institute (BSRI), San Francisco, CA

1.1.3. Central Laboratory

Blood Systems Research Institute (BSRI), San Francisco, CA
American Red Cross, Gaithersburg, MD

1.1.4. Central Repository

SeraCare Life Sciences, Gaithersburg, MD

The WNV/DENV research program will address the blood safety implications of Dengue and WNV, two of the most important arboviruses, and further guide development of rational screening policies for the US and world blood supplies. In addition this program will establish an NHLBI repository of extensively characterized, longitudinal specimens from Dengue and WNV infected donors to advance research into the pathogenesis of these important agents.

1.2 WNV/DENV Study Background and Overview

The emergence of WNV in the US in 1999 and demonstration of transfusion-transmission in 2002 alerted the global blood banking community of the blood safety implications of arbovirus epidemics. Since 2002, systematic research on WNV infected donors and exposed recipients has been conducted and guided donor RNA (NAT) screening and deferral policies that have virtually eliminated WNV transfusion transmission. The unparalleled access to WNV RNA+ donors allowed the capture of individuals in the pre-symptomatic stage of infection. Systematic longitudinal studies of viremic donors have contributed to understanding of the natural history of WNV diseases, and elucidated complex interactions of viral and host genetic, innate and adaptive immune responses that determine progression to symptomatic WNV disease. After the implementation of MP-NAT and improvements in ID-NAT triggering, the risk for WNV transfusion-transmission was drastically reduced.

Although WNV is no longer a major threat to blood safety, several questions remain unanswered and need further studies; e.g., the risk for transfusion transmission by units collected in the tail-end of WNV viremia. Addressing this question will provide data on how to better improve WNV screening and reduce donor deferrals. More importantly, WNV pathogenesis and vaccine development studies require access to pedigreed longitudinal samples from asymptomatic WNV-infected donors.

Dengue is the most important arbovirus in the world, with 50-100 million infections and >25,000 deaths annually in tropical/semi-tropical regions of the world. After decades of absence of in the continental US, clusters of dengue cases have been documented in the southern US over the past several years, and expanded spread is now a real possibility. Although healthcare transmissions have been difficult to ascertain in endemic countries, two clusters of DENV transfusion transmission were recently reported, and high rates of viremia in blood donors have been documented in Central/South America and Puerto Rico (PR), a US territory whose blood supply is run by the American Red Cross. Although DENV is among the highest priority risks to blood safety, routine screening of donors is not currently conducted in epidemic regions due to lack of systematic data on viral dynamics and infectivity of acute viremia that is needed to drive development/licensure of tests and formulation of donor screening guidelines. Sensitive NAT screening will be implemented in PR under a Food and Drug Administration Investigational New Drug (FDA IND), and launch follow-up studies of DENV+ donors similar to those conducted on WNV infected donors. Defining viral and

immune dynamics and the duration of infectivity in primary and secondary infections by different DENV serotypes will accelerate implementation of appropriate DENV donor screening and advance understanding of DENV diagnostics and immunopathogenesis.

The first goal of the study is to enroll 75 to 100 WNV viremic donors into longitudinal follow-up, and characterize viral and immunological parameters including *in vitro* infectivity to address residual questions regarding risk of WNV transfusion transmission from convalescent phase units to further refine NAT screening and deferral policies.

The second goal of the program is to implement DENV NAT screening of PR donors under an FDA IND protocol, interdicting RNA+ units, and to enroll 75-100 DENV viremic donors into follow-up to generate systematic data on the dynamics of viral replication, innate and adaptive immune responses, and infectivity of acute viremia in the context of primary and secondary DENV infections. These data will guide donor screening for DENV and contribute unique insights into viral and host determinants of dengue disease.

Finally, this program will create a comprehensive repository of plasma and PBMC, with corresponding clinical and laboratory data, from WNV+ and DENV+ blood donors identified in the acute phase of infection and followed for 6-months; these samples/data will be transferred to the NHLBI BioLINCC Repository so they will be available to qualified investigators to advance blood safety, diagnostic, pathogenesis and vaccine research related to these important human arboviruses.

1.3 WNV/DENV Study Timeline

WNV and DENV infections occur during seasonal outbreaks. The WNV season spreads from May to October with a peak in July-August, and the DENV season occurs from May to December peaking around September-October.

To meet the study goals and objectives, the study donors will be enrolled immediately after testing positive for WNV or DENV and will then be followed through a collection protocol consisting of 10 visits throughout a one-year period.

WNV+ donors will be enrolled during three consecutive seasons including 2009, 2010 and 2011. Samples from WNV+ donors collected within the UBS network are

shipped to BSRI where plasma aliquoting and Ficoll hypaque cell separations are performed. Aliquots of plasma and PBMCs will be bar code labeled and frozen in liquid nitrogen for further characterization and long-term repository storage. The Freezerworks System will be used to keep track of the specimens and their location in the WNV repository. All participants will be assigned unique study numbers in place of their name. This study number will be used for participants' consent forms, symptom questionnaires, blood draws, and repository aliquots. Linkage between the unique study number and the participant's personal identity will be kept in a locked file cabinet in the research office at BSRI.

After the prospective enrollment and collection of samples from WNV+ donors, different assays will be performed to characterize the repository of samples for viral and immune parameters. Virologic assays such as viral load quantification by real time PCR (index donation and first follow-up samples) and replicate TMA (on all follow-up samples) will be performed. Immunologic analyses will include plaque reduction neutralization titration (PRNT), as well as WNV IgM and IgG quantification by ELISA assays. Forty-two cytokines/chemokines will be measured using multiplex assays from Millipore on BSRI's Luminex platform.

Within these sequential samples, we will focus on the samples from WNV+ donors captured in the tail-end of the viremia (low-level viremia still detectable by TMA with presence of WNV-specific IgG and PRNT) to study their infectivity (low-level viremic "front end" samples will serve as controls).

The DENV+ donors will be enrolled during 2010 and 2011 DENV seasons into a longitudinal clinical and blood sample collection protocol. The ARC Puerto Rico (PR) will adopt the same recruitment and follow-up protocol as BSRI, with the aim of enrolling 75 to 100 DENV+ donors over the 2-year funding period. Once a DENV+ blood donor is identified, the donor will be temporarily deferred and notified. The study coordinator will contact the donor to obtain informed consent and administer a symptom questionnaire over the phone; donors will be asked to participate in the same collection protocol as the one developed for WNV+ donor enrollment.

Blood specimens from enrolled DENV+ donors collected within the ARC PR blood collection sites will be transported and processed into plasma and PBMC aliquots at the main blood center in San Juan on the day of collection. Aliquots of plasma and PBMCs will be frozen down in liquid nitrogen. These samples will be logged into an Access database that will eventually include all laboratory data collected for the DENV repository.

An aliquot of plasma from the index and each bleed will be shipped to BSRI and another to the CDC for virologic and immunologic characterization. Dynamics of viremia and immune responses in acute DENV infection will be studied as follows: genotyping and RNA viral load by RT-PCR; TMA performed in ID and MP-NAT formats; DENV-specific antibodies including IgM, IgG, IgA by ELISA and neutralizing antibodies performed by PRNT; DENV nonstructural protein NS1 will be characterized using the BIORAD PLATELIA™ DENGUE NS1 AG assay. BSRI will perform cytokines/chemokines testing using the same approach as developed for WNV. The CDC Dengue Branch will perform PCR to define the DENV serotype and use mosquito cell culture to determine infectivity.

There are six phases of the study termed as the Launch, Enrollment and Follow-up 1, Characterization 1, Enrollment and Follow-up 2, Characterization 2, and the Close-out. The WNV+ or DENV+ donors are enrolled during the seasonal outbreak (between April and December) and are followed-up to one year post-enrollment. After the Enrollment period and during the follow-up phase, the samples are characterized for viral and immune parameters. The data collected are analyzed and presented during the close-out period.

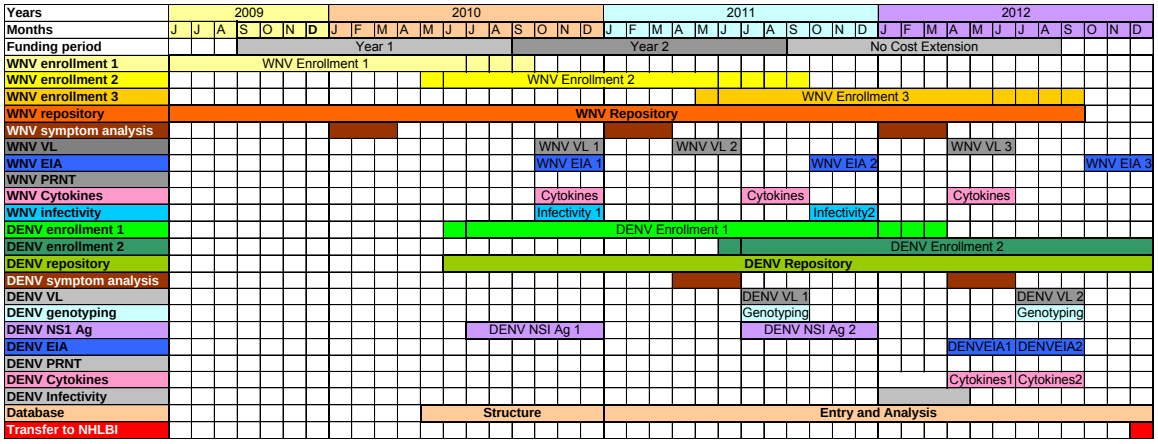


Figure 1B. WNV/DENV Study Timeline

1.4 Computer Systems

For the WNV repository, Freezerworks will be used for the tracking system.

For the DENV repository, an Access database will be used to keep track of the samples and the linked data.

2. ON-SITE DONOR RECRUITMENT

2.1 Target Numbers

The target number for WNV enrollment is 75 to 100 donors. All donors who present for donation at specific recruitment sites and are eligible to participate in the study will be approached. Recruitment will stop once the enrollment targets are met.

2.2 Overview of Activities for Each Study Visit

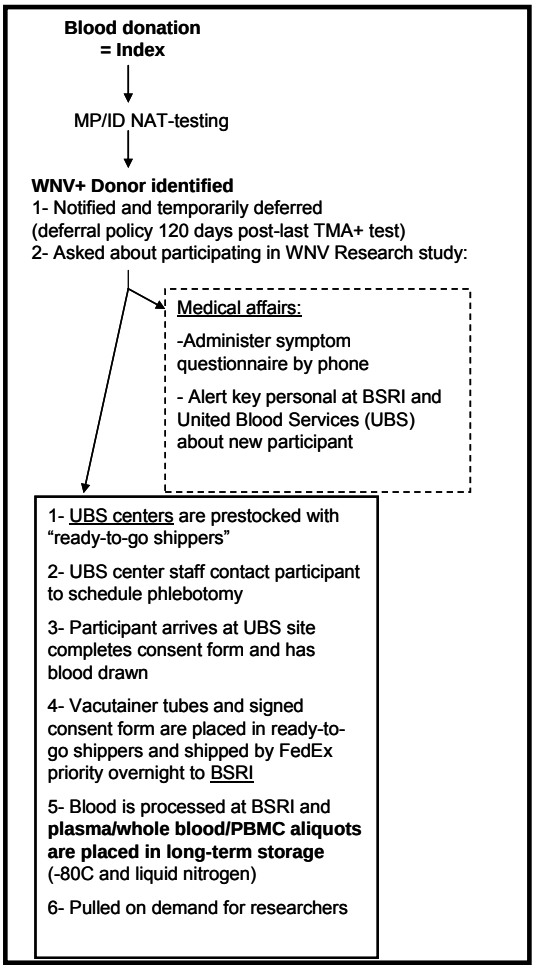


Figure 2A. Workflow at Enrollment Visit

After the eligible donor has been identified during the Index donation, CO Medical Affairs (MA) Donor Counselors will recruit donors by telephone when we inform them of their WNV (+) result. If the donor gives verbal consent to participate in the study, Questionnaire A is administered via telephone by a CO MA Donor Counselors at the time of recruitment while

Questionnaire B is administered at or around week 2 following WNV (+) donation. Then two types of study visits will occur: the Enrollment visit, or first visit, while all the subsequent visits will be considered Follow-up Visits. During the Enrollment visit, the donor needs to consent by filling out and signing the Informed consent document, and give blood. During subsequent Follow-up visits, the donor will only be required to give blood. Below is a summary table of these activities by visit type. Further detail on these items is presented later in this chapter.

Table 2.1 Checklist for each WNV study visit

Visit type	Time post-Index	Consent	Phlebotomy	Questionnaire	reimbursement
Recruitment	Days 0-2	Verbal		√	
Enrollment	Week 1	Signed	√		√
Follow-up 1	Week 2		√	√	√
Follow-up 2	Week 3		√		√
Follow-up 3	Week 4		√		√
Follow-up 4	Week 6		√		√
Follow-up 5	Month 2		√		√
Follow-up 6	Month 3		√		√
Follow-up 7	Month 6		√		√
Follow-up 8	Month 9		√		√
Follow-up 9	Month 12		√		√

2.3 Donor Visit Supplies

BSRI is responsible for supplying their designated study sites with the items listed in this section. Procedures for using each of the supplies listed in **Box 2A** below are detailed further in the sections indicated.

Box 2A

WNV visit Supplies:

*For each donor recruited for the WNVDENV Study, the following supplies are needed:(See also **ROP B VRLRC 0002**)*

- Committee for Human Research (CHR) approved number, size and type of vacutainer tubes used in the phlebotomy of study participants
- One Research Subject Information and Consent form - one form to be signed by the donor and retained by the VRLRC Department at BSRI and one copy for the consenting donor to keep for his/her records (see Exhibit 2).
- One Experimental Subject's Bill of Rights
- One Virology and Immunology WNV Study-Shipping List for Specimens form
- Phlebotomy Instructions
- A completed FedEx airbill for shipment to BSRI
- Insulated outer box
- Certified secondary container (red topped container)

Table 2.2 Sample tubes collected for WNV Study

Type of tube	Enrollment Visit	Follow-up Visits
10 mL EDTA	**7**	**7**
2 mL EDTA	**1**	**1**
9 mL Tempus (6mL RNA Stabilizing Fluid + 3mL blood)	**1**	**1**

2.3 Eligible Donors

Donors will be identified through routine WNV RNA screening of blood donations Study subjects will be contacted if their blood donation tests positive for WNV RNA by the transcription mediated assay (TMA) with a signal to cut-off (S/CO) ≥ 10. Subjects will range in age from 18 to 100 years. Subjects are invited to enroll via

telephone by CO MA Donor Counselors at the time they are informed of their WNV (+) result. Subjects will then be invited to enroll in the study upon presentation to their local blood donation center for Enrollment and subsequent Follow up visits. Pregnant women, donors who are less than 18 years old, and prisoners will be excluded from this study.

Any donor who enrolls, but changes their mind at any time will be withdrawn from the study and de-enrolled (see Section 2.10 for de-enrollment procedures).

Box 2B
WNV Eligibility

- Donors ≥ 18 years of age presenting to give a whole blood donation at index.
- WNV TMA reactive index donation with a S/CO ≥ 10
- Exclusion criteria: pregnancy, age less than 18, prisoners

2.5. Enrolling donors

2.5.1 Approaching donors after the index visit

BSI blood donors who meet the eligibility criteria will be asked if they want to participate in the WNV portion of the Go grant Study at the time they are notified of their WNV test results by an experienced study coordinator. The purpose of the study will be explained to the donor in order for him/her to consider participation. If the donor is willing to participate in the study, verbal consent will be obtained by phone. Once the donor has verbally consented to participate in the study, they will be administered a symptom questionnaire Furthermore, if verbal consent is obtained, a BSI Medical Affairs Study Coordinator designee will email the Central Laboratory Study Coordinator, Central Laboratory and Repository Manager and the UBS center Technical Director. The UBS center will then try to recall the donor. If phone contact is not obtained, up to 3 phone calls will be made, with calls placed at different times of the day. If no contact is made after 3 phone calls, the donor will be deemed lost to follow up and will not be included in the study.

During the enrollment visit the donor will sign a consent form. The recall schedule is based on the current CHR approved proposal.

If a donor initially states that he/she does not wish to participate, no further contact will be made.

2.5.2 Symptom Questionnaire Administration and data entry

Each approached donor from who verbal consent is obtained is required to answer two symptom questionnaires: questionnaire A regards the onset of symptoms developed in the week before donation and the day of donation (See symptom questionnaire A), Questionnaire B regards the onset of symptoms developed in the two weeks after donation. Medical affair staff have been trained in the phone administration of the symptom questionnaires. They fill-in questionnaire A during the first phone interview, and they then administer the second questionnaire two weeks after the first one, entering their initials for track record. Once a symptom questionnaire is completed, Medical Affair staff keep a copy for their records and the original form is sent by FedEx to BSRI and centralized by a research associate from the Epidemiology department and kept in a locked cabinet.

Upon receipt, the questionnaire form is scanned and the image is kept in a dedicated computer folder. During electronic capture, quality control is performed to verify that all entry fields have been completed. If some information is missing, the personal who administered the questionnaire can be contacted for an explanation. .

All fields with entry are captured during the scanning process and the data is automatically transferred into an excel database with restricted access. Each donor is entered by his/her index donation number or donor identification number. Questionnaires A and B are entered in two different tabs. The data is cleaned before any further use of the database.

Box 2C

Approaching a donor for the WNV Study

- A WNV RNA positive donor is contacted by Blood Systems, Inc (BSI) Medical Affairs staff who inform the donor of his/her virologic testing results and the deferral policy.
- The donor is informed on the scope of the study; explain the study at this time and answer questions.
- The donor is invited to participate in the study.
- Ensure that each donor willing to participate gives verbal consent and is aware of the need to sign the consent form at their Enrollment visit.
- If the donor gives "verbal" consent, administer symptom questionnaire (see Box 2D) and verify that the Subject ID is written in the assigned space.
- After the phone interview, designated staff members at both the Blood Collection Site and the VRLRC department are notified by email by a BSI Medical Affairs staff member of the participation of a new donor.

Box 2D

Symptom Questionnaire Administration and Data Entry

- If the donor gives "verbal" consent, the symptom questionnaire is administered on the phone:
- Make sure that the Subject ID is written in the assigned space.
- Medical Affairs staff who administered the symptom questionnaire, initial the questionnaire and send the questionnaires to BSRI via inter-office mail.
- In BSRI, a research associate from the epidemiology department receives the completed questionnaires via inter-office.
- Both sides of the questionnaire are scanned using a TeleForm Scan Station.
- TeleForm Reader processes the images and turns the capture fields into data.
- TeleForm ask for review if it detects any potential errors.
- Once the forms are reviewed, the data scanned from the forms and reviewed, the data is exported to the appropriate Excel spreadsheet.
- The Excel spreadsheet is checked for obvious errors, such as unit ID, date, or cells with garbage text.
- Then the forms are kept in a locked cabinet for record keeping.

Box 2E
Enrolling a donor for the WNV Study

- The donor is contacted by a Blood Collection Site staff member and asked to come to the Blood Collection Site in accordance with the CHR for their Enrollment visit
- The consent form needs to be signed by the participant and a witnessed at the time of participant's first phlebotomy. The witness can be any staff member that watched the participant sign the consent form and identified the participant.
- The participant's donor ID number must be written on the signed informed consent form on the first page, in the upper right-hand corner box.
- Make a copy of the signed informed consent form and give the copy of this form to the donor to retain for his/her records.
- The original signed consent form must be included in the "ready-to-go-shipper" with the results of the participant's first phlebotomy.
- For the phlebotomy: write participant's donor ID number plus the date and time of the phlebotomy on each of the tubes in the "ready-to-go-shipper".
- Obtain blood samples by normal phlebotomy procedures.
- Fill out the Shipping List for Specimens. Note: in order to protect the privacy of the participants, please DO NOT use their name anywhere on this form.
- Schedule the next Follow-up visit
- FAX the Shipping List for Specimens form to BSRI the SAME DAY as the phlebotomy. This alerts BSRI laboratory staff that the shipment is coming and provides the FedEx tracking number to track the shipment during transport, if necessary.
- Ship either the day of phlebotomy or the day after.
- Include the original copy of the Shipping List for Specimens with the shipment.

2.5.3 Obtaining Informed Consent

When approached by Medical Affairs' Staff the donor will have the scope of the study explained and will have the opportunity to ask questions about the study (See Box 2C). Once informed, the donor will be asked if he/she is willing to enroll in the study.

If verbal consent was obtained by BSI Medical Affairs, then the donor will sign the CHR approved consent form the first time the donor has his/her blood drawn

(See Box 2E). The consent form is then sent to the Viral Reference Laboratory and Repository Core (VRLRC) department along with the blood by Fed Ex Priority Overnight at room temperature. The consent form is kept in a locked filling cabinet in the VRLRC department as well as scanned. At the time of the donor's first phlebotomy, the donor is also presented with the BSI Experimental Subject's Bill of Rights.

If verbal consent was given, the symptom questionnaire is administered through by phone and key personal at BSRI and UBS are alerted of a new participant. The donor then will be contacted by UBS staff to schedule the Enrollment visit. During the Enrollment visit, the donor must sign the IRB-approved informed consent form, (See template of an approved Informed Consent section 16). Ensure that each donor willing to participate in the study signs the consent form, prints his/her donor ID number on the form and dates the form correctly. Arrange for a witness to sign the informed consent document. A copy of this signed informed consent form will be given to the donor for his/her own records. As described in Box 2E, the informed consent form must be returned with the tubes of the phlebotomy in the ready-to-go-shipper. The signed informed consent forms are centralized in BSRI by Dr. Leslie Tobler.

2.5.4 Tracking systems

There are 4 components in the tracking system:

- The donor tracking system: collecting information on blood donors (Donor ID, Demographics, History of all donations, Deferral information)
- The WNV tracking system: kept at BSI in Scottsdale collecting information on all WNV+ donors
- The VRLC tracking system: at BSRI, collecting all information on all WNV+ donors enrolled in our study (unit ID number of the index, dates, unites ID number to Follow-up specimens to the index specimen, Testing results for TMA, EIA, etc.)
- Freezerworks for keeping track of the position of the biospecimen when stored
- Another database keeping track of all data related to the characterization of the samples collected form WNV+ donors enrolled in the WNV arm of the Go grant project.

2.5.5 Scheduling a Follow-up Visit

Local centers have the responsibility to keep track of the follow-up visit and to schedule them with the donor. At enrollment, after collecting the completed Informed consent form and the phlebotomy tubes, the local center will schedule the next visit for collection of

follow-up samples.

Enrollment procedures and work flow for Baseline Visit are described in Figure 2A and **Box 2E**.

2.5.6 Follow-up Visits

It is important to flag these donors and inform the Operations Staff about their appointment dates if they have a scheduled visit. Walk-ins may be identified by simply asking them if they are a WNV Study participant. The Operations Staff is required to obtain a ready-to-go-shipper kit for these Follow-up Visits and enter the information on the log form. See the steps required for Follow-up in **Box 2F**.

Box 2F

Steps Involved at Follow-up Visits

- Identify returning WNV Study donors
- Operations Staff will collect blood in the provided tubes. Label tubes with participant's donor ID number plus the date and time of the phlebotomy on each of the tubes in the "ready-to-go-shipper".
- Obtain blood samples by normal phlebotomy procedures.
- Fill out the Shipping List for Specimens provided in the "ready-to-go-shipper". Note: in order to protect the privacy of the participants, please DO NOT use their name anywhere on this form.
- FAX Shipping List for Specimens form to BSRI the SAME DAY that you ship the specimens. This alerts BSRI laboratory staff that the shipment is coming and provides the FedEx tracking number to track the shipment during transport, if necessary.
- Include the original copy of the Shipping List for Specimens with the shipment.
- Schedule the next Follow-up visit

2.7 Labeling Specimen Tubes for blood collection

All centers will need to collect whole blood into seven 10 mL EDTA tubes, one 2 mL EDTA tube (these tubes have lavender tops), and one Tempus tube (blue top). Fill the Tempus tube with 3 mL of blood. Each of these tubes needs to be labeled with the Donor identification number (Donor ID). Label the specimen tubes prior to phlebotomy. The Study Coordinator could write the Donor ID on the tubes while interviewing the donor and have the tubes accompany the donor to the donation table, or alternatively, the labeling could take place at the donation table itself. Once the sample is collected the tubes are sent to VRLRC department at BSRI.

2.8 Documenting Donor's visit Information on Shipment tracking form

Each donor's visit during the study should be documented on the Shipping List for Specimen form. An example of the Shipping List for Specimen form can be found in the Appendix.

In the first line, the collection center must document the blood center location and the FedEx tracking number. The collection center must fill in the left part of the table below, entering the Donor ID number only (not his/her name). Then "Today's Date" and the time of phlebotomy using a 24 hour clock. The phlebotomist should put a "check" mark in the column corresponding to the respective tubes shipped to BSRI. The phlebotomist name should be written and the form should be dated and signed by the phlebotomist.

This shipment tracking form will be used to enter information upon reception of the samples in the participant's tracking database in BSRI.

2.9 Entering the donor visit information into the participant's tracking database

Upon shipment of the samples, the Shipping List for Specimens form is faxed to BSRI to alert personal that samples will arrive. In case the samples are not picked up by FedEx, lost or delivered to the wrong address, the tracking number helps to locate the shipper. Upon arrival, information written on the Shipping List for Specimens form is entered in the participant's tracking database kept at BSRI. This database is supported by

Microsoft Excel, and contains the following column for each donor, and each sample collected from a donor:

1. UBS Donation site
2. Index unit number or index BUI
3. Draw date of index
4. Draw Time
5. Consent form received
6. How index unit was tested, i.e. pool or IDS testing
7. Index TMA results (5 columns are dedicated to results of index testing)
8. Index serology (2 columns are dedicated to these results, i.e. IgM and IgG)
9. Phlebotomy schedule (10 rows are dedicated to this, i.e. week one, week two, week three, week four, week six, month two, month three, month six, month nine, and month twelve)
10. Follow-up phlebotomy ID numbers (Donor ID + time point in the sequence of phlebotomies)
11. Follow-up phlebotomy draw date
12. Follow-up phlebotomy time
13. Days since index
14. Date follow-up phlebotomy received by VRLRC
15. WNV TMA results on follow-up phlebotomies (4 columns dedicated to these results, i.e S/CO1, S/CO2,S/CO3 and final interpretation)
16. TMA aliquots made
17. Repository aliquots made
18. PBMC aliquots made
19. Participant paid

The participant's tracking database is meant to be a useful tool to determine at a glance the number and types of donors enrolled or followed on a given day.

2.10 De-enrolling Donors from the study

Any donor who enrolls but changes their mind will be withdrawn from the study and de-enrolled. Donors may also request to withdraw their samples from the repository.

Staff from the VRLRC lab at BSRI will pull up the subject's record in the participant's tracking database and Freezerworks using the Donor ID and update the "Visit Status" field to "De-Enrolled". The lab will immediately remove and destroy these specimens from their freezer boxes. CO MA Donor Counselors will be informed via email of any donors who de-enrolls.

3. SPECIMEN COLLECTION & PROCESSING

3.1 Overview

It is important for a multi-center study to obtain and collect specimens using standardized methods to insure the integrity of the specimens, the data and any conclusions generated. To meet this benchmark, each blood center is responsible for adhering to and processing all donor samples according to the procedures set forth in this chapter.

Whole blood in lavender top EDTA (10 mL and 2 mL) as well as a blue top Tempus Blood RNA tubes are to be obtained at all study visits:

EDTA Vacutainer tube

Tempus blood RNA tube

- Specimen are shipped by FedEx overnight priority to BSRI
- Upon reception, the blood is processed for plasma and PBMCs aliquots
- Specific plasma aliquots are prepared for laboratory testing to characterize viral load, viral genotype, antibodies and cytokines/chemokines responses
- The rest of the plasma aliquots and the PBMCs aliquots are frozen for long term storage.

A summary of the specimens collected at the donor visits and the actions that are to take place with each component of the specimen are detailed in Table 3.1, which appears on the following page.

The table below summarizes the type of specimen collected, aliquoting, storage procedures, and laboratory testing.

Table 3.1 Specimen Summary

Visit	Time post-enrollment	Collection		Processing		Volume for Laboratory Testing (mL)							Repository	
		Tubes used for collection	Total Volume of blood collected (mL)	Volume for Plasma PBMCs isolation (mL)	Volume for Storage Shipment	VL plasma	TMA plasma	EIA plasma	PRNT plasma	Cytokines plasma	Infectivity plasma	Volume of plasma for laboratory testing	Aliquots of plasma (2 mL)	Aliquots of PBMCs (10.106)
Index	day (-7)	Blood donation bag		N/A	N/A	2	3.1	0.5	2	0.15	0.5	8.25	5	0
Enrollment	0	7x10mL EDTA 1x2mL EDTA 1x3mL Tempus tube	75	72	3 mL in Tempus tube	2	3.1	0.5	2	0.15	0.5	8.25	10	10
Follow-up 1	week 1	7x10mL EDTA 1x2mL EDTA 1x3mL Tempus tube	75	72	3 mL in Tempus tube	2	3.1	0.5	2		0.5	8.1	10	10
Follow-up 2	week 2	7x10mL EDTA 1x2mL EDTA 1x3mL Tempus tube	75	72	3 mL in Tempus tube	2	3.1	0.5	2	0.15	0.5	8.25	10	10
Follow-up 3	week 3	7x10mL EDTA 1x2mL EDTA 1x3mL Tempus tube	75	72	3 mL in Tempus tube	2	3.1	0.5	2		0.5	8.1	10	10
Follow-up 4	week 6	7x10mL EDTA 1x2mL EDTA 1x3mL Tempus tube	75	72	3 mL in Tempus tube		3.1	0.5	2	0.15		5.75	10	10
Follow-up 5	month 2	7x10mL EDTA 1x2mL EDTA 1x3mL Tempus tube	75	72	3 mL in Tempus tube		3.1	0.5	2			5.6	10	10
Follow-up 6	month 3	7x10mL EDTA 1x2mL EDTA 1x3mL Tempus tube	75	72	3 mL in Tempus tube		3.1	0.5	2			5.6	10	10
Follow-up 7	month 6	7x10mL EDTA 1x2mL EDTA 1x3mL Tempus tube	75	72	3 mL in Tempus tube		3.1	0.5	2	0.15		5.75	10	10
Follow-up 8	month 9	7x10mL EDTA 1x2mL EDTA 1x3mL Tempus tube	75	72	3 mL in Tempus tube							0	10	10
Follow-up 9	month 12	7x10mL EDTA 1x2mL EDTA 1x3mL Tempus tube	75	72	3 mL in Tempus tube							0	10	10
10 visits post-Index over a 1 year period for a total of 750 mL of blood collected						Anticipated number of specimen in the WNV repository							105	100

3.2 Laboratory Supplies

Materials and supplies for the collection, processing, storage, and recording of the specimens for the study will be provided from a variety of sources. The next few sections provide a detailed description for all of these materials and supplies.

3.2.1 Organizational Responsibilities

This section provides information regarding which of the collaborating institutions are responsible for the various supplies, tools, and services that are needed for executing the required laboratory procedures.

3.2.1.1 Central Lab, Blood Systems Research Institute

Blood Systems Research Institute will pre-stock the UBS blood centers with "ready-to-go" shippers containing:

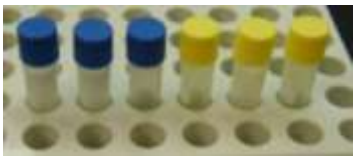
- Committee for Human Research (CHR) approved
- 7 x 10 ml EDTA Vacutainer™ tubes used in the phlebotomy
- 1 x 2 mL EDTA Vacutainer™ tubes used in the phlebotomy
- 1 x 3 ml Tempus Blood RNA tube used in the phlebotomy
- A blank informed consent document
- A California Study Bill of Rights
- Phlebotomy instructions for the collection staff
- A Virology and Immunology WNV- 2009 Study Shipping List for Specimens

- A completed Fed Ex air bill for return FedEx Priority
- 4G outer box and polypropylene secondary container
- EXAKT-PAK overpack
- 20x15x15 ULINE secondary overpack
- ULINE Industrial tape

Once the blood has been drawn at the UBS center the samples are shipped to Blood Systems Research Institute by FedEx overnight priority. The samples are processed at Blood Systems Research Institute upon reception. For the processing of the samples the following materials are required (See **ROP C VRLRC 003 and Fa VRLRC 008**):

<u>Tubes:</u>

- 225-mL Graduated Conical Polypropylene tube with Cap (sterile)
- 50-mL Polypropylene Conical Tube (non-pyrogenic, sterile)



- Wheaton 2.0ml Cryovials for Plasma Aliquots (sterile)
- Wheaton 2.0ml Cryovials for PBMC Aliquots (sterile)
- Coulter Vials

- Vi-Cell Sample Vials

<u>Pipettes:</u>

- 25-mL Individually Wrapped Disposable Plastic Pipettes (sterile, plugged)
- 10-mL Individually Wrapped Disposable

Plastic Pipettes (sterile, plugged)

- 5-mL Individually Wrapped Disposable Plastic Pipettes (sterile, plugged)
- Transfer Pipettes for Processing Plasma and PBMC (sterile)
- 20ul Pipette Tips
- 1000ul Pipette Tips
- Pasteur Pipettes

Filters:

- 150-ml Filters (0.2 micron filter)

Reagents:

- Sterile D-PBS w/o Calcium & Magnesium Salts (UCSF Cell Culture Facility)
- Fetal Bone Serum (FBS), Refiltered, Heat Inactivated (UCSF Cell Culture Facility)
- Ficoll-PaqueTM Plus (endotoxin tested) (GE Healthcare Bio-Sciences)
- Dimethyl sufoxide (DMSO), minimum 99.5% GC (Sigma-Aldrich)
- Isoton II Diluent (Beckman Coulter)
- Zap-Oglobin II Lytic Reagent (Beckman Coulter)

Boxes:

- 2" Freezers Boxes with drains for LN2 for PBMCs aliquots are 5” x 5” x 2” with 9 x 9 (81 slots)
- 2" Freezer Boxes without drains for Plasma Aliquots are 5” x 5” x 2” with 9 x 9 (81 slots)

Labels (See **ROP V VRLRC 0024**):

- Paperwork, Aliquots and Processing Labels
- Label sets for freezer boxes

Equipment:

- Beckman Coulter Vi-Cell XR

- Coulter Particle Counter Z1 (See **ROP O VRLRC 0016**)

- Sorvall Lengend RT Centrifuge (See **ROP Fa VRLRC 008**)

- Autoclave

- Vacuum Pump
- Glass Master Pipette
- Cool Cells (Biocision) (See **ROP G VRLRC 0010**)

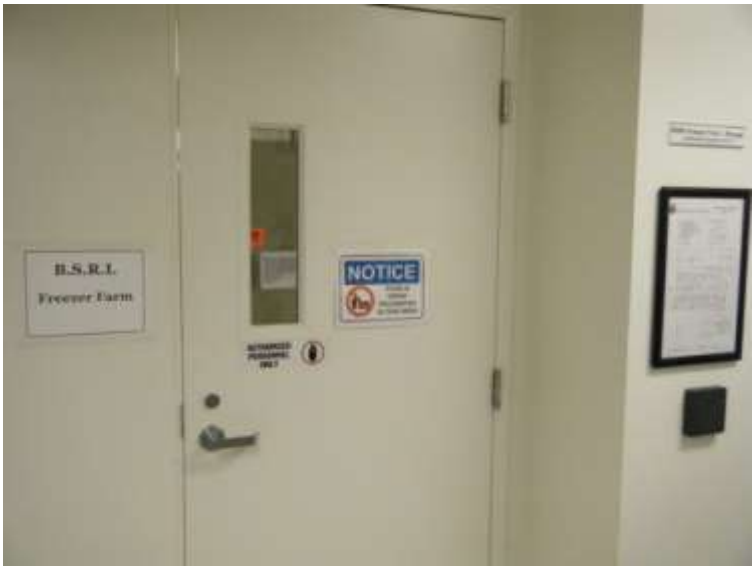
Equipment controls:

- Para 4 Multi-Parameter Assayed Hematology Control with LNH Coulter Controls (Streck)
- Para 4 INTRO Coulter Controls (Streck)

- VI-Cell Reagent Quad Pack (Beckman Coulter)
- VI-Cell Concentration Control (Beckman Coulter)

- VI-Cell Focus Control (Beckman Coulter)

Freezers:



- LN2 Freezers for PBMC Aliquots

(See **ROP Qb VRLRC 0019, G VRLRC 0010, R VRLRC 0020, and S VRLRC 0021**)

- -80 Mechanical Freezers for Plasma and Whole Blood Aliquots

(See **ROP Qa VRLRC 0018, G VRLRC 0010, and S VRLRC 0021**)

- Refrigerator

Miscellaneous:

- Absorbent pads
- Dry Ice Trays (See **ROP Qa VRLRC 0018 and Qb VRLRC 0019**)

- Dry Ice
- Bleach
- Cryovial racks
- Test tube racks
- BloodBlocs

Containers:

- Shipping containers and all associated items, labels and shipping costs for any specimens going to other designated testing facility
- Re-usable or disposable shipping containers for specimen transport to different sites in charge of the laboratory testing

Personnel protection equipment

(See **Qb VRLRC 0019 and BSRI PPE SM0022**)

- Gloves
- Disposable Lab Coats
- Goggles
- Face shield




Forms:

- Weekly Reagent Control Record
- Processing Form
- Shipping Form
- Coulter Book
- Lab Supply Order Form

Training:

- Provide training and support for systems and study activities

Computer system

- 6 computers (5 x desktop and 1x laptop computer) with Microsoft Windows XP or Windows 2000 operating system
- All computers have internet access
  - Internet Explorer (6 Service Pack 1 (SP1) web browser with 128 bit encryption);
- Software: Microsoft Word, Excel, Access
- Repository software (not sure yet what we will be using)
- Two printers (one with scanning and faxing capability)

3.2.1.2 UBS centers

- Provide samples using the Vacutainer tubes included in the "ready-to-go" shipper
- Ship the samples and associated forms to Blood Systems Research Institute
- Fax shipping forms

3.2.1.3 Central Repository, SeraCare

- Provide support system for repository database management

3.3 Collection of Specimens

Donors enrolled in the study have signed a consent indicating that they will return to donate 10 times throughout the one year study period. You will therefore be linking the multiple visits and the specimens associated with each visit through entries in Freezerworks and the database management system.

3.3.1 Sample Volume Requirements

See Table 3.1 on page 3-2 for blood collection tube volumes by visit type. All specimens collected with anticoagulants, such as EDTA, must have sufficient volume to insure that there are no improper dilutional effects. An improper ratio of anticoagulant to whole blood (see manufacturers insert) may interfere with the analytes being measured.

3.3.2 Specimen Integrity

The integrity and stability of certain analytes found in biological specimens can be affected by storage conditions such as time, temperature, or poor collection technique. Improper collection or storage can especially adversely affect cell viability, cell function, cytokine secretion and liability, etc. To avoid specimen, and hence analyte deterioration, research staff should process the specimen **as soon as possible** after a specimen is received. The goal is to have the lavender tops collected for CBC analysis transferred to the testing laboratory so that all specimens can be **tested within 24 hours** of the collection time. Then other whole blood tubes are to be shipped overnight and processed into the plasma and cellular aliquots and **stored in the freezer within a maximum of 48 hours**. Optimally, these specimens should be processed in less than 48 hours.

3.4 WNV Study Specimen Labeling System (See ROP V VRLRC 0024)

The use of Study ID labels in conjunction with the Freezerworks system (i.e. addition of sequence number to sequential BSI number) will facilitate the tracking of blood collection tubes, cryovials and shipments.

3.4.1 Subject ID Labels

3.4.2 Recording WNV Specimens in Freezerworks

3.5 Processing Specimens

Laboratory staff should follow Universal Precautions and OSHA Bloodborne Pathogen Rules throughout the following sample processing procedure.

3.5.1. Reception of specimens (See ROP C VRLRC 003, H VRLRC 0011)

Upon reception of new specimens, universal Safety Precautions need to be used. The package is delivered to the appropriate laboratory. The content of each package is carefully unpacked and the original box is retained for reuse and return to the shipping area within the VRLRC. The sample identification numbers must match with the invoice that arrived with the package. The specimen identification numbers are entered in the VRLRC study specific log sheet

along with any comments and date. The invoice(s) and study specific log sheet(s) are placed into the study specific log book. The working labels are created using appropriate labeling program.

3.5.1. Aliquoting Specimens for Testing and Long Term Storage

(See ROP Fa VRLRC 008)

1. Centrifuge the EDTA purple top(s) at 2500 g for 10-15 minutes at room temperature. Following centrifugation, the specimens should be separated into plasma and cells:

2. While the tubes are being centrifuged, label the appropriate aliquot cryovials with the Sample ID labels associated with the parent EDTA tubes and place the vials in a rack.

3. The plasma is removed and aliquoted into the appropriate cryovials and immediately frozen. This process should occur within **48** hours of collection.

4. Once plasma has been removed, replace plasma volume with Ca++, Mg++ free PBS to bring the blood volume back to its original volume. Note: subsequent dilutions of blood throughout this procedure should be performed after the blood has been returned to its original volume.

5. After replacing the plasma volume, gently mix tubes by inversion and process for PBMC's as follows. Pour the blood from either the ACD or EDTA tubes collectively into either a 50 mL or 225mL conical collection tube. The choice of tube size is based on the amount of blood being processed.

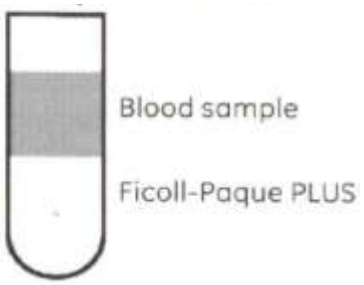
6. Wash EDTA tubes sequentially with an equal volume of PBS to obtain the blood clinging to the sides of each tube. Place the PBS wash into the collection tube holding the blood. The ratio should be 1 volume of Ca++, Mg++ free PBS to 1 volume of blood + PBS. Gently mix the PBS – Blood

mixture in either a 50mL conical or a 225mL conical tube using a sterile 25mL pipette. The volume of PBS-Blood will determine the number of 50mL centrifuge tubes to prepare for the overlay separation using a density gradient.

7. Thoroughly mix density gradient before using.

8. Prepare each 50mL centrifuge tube with either 7mL or 10mL of either Lymphoprep (AXIS-SHIELD PoC AS) or Ficoll-PaqueTM PLUS (Amersham Biosciences).

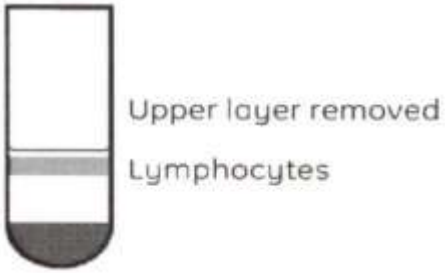
9. Slowly overlay approximately either 20mL or 30mL of the PBS – Blood solution (must always be 3 parts blood+PBS:1 part density gradient) into each tube with density gradient



10. Centrifuge the tube(s), no brake at room temperature for 45 minutes using either the Sorvall RT6000B Refrigerated Centrifuge at 1400 rpm (400g) or the Sorvall Legend RT at 1355 rpm (400g) (see RPM versus g force conversion table in the VRLRC Laboratory Processes and Protocols binder).

11. Once centrifugation is done, do not leave tubes in the centrifuge for an extended period of time. **The density gradient is toxic to the lymphocytes**.

12. Very carefully aspirate off (with vacuum pump and glass Pasteur pipette) the upper layer (plasma+ PBS layer) leaving the lymphocyte layer undisturbed at the interface



13. Collect the PBMC interface layer from each tube and transfer into a fresh sterile conical 50 mL centrifuge tube. **Note: Including excess Ficoll-Paque PLUS causes granulocyte contamination; Including excess supernatant results in platelet contamination**. Add Ca++, Mg++ free PBS wash media up to the 50mL mark. (1st wash)

14. Centrifuge for 10 minutes at 1070 rpm (250g) when using the Sorvall RT6000B Refrigerated Centrifuge. Or when using the Sorvall Legend RT spin for 10 minutes at 1070 rpm (250g). **This step removes platelets that are in the lymphocyte layer**.

15. Aspirate off Ca++, Mg++ free PBS wash media and re-suspend the pellet in 25 mL of Ca++, Mg++ free PBS. (2nd wash).

16. Centrifuge. When using the Sorvall RT6000B Refrigerated Centrifuge, spin for 10 minutes at 1070 rpm or 250g. When using the Sorvall Legend RT, spin for 10 minutes at 1070 rpm or 250g.

17. Aspirate off PBS wash media and gently re-suspend the pellet in Ca++, Mg++ free PBS media.

18. **Cell count using Coulter Counter**: From this cell-PBS suspension, take 10µL and add to the labeled Coulter counting vial containing 10 mL of Isoton solution (add 3 drops of Zap-oglobin™ II Lytic Reagent to remove red cells – Coulter only).

19. Follow the Coulter instrument procedure to count cells; apply raw counts, and dilution factors to get total number of cells per volume of PBS used to

resuspend the cell pellet after the second wash. Divide the total number of cells by the number of cells needed for each aliquot to get the number of cryovials that can be frozen down.

20. Centrifuge and gently remove Ca^{++}, Mg^{++} free PBS without disturbing the pellet.

21. Preparing PBMCs for Liquid Nitrogen Storage: add Freeze Media dropwise for the first 2 to 3 mL and then gently add the remaining volume.

22. Gently resuspend the cells in the freeze medium. Aliquot into the proper number of labeled cryovials and freeze cells in accordance with the CoolCell procedure. **The use of the CoolCell allows for optimal freezing at 1° per hour).**

23. Document the freezer box number and freezer box positions on the laboratory specific batch record and store the laboratory specific batch record in front of the Virology and Immunology WNV Study Shipping List for Specimens Shipping List form in the study specific binder.

3.6 Storing Specimens

3.7 Entering information into Freezerworks (See ROP VRLRC 0025)

3.8 Destroying Samples Due to De-enrollment

If a donor wishes to have his specimens removed from frozen storage, they need to contact ***Dr. Leslie Tobler at*** the BSRI Viral Reference Laboratory and Repository at 415-749-6606 or via email at ltobler@bloodsystems.org. If the specimens have already been transferred to the biorepository at the NHLBI, every effort will be made to have the specimens removed, but we cannot guarantee our ability to do so in all circumstances.

The steps outlined below are to be taken. Additionally, at the conclusion of the study, steps will be taken for interim samples that are going to be tested, be destroyed or transferred to the Central Repository for long term repository storage. More detailed instructions will be provided near the end of the study for this process.

This will be done utilizing the following procedures:

1. Pull those samples from the boxes in the freezers and discard through current and appropriate guidelines for disposal of biological waste.
2. Record in the BSRI II that those samples no longer exist
3. If specimens have already been shipped to another location then the Coordinating Center must be notified so that appropriate action can be taken to destroy specimens and clean all databases of de-enrolled subjects and specimens.

6. SPECIMENS SHIPPING PLANS AND PROCEDURES

6.1 Overview

For the WNV DENV study, different specimens shipping plans are to be considered.

During the enrollment and follow-up period of WNV+ donors, specimens will be shipped from the phlebotomy centers to BSRI within 24 hours after phlebotomy. The samples will be processed at BSRI within 48 hours after phlebotomy with plasma and PBMCs aliquots derived for long term storage as part of the WNV repository. Some plasma aliquots will be placed in freezer boxes and saved in the freezers before being shipped in batches to different locations for viral and immune parameters characterization.

During the enrollment and follow-up period of DENV+ donors, specimens will be centralized at the ARC facility in San Juan, PR, where they will be processed. Derived aliquots of plasma and PBMCs will be frozen for long term storage as part of the DENV repository. Some of the plasma aliquots will be placed in freezer boxes and saved in the freezers before being shipped in batches to different location for viral and immune parameters characterization.

BSRI will retain possession of the repository of specimens collected from WNV+ donors while ARC will retain possession of the repository of specimens collected from DENV+ donors until the end of the WNV DENV Study. After completion of the study, both repositories will be delivered to Sera Care facility.

Additional information will be distributed regarding this process of specimen transfer to the NHLBI Repository, which should occur in the summer of 2012.

6.2 Shipment of "Ready-to-go" shippers for specimen collection from WNV+ donors (See ROP A VRLRC 0001)

The blood centers will be pre-stocked with "ready-to-go" shippers based on their activity during the previous years. The pre-stocking phase will occur in June, before the seasonal outbreak. "Ready-to-go" shippers will be sent as well on a case by case basis directly to the donor. After each interview of a WNV+ donor who agreed to participate in the study, BSRI will be requested to ship the "ready-to-go" shipper to the donor or to the closest blood center. For each shipper returned with specimen, another shipper will be sent to the blood center.

Request for BSRI to ship the Sample Kit/Shipper		
Step	**Timeframe**	**Action**
1	Request Sample/Kit Shipper to be mailed to local UBS blood center	▪ Email the subject's name, phone number and address to the Study Coordinator at BSRI ▪ The Study Coordinator at BSRI will contact the subject to schedule an appointment for a blood draw at his/her local UBS blood center. The Study Coordinator will then email the contact person at the center to coordinate schedules.
2	Sample Kit/Shipper is mailed to local UBS blood center	▪ The Study Coordinator at BSRI will mail the "ready-to-go" shipper following by FedEx to the contact person at the local UBS blood center or to the donor ▪ After the Sample Kit/Shipper is received and the specimens are collected, the contact person will be responsible for mailing the Sample Kit/Shipper back to BSRI and faxing the Specimen Shipping List to the Laboratory Coordinator.
3	Sample Kit/Shipper is mailed back to BSRI	▪ The Study Coordinator at BSRI will track the Sample Kit/Shipper using its FedEx tracking number.
4	Sample Kit/Shipper is received at BSRI	▪ When the Sample Kit/Shipper is received at BSRI, the Study Coordinator at BSRI will arrange for specimen to be processed
5	Another Sample Kit/Shipper is mailed back to the blood center	▪ The study coordinator will mail the "ready-to-go" shipper following by FedEx to the contact person at the local UBS blood center

6.3 Shipping specimens from WNV+ donors back to BSRI

At each donor visit and after the phlebotomy, the blood center staff is responsible for placing the blood tubes in the "ready-to-go" shipper and placing the shipper for FedEx pickup. This procedure for packaging/shipping samples is detailed below.

Step	Shipment from phlebotomy center to BSRI
1	Take the plastic container from the shipper
2	Remove cap from the plastic container
3	Insert filled lavender-top and PAXgene tubes into the holes in the gray foam insert. Fill the center holes first and work outward
4	Replace the top gray foam disk
5	Make sure the O-ring is seated in the groove below the threads of the white container
6	Seal the container by screwing the cap on the container tightly. If any part of the O-ring is showing, the cap is not correctly sealed. Unscrew the cap and correctly seat it on the container
7	Place the sealed container upright in the insulated shipper. Also, place signed consent form in shipper (if this is the first blood draw)
8	Place the top piece of white EPS foam on top of the white EPS sides. The top EPS foam must sit flush on top of the EPS sides
9	Place faxed shipping form and signed consent form (only if this is the participant's first phlebotomy) on top of the white EPS foam lid
10	Close the outer box flaps and seal the top seam of the box with postal-lock tape
11	Using the postal-lock tape seal both side seams making sure that half the tape is on the top of the box and the other half is on the side of the box
12	The UN3373/Diagnostic Specimen and Room Temperature labels should already be affixed to the outside of the shipper for your convenience. Please note the FedEx air-bill must NOT BE wrapped around any of the corners
13	Ship specimens at Room Temperature (DO NOT add ice or gel packs to shipper)
14	Place shipper for Fed Ex pick-up. If you do not have regular FedEx pick-ups, call FedEx and schedule a same day pick-up

Step	Reception of specimens at BSRI
1	Use Universal Safety Precautions
2	Receive and deliver FedEx package(s) to the appropriate person
3	Unpack the contents of each package carefully
4	Retain original box for reuse and return to the shipping area within the VRLRC
5	Verify sample identification numbers with the invoice that arrives with the package
6	Copy specimen identification numbers along with any comments onto a VRLRC study specific log sheet
7	Date VRLRC study specific log sheet
8	Place invoice(s) and study specific log sheet(s) into the study specific log book
9	Make "in house" working labels using appropriate labeling program. Note, the labeling of tubes is study specific

6.4 Instructions for shipping plasma samples from BSRI to the testing labs

(See ROP H VRLRC 0011)

6.4.1. Materials required for shipment of frozen samples on dry ice

- Saf-T-PakTM Compliance Training for Shipping Class 6 Division 6.2 – Infectious Substances
- -80°C frozen infectious samples
- Insulated shipper for temperature sensitive infectious specimens (The shipping container will maintain a temperature of between 0º C and minus 44º C for 83 hours when using 7.8 kg of dry ice)
- Inner box (optional)
- Polystyrene cooler and lid
- Dry ice
- Hazard and handling labels:
  - Dry ice label (class 9 label)
  - A label that reads “Biological Substance, Category B UN3373”
  - Shipper’s name, address and phone number as well as the consignee’s name, address and phone number (on outside of outer box).
- Completed FedEx air bill form

Example of the items provided with Saf-T-Pak STP 320 (Diagnostic Specimens)

6.4.2 Procedures Using the Saf-T-Pak STP 320

Step	Shipment of frozen specimens on dry ice
1	Each tube must be placed inside a certified secondary container
2	Remove lid from polystyrene cooler
3	Place inner box into the polystyrene cooler

4	Amount of dry ice added is determined by the length of the shipment
5	The Place the Styrofoam lid onto the inner Styrofoam container (do not tape the styrofoam lid)
6	Partially seal the cardboard box so that the dry ice vapor can vent
7	Complete the FedEx air bill: 1) BSRI shipping address, 2) amount of dry ice used, 3) Section 2 – fill in the internal billing number 4) Section 4a – Check the "FedEx Priority Overnight" box, 5) Section 5 – Check the "other" box, 6) Section 6 – Check the box that says, "Yes Shipper's Declaration not required" and check the "Dry Ice" box and write "1" in the first blank line and the "kg" amount of dry ice used on the second line, i.e. 1x9 kg, 7) Section 7 – Check sender. Fill in the dry ice label on the outer box with the amount of dry ice used
8	Fill in the sender's and consignee's name and address on the outer box or use preprinted address stickers
9	Include with the shipment a listing of the specimen ID numbers contained in the shipment
10	Email the list of specimen ID numbers to the recipient
11	Alert the recipient a day in advance of the incoming shipment. Provide the recipient with the tracking number
12	Shipments may only be shipped on Monday through Thursday

6.5 Shipment of the WNV repository to NHLBI

The WNV specimens being held for long term storage will remain at BSRI in LN2 freezers at -196 °C until they will be shipped to the NHLBI Repository SeraCare BioServices. Further information on this process will be communicated as that date draws near.

Table 6.1 WNVDENV Shipping Schedule

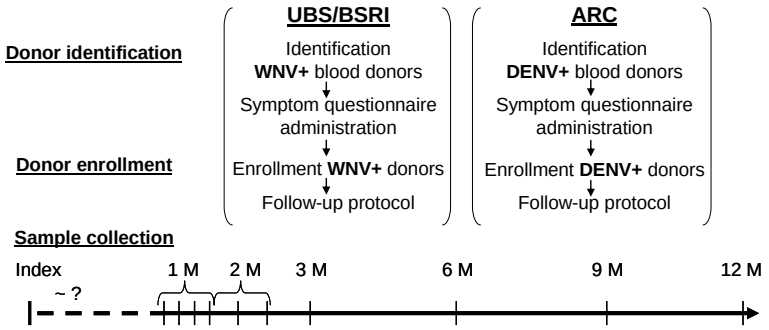
Phase	From	To	Type of specimen	Volume (mL)	Container	Shipping temperature	Number of batches	Time-points	Schedule
Pre-stocking with ready-to-go shippers	BSRI	UBS/BCP blood centers	Kit with EDTA vacutainer tubes and forms	N/A	EXAKT-PAK shipper for the transport of Biological Subtance, Category B	Ambiant	N/A	Pre-season	June and on-going (get one send one)
WNV collection	UBS/BCP blood centers	Viral Reference Laboratory and Repository Core atBSRI	EDTA anticoagulated Blood	75	7x10 mL EDTA (lavender top) 1 x 2 mL EDTA (lavender top) 1x3 mL Tempus	Ambiant	N/A	Study protocol	Study Participant visit
DENV collection	ARC collection sites in Peurto Rico	ARC San Juan	Blood	73	7x10 mL EDTA (lavender top) 1x3 mL Tempus	Ambiant	N/A	Study protocol	Study Participant visit
DENV CBC count	ARC collection sites in Peurto Rico	ARC San Juan	EDTA anticoagulated	10mL	10 mL EDTA tube	Ambiant	N/A	Index to M1	Study Participant visit
WNV TMA	BSRI	BSL Scottsdale	Plasma	3mL	1 x 12mm x 75mm tube (1.3 mL) and 1 x 2 mL cryovial tube (1.8 mL)	Dry ice (−78.5 °C)	approx.3 per season	Index to M6	?
WNV EIA	BSRI	Focus Cypress	Plasma	0.5	2 mL cryovial tube	Dry ice (−78.5 °C)	2	Index to M12	Completion M6 & M12
WNV PRNT	BSRI	CDC Fort-Collins	Plasma	1.8	2 mL cryovial tube	Dry ice (−78.5 °C)	1	Index	January 2011 & 2012
WNV Cytokines	BSRI	BSRI	Plasma	0.15	2 mL cryovial tube	Dry ice (−78.5 °C)	1	Index to M6	April 2010 & 2011 & 2012
WNV infectivity	BSRI	CDC PR	Plasma	0.5	2 mL cryovial tube	Dry ice (−78.5 °C)	1	Index to M1	February 2011 & 2012
DENV VL	ARC San Juan	BSRI / CDC PR	Plasma Blood	2	2 mL cryovial tube	Dry ice (−78.5 °C)	1	Index to M1	At the end of season for batch testing
DENV genotyping	ARC San Juan	BSRI / CDC PR	Plasma	2	2 mL cryovial tube	Dry ice (−78.5 °C)	1	Index	January 2011 & 2012
DENV TMA	ARC San Juan	ARC	Plasma	3 mL	2 mL cryovial tube	Dry ice (−78.5 °C)	approx.3 per season	Index to M6	?
DENV EIA	ARC San Juan	CDC PR	Plasma	0.5	2 mL cryovial tube	Dry ice (−78.5 °C)	2	Index to M12	At the end of the season for batch testing
DENV PRNT	ARC San Juan	CDC PR	Plasma	2	2 mL cryovial tube	Dry ice (−78.5 °C)	1	Index	January 2011 & 2012
DENV Cytokines	ARC San Juan	BSRI	Plasma	0.15	2 mL cryovial tube	Dry ice (−78.5 °C)	1	Index to M6	April 2011 & 2012
DENV infectivity	ARC San Juan	CDC PR	Plasma	0.5	2 mL cryovial tube	Dry ice (−78.5 °C)	1	Index to M1	February 2011 & 2012
WNV repository	BSRI	Sera Care	Plasma & PBMC	N/A	LN2 freezer	LN2 (−196 °C)	N/A	N/A	September 2012
DENV repository	ARC San Juan	Sera Care	Plasma & PBMC	N/A	LN2 freezer	LN2 (−196 °C)	N/A	N/A	September 2012

8. CHARACTERIZATION OF WNV+ BIOSPECIMENS

8.1 Overview

Once the samples have been collected and processed in plasma and PBMC aliquots different assays will be used to characterize those samples for viral and immunologic parameters.

As the assays will be run by different laboratories (Figure 1), some shipping plans have been developed in C6.



Sample characterization	Time-point	WNV	DENV
CBC/Platelets count)	Index	N/A	ARC
Blood processing (plasma/PBMC)	0 →12 M	BSRI	ARC
Viral load	0 → 1 M	BSRI	BSRI/CDC
TMA Index (neat and MP dilutions)	Index	BSL	ARC
TMA Follow-up (neat x 3)	0 → 6 M	BSL	ARC
NS1 Antigen	0 → 3 M	N/A	ARC
Genotyping	Index	N/A	BSRI/CDC
Antibody testing: EIA	0 → 6 M	Focus	CDC
Antibody testing: PRNT	0 → 6M	CDC	CDC
Cytokines/chemokines testing	0 → 6 M	BSRI	BSRI
Infectivity studies	0 → 1 M	CDC	CDC

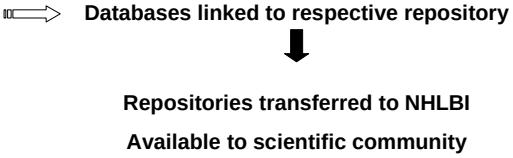


Figure 8A. Overview of the study flow work and sites for characterization assays

8.2 WNV+ biospecimen characterization

This study will involve prospective enrollment and frequent follow-up of a representative group of donors detected in the very early asymptomatic phase of WNV viremia.

We will perform virologic assays such as viral load quantification by real time PCR (index donation and first follow-up samples) in BSRI and replicate TMA (on all follow-up samples) in BSL laboratory.

Immunologic analyses will include: plaque reduction neutralization titration (PRNT) which is the most specific method for determining the presence of virus-specific antibodies and this will be done by the CDC in Fort-Collins. CO.

WNV IgM and IgG quantification will be done by ELISA assays at Focus, Cypress, CA.

Forty-two cytokines/chemokines will be measured using multiplex assays from Millipore (a combination of the Human Panel I standard sensitivity 39-plex assay with the human high sensitivity 13-plex assay) on BSRI's Luminex platform in San Francisco, CA.

Within these sequential samples, we will focus on the samples from WNV+ donors captured in the tail-end of the viremia (low-level viremia still detectable by TMA with presence of WNV-specific IgG and PRNT) to study their infectivity (low-level viremic "front end" samples will serve as controls). We will use serial dilutions of the plasma derived from these samples for infectivity studies done by the CDC in Puerto Rico. Vero cell lines and human monocytes will be inoculated in parallel and cultures monitored for cytopathic effect and for production of WNV virions by RT-PCR as described.

Procleix® WNV Assay

For *In Vitro* Diagnostic Use
5000 Test Kit

CONTENTS

SELECT THE APPROPRIATE SECTION FOR THE INSTRUMENT PLATFORM BEING USED

GENERAL INFORMATION

INTENDED USE

The PROCLEIX® WNV Assay is a qualitative *in vitro* nucleic acid assay system for the detection of West Nile Virus (WNV) RNA in plasma specimens from individual human donors, including volunteer donors of whole blood and blood components, and other living donors. It is also intended for use in testing plasma specimens to screen organ donors when specimens are obtained while the donor's heart is still beating, and in testing blood specimens to screen cadaveric (non-heart-beating) donors. It is not intended for use on cord blood specimens.

The assay is intended for use in testing individual donor samples. It is also intended for use in testing pools of human plasma comprised of equal aliquots of not more than 16 individual donations from volunteer donors of whole blood and blood components.

This assay is not intended for use as an aid in the diagnosis of West Nile Virus infection.

SUMMARY AND EXPLANATION OF THE TEST

WNV is a mosquito-borne flavivirus that is associated with human disease ranging from mild flu-like symptoms to severe neurological disease[1,2]. Most WNV infections are asymptomatic and approximately 20% lead to a mild illness known as West Nile virus fever. Less than 1% of infections are estimated to cause serious neurological disease, with advanced age being the most significant risk factor[3].

WNV was first isolated and identified in 1937 from a febrile person in the West Nile district of Uganda. Prior to 1999, the presence of the virus had not been documented in North America and was found only in the Eastern Hemisphere with wide distribution in Africa, Asia, the Middle East, and Europe[4]. Since the 1999 outbreak in Queens, New York, the virus has continued to expand westward in the United States. During the years 2000 and 2001, geographic spread to about half of the United States was documented via avian mortality surveillance; the virus is now thought to be permanently established in North America[5,6]. A large number of avian species serve as reservoir hosts for the virus, whereas humans and animals, such as horses and other mammals, are believed to be incidental hosts[7].

As of December 28, 2004, 2448 human WNV cases were reported to the CDC for the 2004 calendar year, 87 of which resulted in death. This compares to 9862 human cases, including both mild and severe disease cases, with 264 deaths for 2003, and 4156 WNV cases with 284 fatalities for 2002[8]. The principal route of human WNV infection is through the bite of an infected mosquito, predominantly by the bite of the *Culex, sp.* of mosquitoes. However, in 2002, new mechanisms of person-to-person transmission were documented, including possible mother to infant infection through breast milk, transplacental infection, possible dialysis-related transmission, and transmission through organ donation and blood transfusion. During 2003, twenty-three suspected cases of WNV transfusion-associated transmission (TAT) were reported to CDC; of these, six cases were classified as confirmed TAT cases. As of September 2004, one TAT case has been reported for the year[9-15].

In most human infections, WNV multiplies to a relatively low level producing a transient viremia that can be detected in whole blood, plasma, and serum. Current diagnostic methods for WNV include Immunoglobulin M (IgM) enzyme immunoassays, Plaque Reduction Neutralization assays, and nucleic acid testing (NAT) methods. IgM antibody can be detected in serum or cerebrospinal fluid (CSF) collected within eight days of illness onset but NAT methods are capable of detecting infection prior to the presence of antibodies during the viremic phase. Because serologically based assays detect host immune response after this primary viremic phase and IgM can remain in the body for long periods of time, these tests may not be appropriate for blood screening[3,7].

Screening of whole blood donations with NAT has been in place in the United States since early 1999 and licenses were granted for HIV-1 and HCV screening in 2002[16]. The PROCLEIX WNV Assay uses the same technology as the PROCLEIX® HIV-1/HCV Assay to detect WNV RNA and has been utilized in the United States for prospective blood screening since June 19, 2003 and was licensed in 2005.

PRINCIPLES OF THE PROCEDURE

The PROCLEIX® WNV Assay involves three main steps, which take place in a single tube: sample preparation; WNV RNA target amplification by Transcription-Mediated Amplification (TMA)[17]; and detection of the amplification products (amplicon) by the Hybridization Protection Assay (HPA)[18].

During sample preparation, RNA is isolated from specimens via the use of target capture. The specimen is treated with a detergent to solubilize the viral envelope, denature proteins and release viral genomic RNA. Oligonucleotides ("capture oligonucleotides") that are homologous to highly conserved regions of WNV are hybridized to the WNV RNA target, if present, in the test specimen. The hybridized target is then captured onto magnetic microparticles that are separated from the specimen in a magnetic field. Wash steps are utilized to remove extraneous components from the reaction tube. Magnetic separation and wash steps are performed with a target capture system.

Target amplification occurs via TMA, which is a transcription-based nucleic acid amplification method that utilizes two enzymes, MMLV reverse transcriptase and T7 RNA polymerase. The reverse transcriptase is used to generate a DNA copy (containing a promoter sequence for T7 RNA polymerase) of the target RNA sequence. T7 RNA polymerase produces multiple copies of RNA amplicon from the DNA copy template. The PROCLEIX WNV Assay utilizes the TMA method to amplify regions of WNV RNA.

Detection is achieved by HPA using single-stranded nucleic acid probes with chemiluminescent labels that are complementary to the amplicon. The labeled nucleic acid probes hybridize specifically to the amplicon. The Selection Reagent differentiates between hybridized and unhybridized probes by inactivating the label on unhybridized probes. During the detection step, the chemiluminescent signal produced by the hybridized probe is measured in a luminometer and is reported as Relative Light Units (RLU).

Internal Control is added to each test specimen, control (if used), and assay calibrator via the working Target Capture Reagent. The Internal Control in the PROCLEIX WNV Assay controls for specimen processing, amplification and detection steps. Internal Control signal is discriminated from the WNV signal by the differential kinetics of light emission from probes with different labels[19]. Internal Control-specific amplicon is detected using a probe with rapid emission of light (flasher signal). Amplicon specific to WNV is detected using probes with relatively slower kinetics of light emission (glower signal). The Dual Kinetic Assay (DKA) is a method used to differentiate between the signals from flasher and glower labels[19]. When used for the detection of WNV, the PROCLEIX WNV Assay differentiates between Internal Control and WNV signals.

REAGENTS

PROCLEIX® WNV Assay Kit: P/N 301187 – 5000 Test Kit

CONTENTS

Reagent Name	Number of vials/ Volume per vial
Internal Control Reagent *A HEPES buffered solution containing detergent and an RNA transcript.* *Store **unopened reagent** at –15° to –35°C.*	**10 x 5 mL**

CONTENTS

Reagent Name	Number of vials/ Volume per vial
Target Capture Reagent *A HEPES buffered solution containing detergent, capture oligonucleotides and magnetic microparticles. Store at 2° to 8°C (do not freeze). Internal Control Reagent must be added to Target Capture Reagent before use in the assay.*	**10 x 280 mL**
Amplification Reagent *Primers, dNTPs, NTPs and co-factors in TRIS buffered solution containing PROCLIN 300 as preservative.* *Store **unopened reagent** at –15° to –35°C.*	**10 x 50 mL**
Enzyme Reagent *MMLV Reverse Transcriptase and T7 RNA Polymerase in HEPES/TRIS buffered solution containing 0.05% sodium azide as preservative.* *Store **unopened reagent** at –15° to –35°C.*	**10 x 18 mL**
Probe Reagent *Chemiluminescent oligonucleotide probes in succinate buffered solution containing detergent.* *Store **unopened reagent** at –15° to –35°C.*	**10 x 75 mL**
Selection Reagent *Borate buffered solution containing surfactant.* *Store at 15° to 30°C.*	**10 x 180 mL**
PROCLEIX® WNV Negative Calibrator *A HEPES buffered solution containing detergent.* *Store at –15° to –35°C.* C0	**90 x 2 mL**
PROCLEIX® WNV Positive Calibrator *A HEPES buffered solution containing detergent and a WNV RNA transcript.* *Store at –15° to –35°C.* C1	**90 x 2 mL**

STORAGE INSTRUCTIONS

A. Room temperature is defined as 15° to 30°C.

B. The Probe Reagent is light sensitive. Protect this reagent from light during storage and preparation for use.

C. Do not use reagents or fluids after the expiration date.

D. Do not use assay-specific reagents from any other PROCLEIX® assay.

E. If a precipitate forms in the Target Capture Reagent (TCR) during storage, see instructions under REAGENT PREPARATION. DO NOT VORTEX. DO NOT FREEZE TCR.

Note: If after removing the TCR from storage at 2° to 8°C, the precipitate is allowed to settle to the bottom of the container, the likelihood of the formation of a gelatinous precipitate is increased substantially.

F. Do not refreeze Internal Control, Amplification, Enzyme, and Probe Reagents after the initial thaw.

G. Calibrators are single use vials and must be discarded after use.

H. If precipitate forms in the Wash Solution, Amplification Reagent, Selection Reagent, Probe Reagent, Negative Calibrator, or Positive Calibrator, see instructions under REAGENT PREPARATION.

I. Changes in the physical appearance of the reagent supplied may indicate instability or deterioration of these materials. If changes in the physical appearance of the reagents are observed (e.g., obvious changes in reagent color or cloudiness are indicative of microbial contamination), they should not be used.

J. Consult the following table for storage information.

Reagent/Fluid	Unopened Storage	Opened/Thawed Stability (up to expiration date)
Internal Control Reagent (IC)	–15° to –35°C until the expiration date	Prior to combining with TCR, 8 hours at RT*
Target Capture Reagent (TCR), wTCR**	2° to 8°C until the expiration date	30 days at 2° to 8°C; 80 hours at RT***
Probe Reagent	–15° to –35°C until the expiration date	30 days at 2° to 8°C; 80 hours at RT***
Amplification Reagent	–15° to –35°C until the expiration date	30 days at 2° to 8°C; 80 hours at RT***
Enzyme Reagent	–15° to –35°C until the expiration date	30 days at 2° to 8°C; 80 hours at RT***
Selection Reagent	RT until the expiration date	30 days at RT
Calibrators	–15° to –35°C until the expiration date	8 hours at RT
Auto Detect Reagents	RT until the expiration date	30 days at RT
Buffer for Deactivation Fluid	RT until the expiration date	30 days at RT
Deactivation Fluid	N/A	30 days at RT
Oil	RT until the expiration date	30 days at RT
Wash Solution	RT until the expiration date	30 days at RT

* RT = Room Temperature
** Stability time for TCR includes both before and after adding Internal Control
*** The 80 hours must occur within the 30 days.

SPECIMEN COLLECTION, STORAGE AND HANDLING

Note: Handle all specimens as if they are potentially infectious agents.

Note: Take care to avoid cross contamination during the sample handling steps. For example, discard used material without passing over open tubes.

LIVING DONOR BLOOD SPECIMENS

A. Blood specimens collected in glass or plastic tubes may be used.

B. Plasma collected in K_2EDTA, K_3EDTA, ACD, heparin, or sodium citrate, or in Becton Dickinson EDTA Plasma Preparation Tubes (BD PPT™), may be used. Follow sample tube manufacturer's instructions. Specimen stability is affected by elevated temperature.

Specimens may be stored for a total of 8 days from the time of collection to the time of testing with the following conditions:

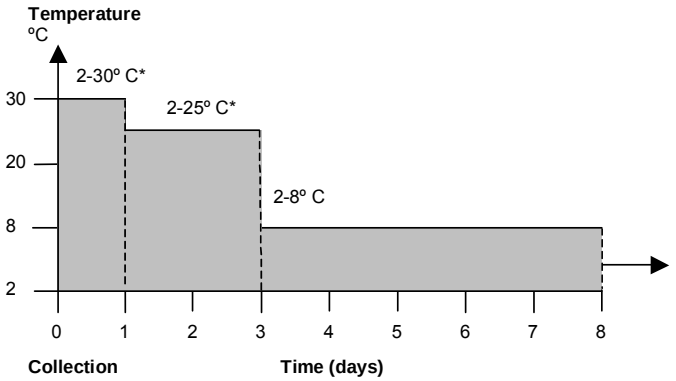
Specimens must be centrifuged within 72 hours of draw.

For storage above 8°C, specimens may be stored for 72 cumulative hours at up to 25°C, and up to 30°C for 24 cumulative hours during the 72 hours. This includes time on the instrument.

Other than noted above, specimens are stored at 2° to 8°C.

Refer to the example storage temperature table below.

Plasma separated from the cells may be stored for up to 9 months at ≤ –20°C or up to 15 months at ≤ –70°C before testing. Do not freeze whole blood.



*The 2-30° and 2-25°C periods indicated above may occur at any time.

C. Additional specimens may be taken from whole blood or plasma units containing CPD, CP2D, or CPDA-1 anticoagulants collected according to the collection container manufacturer's instructions.

Specimens may be stored for a total of 5 days from the time of collection to the time of testing with the following conditions:

Specimens must be centrifuged within 72 hours of draw.

For storage above 8°C, specimens may be stored for 72 cumulative hours at up to 25°C, and up to 30°C for 24 cumulative hours during the 72 hours. This includes time on the instrument.

Other than noted above, specimens are stored at 2° to 8°C.

Plasma separated from the cells may be stored for up to 9 months at ≤ –20°C or up to 15 months at ≤ –70°C before testing.

D. No adverse effect on assay performance was observed when plasma was subjected to three freeze-thaw cycles.

E. Specimens with visible precipitates or fibrinous material should be clarified by centrifugation for 10 minutes at 1000 to 3000 x g prior to testing. Do not test specimens that do not have sufficient sample volume above the gel separator or red cell interface.

F. Mix thawed plasma thoroughly and centrifuge for 10 minutes at 1000 to 3000 x g before testing. Centrifugation times and speeds for thawed BD PPT tubes must be validated by the user.

G. If specimens are to be shipped, they should be packaged and labeled in compliance with applicable federal and international regulations covering the transport of clinical specimens and etiologic agents.[20]

H. False positive results may occur if cross-contamination of specimens is not adequately controlled during specimen handling and processing.

I. Specimen Pooling

The pooling software, used in combination with a front end pipettor, performs sample scanning and pooling operations that combine aliquots from individual samples into a single Master Pool Tube, which may be used for further testing.

Note: Only specimens from donors of whole blood or blood components may be pooled.

CADAVERIC BLOOD SPECIMENS

Note: A serum or plasma specimen collected from a donor prior to death may be tested instead of a cadaveric blood specimen using either the instructions for cadaveric donor specimens or the instructions for living donor blood specimens.

A. Cadaveric blood specimens can be collected in clot or EDTA anti-coagulant tubes. Follow sample tube manufacturer's instructions.

B. For collection of specimens from cadaveric donors, follow general standards and/or regulations. Specimen stability is affected by elevated temperature.

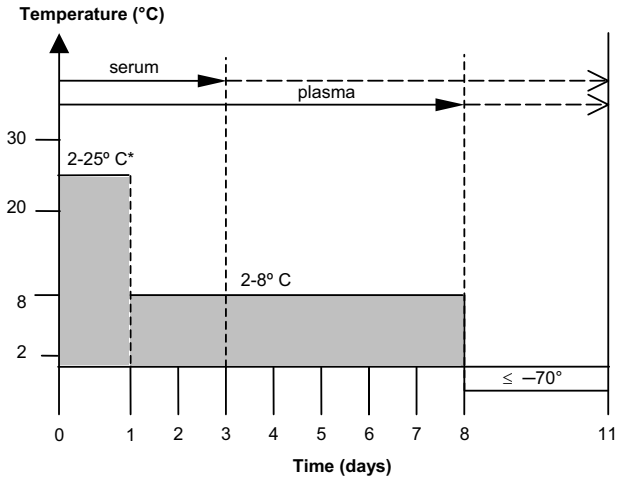
C. Plasma (EDTA collection tubes) may be stored for up to 24 hours at 2° to 25°C. Specimens may be stored for an additional 7 days at 2° to 8°C following centrifugation. Plasma separated from the cells may be stored for up to 11 days at ≤ –70°C before testing. Do not freeze whole blood.

D. Whole blood (clot tubes) and serum may be stored for up to 24 hours at 2° to 25°C. Specimens may be stored for an additional 2 days at 2° to 8°C following centrifugation. Serum removed from the clot may be stored for up to 11 days at ≤ –70°C before testing. Do not freeze whole blood.

E. No adverse effect on assay performance was observed when plasma and serum were subjected to three freeze-thaw cycles.

F. Specimens with visible precipitates or fibrinous material should be clarified by centrifugation for 10 minutes at 1000 to 3000 x g prior to testing. Do not test specimens that do not have sufficient sample volume above the gel separator or red cell interface.

G. Mix thawed plasma or serum thoroughly and centrifuge for 10 minutes at 1000 to 3000 x g before testing. Centrifugation times and speeds for thawed BD PPT tubes must be validated by the user.



*The 2–25°C period indicated above may occur at any time.

H. If specimens are to be shipped, they should be packaged and labeled in compliance with applicable federal and international regulations covering the transport of clinical specimens and etiologic agents.[20]

I. False positive results may occur if cross contamination of specimens is not adequately controlled during specimen handling and processing.

J. Cadaveric blood specimens may be diluted to overcome potential sample inhibitory substances or specimen shortage. Plasma and/or serum may be diluted 1:5 in saline (0.9% sodium chloride), i.e. 100 µL sample plus 400 µL saline. Diluted specimens should be inverted several times to mix and then may be used in standard assay procedure by pipetting the 500 µL of the diluted specimen into the TTU containing TCR.

Note: If a front-end pipettor will be used to pipette the samples, the minimum volume for the diluted sample should be 1100 µL (220 µL neat sample plus 880 µL saline).

Note: Studies performed to validate these conditions were performed on negative cadaveric specimens spiked with virus. The stability of WNV *in vivo* post-mortem was not assessed.

PROCLEIX® SYSTEM USERS

MATERIALS PROVIDED

PROCLEIX® WNV Assay 5000 Test Kit P/N 301187

Internal Control Reagent
Target Capture Reagent
Amplification Reagent
Enzyme Reagent
Probe Reagent
Selection Reagent
PROCLEIX® WNV Negative Calibrator
PROCLEIX® WNV Positive Calibrator

MATERIALS REQUIRED BUT PROVIDED SEPARATELY

PROCLEIX® Assay Fluids P/N 301116

Wash Solution
Oil
Buffer for Deactivation Fluid

PROCLEIX® Auto Detect Reagents P/N 301120

Auto Detect 1
Auto Detect 2

Disposables

(Disposables are single use only, do not reuse. Use of other disposables is not recommended.)

Ten-Tube Units (TTUs) P/N TU0040
Ten Tip Cassettes P/N 104578
Sealing Cards P/N 102085

Equipment/Software

PROCLEIX® System:

TECAN GENESIS RSP instrument (front end pipettor), PROCLEIX® Assay Software, and operator's manual; or PROCLEIX® Worklist Editor software and operator's manual

PROCLEIX® TCS (target capture system) and operator's manual

PROCLEIX® HC+ Luminometer, PROCLEIX® System Software, and operator's manual

Multi-tube Vortex Mixer (Vortexer)

Water bath

Dedicated fixed or adjustable repeat pipettors capable of delivering 25-500 µL of liquid with a ± 5% accuracy and a coefficient of variation of ≤ 5%.

Other

PROCLEIX® System Quick Reference Guide (PROCLEIX® System QRG)
Any applicable technical bulletins

OTHER MATERIALS AVAILABLE FROM CHIRON FOR USE WITH PROCLEIX® WNV ASSAY

PROCLEIX® WNV Assay Calibrators P/N 301186

PROCLEIX® WNV Negative Calibrator
PROCLEIX® WNV Positive Calibrator

PROCLEIX® Oil P/N 302441

General Equipment/Software

PROCLEIX® Reagent Preparation Incubator (RPI), independent temperature monitor (ITM), and operator's manual

PROCLEIX® CPT Pooling Software and operator's manual

For instrument specifics and ordering information, contact Chiron Customer Support.

MATERIALS REQUIRED BUT NOT PROVIDED

Repeat pipettor tips (1.25 mL, 5.0 mL, 10 mL, 12.5 mL)

If using the Manual Sample Pipetting Method: Filtered fixed pipettor tips capable of delivering 500 µL (for samples) and repeat pipettor tips capable of delivering 400 µL (for wTCR)

If using the TECAN GENESIS RSP instrument: Disposable 1000 µL conductive filter tips in rack approved for use with equipment and Front End Pipettor reagent troughs

Bleach

For use in final concentrations of 5% sodium hypochlorite and 0.5% sodium hypochlorite

Bleach alternative (optional)

Contact Chiron Technical Support for a list of bleach alternatives and instructions for use.

Sterile, polypropylene conical tubes with sealing caps. Freestanding tubes are recommended in two different sizes (5 mL to 10 mL tube and ≥ 30 mL tube). The tubes must be able to accommodate the diameter of a repeat pipettor tip.

PRECAUTIONS

A. For *In Vitro* diagnostic use.

B. When performing testing with different PROCLEIX® Assays using shared instrumentation, ensure appropriate segregation is maintained to prevent mix-up of samples during processing (e.g., use of colored TTU racks). In addition, verify that the correct set of reagents is being used for the assay that is being run.

C. Specimens may be infectious. Use Universal Precautions when performing the assay[21]. Proper handling and disposal methods should be established according to local, state and federal regulations[22,23]. Only personnel qualified as proficient in the use of the PROCLEIX® WNV Assay and trained in handling infectious materials should perform this procedure.

D. Use routine laboratory precautions. Do not pipette by mouth. Do not eat, drink or smoke in designated work areas. Wear disposable gloves and laboratory coats when handling specimens and kit reagents. Wash hands thoroughly after handling specimens and kit reagents.

E. To reduce the risk of invalid results, carefully read the entire package insert for the PROCLEIX WNV Assay and instrument and software operator's manuals prior to performing an assay run.

F. Material Safety Data Sheets are available upon request.

G. Avoid contact of Auto Detect Reagents 1 and 2 with skin, eyes and mucous membranes. Wash with water if contact with these reagents occurs. If spills of these reagents occur, dilute with water before wiping dry and follow appropriate site procedures.

H. Dispose of all materials that have come in contact with specimens and reagents according to local, state and federal regulations[22,23]. Thoroughly clean and disinfect all work surfaces.

I. Working TCR (wTCR) remaining in the reagent trough after the completion of the run must be discarded.

J. Use only supplied or specified required disposables.

K. Do not use this kit after its expiration date. DO NOT interchange, mix, or combine reagents from kits with different master lot numbers.

L. Avoid microbial and ribonuclease contamination of reagents.

M. Store all assay reagents at specified temperatures. The performance of the assay may be affected by use of improperly stored assay reagents. See STORAGE INSTRUCTIONS and REAGENT PREPARATION.

N. Store all specimens at specified temperatures. The performance of the assay may be affected by use of improperly stored specimens. See SPECIMEN COLLECTION, STORAGE AND HANDLING for specific instructions.

O. Only combine assay reagents or fluids as instructed to by the PROCLEIX WNV Assay package insert.

P. Refer to precautions in the appropriate package inserts, operator's manuals, and the PROCLEIX® System QRG.

REAGENT PREPARATION

These steps should be performed prior to beginning Target Capture in an area that is free of template and amplicon.

A. Room temperature is defined as 15° to 30°C.

B. Verify that reagents have not exceeded the expiration date and/or storage stability times.

C. Remove a bottle of Selection Reagent from room temperature storage.

1. The Selection Reagent must be at room temperature before use.
2. If Selection Reagent has been inadvertently stored at 2° to 8°C or the temperature of the laboratory falls to between 2° and 15°C, precipitate may form.
3. If precipitate forms in the Selection Reagent, heat at 60° ± 1° C for no more than 45 minutes, shaking the bottle frequently (every 5 to 10 minutes). Once all precipitate has gone back into solution, place the bottle in a room temperature water bath and allow the bottle to equilibrate for at least 1 hour. Alternatively, use the Reagent Preparation Incubator (RPI) as described in the PROCLEIX® System QRG. Do not use the Selection Reagent until it has equilibrated.
4. If foam is present, carefully remove it with sterile swabs or sterile pipettes. Use a new swab or pipette for each vial.
5. Do not use if precipitate or cloudiness persists.
6. Record the date that it was first opened (OPEN DATE) on the space provided on the label.

D. Warm all reagents to room temperature and mix thoroughly prior to use. A dedicated water bath at room temperature or the RPI may be used to aid this process. If using the RPI to warm the TCR, Probe Reagent, Enzyme Reagent, and Amplification Reagent, refer to the PROCLEIX System QRG.

1. If using a water bath, thaw reagents upright.
2. Amplification and Probe Reagents may be mixed by vortexing.
3. Enzyme Reagent should be mixed thoroughly by gentle inversion, taking care to avoid excessive foaming.
4. Record the date of thaw (THAW DATE) for each reagent on the space provided on the label.

E. Ensure that precipitates are dissolved. Do not use a reagent if gelling, precipitate or cloudiness is present.

F. DO NOT heat Probe Reagent above 30°C if using a water bath. DO NOT heat Probe Reagent above 35°C if using the RPI. Refer to the PROCLEIX System QRG.

G. Probe Reagent is light-sensitive. Protect this reagent from light during storage and preparation for use.

H. Precipitate will form in the Probe Reagent when stored at 2° to 8°C. Probe Reagent may be warmed in a water bath to facilitate dissolution of precipitate, but temperature in the bath should not exceed 30°C. The Probe Reagent may take up to 4 hours with periodic mixing to allow complete dissolution of precipitate if thawing is conducted on the lab bench. Alternatively, use the RPI to thaw the Probe Reagent at an average temperature of 32° ± 2°C, not to exceed 35°C. Refer to the PROCLEIX System QRG. Ensure that precipitates in the Probe Reagent are dissolved. Do not use if precipitate or cloudiness is present.

I. Prepare working Target Capture Reagent (wTCR):

1. Remove TCR from 2° to 8°C storage. IMMEDIATELY upon removing from storage, mix vigorously (at least 10 inversions). DO NOT VORTEX.
2. After mixing, place the TCR bottle at 22° to 30°C. Approximately every 10 minutes shake the bottle until all precipitate has disappeared. TCR precipitate should normally dissolve in about 30 minutes. Alternatively, use the RPI to thaw the TCR at an average temperature of 32° ± 2°C, not to exceed 35°C. Refer to the PROCLEIX System QRG.

   Note: If a gel is observed after performing this procedure, a new bottle must be used according to the handling recommendations above. Return the bottle with gel back to 2° to 8°C storage for subsequent use.
3. Thaw one vial of Internal Control (IC) Reagent up to 24 hours at 2° to 8° C or up to 8 hours at room temperature. **Do not use the RPI to thaw Internal Control Reagent**.
4. Mix the Internal Control Reagent thoroughly by inversion or vortexing.
5. When the Internal Control Reagent and TCR have reached room temperature, mix TCR thoroughly by inversion. Pour the entire vial of Internal Control Reagent into the TCR bottle. This is now the working Target Capture Reagent (wTCR). Mix thoroughly.
6. Use the space indicated on the TCR bottle to record the date Internal Control Reagent was added and lot number used (IC LOT). Record the expiration date of the wTCR in the space provided on the label.

J. Thaw calibrators at room temperature. **Do not use the RPI to thaw calibrators.**

1. These are single use vials and must be thawed prior to each run.
2. Mix calibrators gently by inversion to avoid foaming.
3. If foam is present, carefully remove it with sterile swabs or sterile pipettes. Use a new swab or pipette for each vial.

K. Wash Solution is shipped at ambient temperature and stored at room temperature. Precipitates may form in the Wash Solution during shipment or during storage when temperatures fall to between 2° and 15°C. Wash Solution may be warmed to facilitate dissolution of precipitate. **Do not use the RPI to warm the Wash Solution**. Temperature should not exceed 30°C. Ensure that precipitates in the Wash Solution are dissolved prior to use. Do not use if precipitate or cloudiness is present.

L. For Wash Solution, Oil, Selection Reagent, Buffer for Deactivation Fluid, Auto Detect 1, and Auto Detect 2, record the date the reagent was first opened (OPEN DATE) in the space provided on the label.

M. To prepare Deactivation Fluid, mix one part Buffer for Deactivation Fluid with one part 5% sodium hypochlorite. Record the date the Deactivation Fluid was prepared.

PROCEDURAL NOTES

Note: Refer to the PROCLEIX® System QRG for maintenance procedures and information about software operation.

A. To reduce the risk of invalid results, carefully read the entire package insert for the PROCLEIX® WNV Assay prior to performing an assay run. This package insert must be used with the PROCLEIX® System QRG and any applicable technical bulletins.

B. RUN SIZE

1. Kit size is based on an average run size of 55 tests. Smaller run sizes will result in a lower number of tests performed per kit.
2. Each run will yield up to 100 test results, including results for three replicates of the Positive Calibrator and three replicates of the Negative Calibrator.

C. EQUIPMENT PREPARATION

1. Three dedicated circulating water baths must be used: one for target capture and pre-amplification (60° ± 1°C), one for amplification (41.5° ± 1°C) and one for hybridization and selection (61° ± 2°C). An additional container of water is required to be maintained at 23° ± 4°C for the step preceding detection.
2. Equilibrate circulating water baths to 60° ± 1°C for target capture and 41.5° ± 1°C for amplification incubations.
3. If using a front end pipettor, set up according to instructions in the PROCLEIX System QRG.
4. Prepare the target capture system for use according to instructions in the PROCLEIX System QRG.
5. Wipe work surfaces and pipettors daily with diluted bleach (0.5% sodium hypochlorite in water). Allow bleach to contact surfaces and pipettors for at least 15 minutes and then follow with a water rinse. **A bleach alternative may be used in pre-amplification/ RPI areas only. Do not use bleach alternatives in amplification areas or in areas suspected to be contaminated with amplification products. Do not use deactivation fluid on surfaces.**
6. Equilibrate a circulating water bath to 61° ± 2°C for hybridization and selection incubations. Prepare a container of water at 23° ± 4°C for cool down prior to detection.
7. Prepare the luminometer according to instructions in the PROCLEIX System QRG.

D. REAGENTS

1. Add all reagents using a repeat pipettor capable of delivering specified volume with ± 5% accuracy and a precision of ≤ 5% CV. Check pipettor functionality monthly and calibrate regularly.
2. To minimize waste of Amplification, Oil, Enzyme, Probe, and Selection Reagents, aliquot each reagent for a given run size. Aliquoting must be performed after reagent preparation using sterile, polypropylene conical tubes with sealing caps in an area that is template and amplicon free. The aliquoting area must be wiped down with diluted bleach (0.5% sodium hypochlorite in water) before and after the aliquoting process. **A bleach alternative may be used in pre-amplification/RPI areas only. Do not use bleach alternatives in amplification areas or in areas suspected to be contaminated with amplification products.** The aliquoted reagents must be used the same day the aliquoting was performed. DO NOT store reagents in the aliquot conical tubes.
3. A color change will occur in the reaction tube after the addition of each of the following reagents: Amplification Reagent, Enzyme Reagent, Probe Reagent, and Selection Reagent.

E. RUN CONFIGURATION

A set of calibrators must be used at the beginning of each worklist. A set of calibrators consists of one vial each of Negative Calibrator and Positive Calibrator. Each calibrator is run in triplicate.

F. WORK FLOW

1. To minimize the possibility of laboratory areas from becoming contaminated with amplicon, the laboratory area should be arranged with a uni-directional workflow. Proceed from reagent preparation to sample preparation to amplification and then to detection areas. Samples, equipment and reagents should not be returned to the area where a previous step was performed. Also, personnel may not move from the dedicated Hybridization Protection Assay (HPA) area back into previous work areas without proper anti-contamination safeguards.
2. Perform reagent preparation in a clean (amplicon- and template-free) area.
3. Perform Sample Preparation, Target Capture and pre-Amplification steps in an amplicon-free area.
4. Perform HPA in an area separate from the reagent preparation and amplification areas.
5. Upon completion of pipetting specimens (individual samples or pools) into TTUs, the TTUs are removed from the deck and loaded into a TTU rack. If the operator needs to pipette the same specimens (individual samples or pools) for a different PROCLEIX® Assay, the empty calibrator tubes and TCR trough must be discarded but the specimens may be left on the deck. The operator should then change gloves after emptying the used empty calibrator tubes and TCR trough. Clean TTUs should then be loaded into the TTU carriers on the deck. See PROCLEIX® System Users, PRECAUTIONS, Step B, for additional information.

   Proceed with Section A, Sample Preparation under ASSAY PROCEDURE.

G. ENVIRONMENTAL CONDITIONS

1. The Target Capture, Amplification, HPA and Selection steps are temperature dependent. Therefore, it is imperative that the water baths are maintained within the specified temperature range. Use a calibrated thermometer.
2. Room temperature is defined as 15° to 30°C.
3. Detection is sensitive to temperature. The laboratory temperature in the detection area must be 21° to 27°C.
4. The operational conditions of the room in which the RPI runs must be within a temperature of 15° to 25°C.
5. Refer to instrument and software operator's manuals for additional environmental conditions requirements.

H. TIME

The Target Capture, Amplification, and HPA steps are all time dependent. Adhere to specific times outlined in ASSAY PROCEDURE.

I. VORTEXING

Proper vortexing is important to the successful performance of the PROCLEIX WNV Assay. Vortex equipment speed settings may vary. The vortexer speed should start at a low level and increase until the speed is adequate to achieve the desired results without allowing the reaction mixture to touch the sealing cards. **For each step that requires vortexing, it is critical that the content of the tubes be well-mixed.**

J. PIPETTING

1. All the pipettes used in the Target Capture, Amplification and HPA steps must be dedicated for these purposes only to avoid cross contamination.
2. Take care to deliver reagents, excluding working Target Capture Reagent (wTCR), to each tube without inserting pipette tip into the tube or touching the rim of the tube to minimize the chance of carryover from one tube to another.

3. When adding Oil, Probe Reagent, and Selection Reagent, angle the pipette tip toward the sides of the tube, not straight to the bottom, to avoid splashback.

K. MANUAL SPECIMEN PIPETTING

1. When using the manual sample/wTCR pipetting method, improper pipetting technique will affect the results of the assay. In order to avoid the loss of Positive ID Tracking, verification of correct sample ID by a second individual is recommended.
2. Ensure that the TTU is oriented in the rack with the pointed end on the left side and the rounded end on the right side of the rack. Pipette the first calibrator into the first tube next to the pointed end of the TTU. Samples are pipetted from left to right.
3. Use a new pipette tip for each sample and dispose of the tip in a biological waste container after use. Take care to avoid cross contamination by pipetting the specimens and discarding the used pipette tips without passing over open tubes or touching laboratory surfaces or other pieces of equipment.
4. To avoid the risk of contamination, clean and decontaminate manual sample pipettors between assay runs.
5. Ensure proper sample placement into the correct TTU position as indicated on the manual work list record.

L. DECONTAMINATION

1. The extremely sensitive nature of the test makes it imperative to take all possible precautions to avoid contamination. Laboratory bench surfaces and pipettes must be decontaminated daily with diluted bleach (0.5% sodium hypochlorite in water). Allow bleach to contact surfaces for at least 15 minutes and then follow with a water rinse. Chlorine solutions may pit equipment and metal. Thoroughly rinse bleached equipment to avoid pitting.
2. **A bleach alternative may be used in pre-amplification/RPI areas only. Do not use bleach alternatives in amplification areas or in areas suspected to be contaminated with amplification products.**
3. Reaction tubes must be decontaminated with Deactivation Fluid as described in the PROCLEIX System QRG.
4. Follow instructions provided in the PROCLEIX System QRG for instrument decontamination and maintenance procedures.

M. SEALING CARDS

1. When applying sealing cards, cover the TTUs with the sealing card and press gently to ensure complete contact with all of the tubes. Always use a new sealing card. DO NOT re-use sealing cards.
2. When removing sealing cards, carefully lift and peel in one continuous motion to avoid aerosols and cross contamination. Immediately dispose of card in appropriate waste container.

ASSAY PROCEDURE

PROCLEIX® WNV ASSAY ON INDIVIDUAL DONOR SPECIMENS OR POOLED SPECIMENS

All specimens (individual donations or pooled specimens) should be run in singlet in the PROCLEIX® WNV Assay.

PROCLEIX® WNV Assay Calibrators are to be used with the corresponding master lot of the PROCLEIX WNV Assay. The operator must check to ensure that the PROCLEIX WNV Assay Calibrators are used with the corresponding master lot of kit reagents as indicated on the PROCLEIX WNV Assay master lot sheet in use.

Specimens from other living donors (except whole blood or blood components) and from cadaveric donors must be tested neat using the individual donor testing method only. If the initial test result from a cadaveric blood specimen is invalid, the specimen may be diluted to overcome potential inhibitory substances as described in SPECIMEN COLLECTION, STORAGE AND HANDLING, Cadaveric Blood Specimens, and retested in singlet.

To run the PROCLEIX WNV Assay for the detection of WNV RNA, follow the steps below for Target Capture, Amplification and Hybridization Protection Assay.

Note: For instrument and software steps, refer to the PROCLEIX® System QRG.

Note: Continuous Process Flow: All process steps described below are intended to be completed in a continuous flow with a minimal, if any, delay between steps.

A. SAMPLE PREPARATION/TARGET CAPTURE

Sample Preparation

The PROCLEIX® WNV Assay has been validated using manual pipetting and a front end pipettor. The use of manual pipetting requires additional operator training and demonstration of proficiency. Repeat pipettors used in this step must be dedicated for use only in the TARGET CAPTURE steps.

IF USING THE MANUAL SAMPLE PIPETTING METHOD:

For sample tracking, an electronic worklist must be created using the PROCLEIX® Worklist Editor software. Refer to the PROCLEIX® System QRG for instructions, or contact Chiron Technical Support. Verification of correct sample ID on the worklist with the specimen tubes and with the detailed assay run report by a second individual is recommended. The assay results within the run report will be marked *M* indicating that the specimens were manually pipetted.

1. Load sufficient Ten Tube Units (TTUs) for the run into a TTU rack.
2. Thoroughly mix the wTCR immediately before use to resuspend microparticles.
3. Refer to the worklist and carefully pipette 400 µL of wTCR to each tube that will contain a sample. To dispense, insert the tip approximately one quarter of the way into the tube at an angle and pipette wTCR down the side of the tube. Take care to avoid touching the rim or the side of the tube with the pipette tip. Always pipette the wTCR first, followed by the sample.
4. Pipette samples.
   a. Refer to the worklist to identify the TTU number with the corresponding calibrator and test specimen identification numbers.
   b. Aspirate 500 µL of each calibrator, external quality control or test specimen from its collection tube using a single channel pipettor with corresponding filtered disposable tip. Insert only the end of the pipette tip into the sample. Do not disturb the sediment, if any.
   c. To dispense, insert the pipette tip halfway into the tube taking care not to touch the sides of the upper half of the tube with the pipette tip. At an angle, pipette the sample down the side of the bottom half of the tube. Hold down the plunger of the pipettor while removing it from the tube. Take care to avoid touching the rim or the side of the tube with the pipette tip when removing it from the tube.
5. Replace the pipette tip with a new tip and repeat Step 4 until all samples have been pipetted.
6. Visually inspect tubes to ensure proper sample volume and wTCR volume have been dispensed.
7. Cover the TTUs with sealing cards. See PROCLEIX® System Users, PROCEDURAL NOTES. Proceed to the Target Capture section.

IF USING A FRONT END PIPETTOR:

1. Prepare front end pipettor for automatic pipetting of calibrators, samples, and wTCR; refer to the PROCLEIX® System QRG.
2. Instrument will add 400 µL of wTCR to reaction tubes.

3. Instrument will add 500 µL each of calibrators and test samples into assigned reaction tubes.
4. When all samples have been pipetted, transfer the TTUs to a TTU rack. Cover the TTUs with sealing cards. See PROCLEIX® System Users, PROCEDURAL NOTES, Step M, SEALING CARDS for additional information.
5. Proceed to the Target Capture section.

Target Capture

1. Vortex the rack of TTUs a minimum of 20 seconds and until magnetic microparticles are resuspended. See PROCLEIX® System Users, PROCEDURAL NOTES on vortexing.
2. The rack may remain at room temperature up to 75 minutes prior to proceeding to the 60° ± 1°C incubation.
3. Incubate the tubes in a water bath at 60° ± 1°C for 20 minutes ± 1 minute.
4. Remove the rack of TTUs and transfer to the Target Capture area.
5. Incubate the rack of TTUs on the lab bench at room temperature for 14 to 20 minutes.
6. Transfer the rack of TTUs to the target capture system (TCS) for 9 to 20 minutes.
7. Carefully remove and dispose of the sealing cards.
8. To aspirate and wash, refer to the Target Capture section of the PROCLEIX System QRG.
9. Cover the TTUs with sealing cards.
10. Vortex to resuspend the microparticle pellets, then inspect the reaction tubes to make sure that all of the magnetic particles have been uniformly suspended.
11. Place the rack of TTUs on the TCS for 4 to 10 minutes.
12. Carefully remove and dispose of the sealing cards.
13. Repeat Steps 8 through 12.
14. Completely aspirate the solution from each tube. Refer to the Target Capture section of the PROCLEIX System QRG.
15. Cover the TTUs with sealing cards.
16. Proceed directly to Amplification.

B. AMPLIFICATION

Do not use bleach alternatives in this area.

The repeat pipettors used in this step must be dedicated for use only in AMPLIFICATION steps.

1. Carefully remove and dispose of the sealing cards.
2. Add 75 µL of Amplification Reagent to each tube (a color change can be observed in the reaction tube). See PROCLEIX® System Users, PROCEDURAL NOTES on pipetting.
3. Add 200 µL of Oil to each tube.
4. Cover the TTUs with sealing cards.
5. Vortex the rack of TTUs a minimum of 20 seconds until well-mixed and all microparticles are resuspended. Ensure that magnetic particles are no longer adhering to the walls of the tube, and are uniformly resuspended.
6. Incubate the TTUs in a water bath at 60° ± 1°C for 10 minutes ± 1 minute.
7. Incubate the TTUs in a water bath at 41.5° ± 1°C for 9 to 20 minutes.
8. Leaving the rack of TTUs at 41.5° ± 1°C, carefully remove and dispose of the sealing cards. Immediately add 25 µL of the Enzyme Reagent into each tube (a color change can be observed in the reaction tube). Place new sealing cards over the TTUs.
9. Remove the rack of TTUs from the water bath and shake to mix. DO NOT VORTEX. Minimize the time the tubes are out of the water bath.
10. Incubate the rack of TTUs in the water bath at 41.5° ± 1°C for 60 minutes ± 5 minutes.
11. Remove the rack of TTUs from the water bath and transfer it to the HPA area. Rack may remain at room temperature for up to 30 minutes prior to the addition of Probe Reagent.

C. HYBRIDIZATION PROTECTION ASSAY (HPA)

A separate, dedicated location for the Hybridization Protection Assay (HPA) step is recommended to minimize amplicon contamination in the assay. This dedicated area should be on a separate bench in a separate area from the reagent and sample preparation and amplification areas. **Do not use bleach alternatives in this area.**

The repeat pipettor used in this step must be dedicated for use only in HYBRIDIZATION PROTECTION ASSAY.

1. Hybridization
   a. Carefully remove and dispose of the sealing cards. See PROCLEIX® System Users, PROCEDURAL NOTES on pipetting.
   b. Add 100 µL of Probe Reagent into each tube (a color change can be observed in the reaction tube). See PROCLEIX® System Users, PROCEDURAL NOTES.
   c. Cover the TTUs with sealing cards. Vortex the rack of TTUs a minimum of 20 seconds and until contents are well-mixed. To avoid possible contamination, do not allow reaction mixture to come in contact with the sealing card. See PROCLEIX® System Users, PROCEDURAL NOTES on vortexing.
   d. Incubate the rack of TTUs in a dedicated water bath at 61° ± 2°C for 15 minutes ± 1 minute.
2. Selection
   a. Remove the rack of TTUs from the 61° ± 2°C water bath. Carefully remove and dispose of the sealing cards.
   b. Add 250 µL of Selection Reagent to each tube (a color change can be observed in the reaction tube).
   c. Cover the TTUs with sealing cards. Vortex the rack of TTUs a minimum of 20 seconds and until contents are well-mixed. To avoid possible contamination, do not allow reaction mixture to come in contact with the sealing card. Return the rack of TTUs to the 61° ± 2°C water bath for 10 minutes ± 1 minute.
   d. Cool the rack of TTUs in a 23° ± 4°C container of water for a minimum of 10 minutes while preparing for Detection.
   e. Remove the rack of TTUs from the 23° ± 4°C container of water onto absorbent material.
3. Detection

Note: Tube readings should be completed within 75 minutes after completing the selection reaction.

For detection and decontamination, refer to the PROCLEIX® System QRG.

QUALITY CONTROL PROCEDURES

I. ACCEPTANCE CRITERIA FOR THE PROCLEIX® WNV ASSAY

A. A run is valid if the minimum number of calibrators is valid and calibrators meet acceptance criteria (see section II below).

1. In a PROCLEIX® WNV Assay run, at least four of the six calibrator replicates must be valid. At least two of the three Negative Calibrator replicates and two of the three Positive Calibrator replicates must be valid.

2. The PROCLEIX® System Software will automatically invalidate a run if less than the minimum number of calibrator replicates is valid. All specimens in an invalid run due to calibrators must be retested.
3. Cutoff values will be automatically calculated for Internal Control (flasher) and analyte (glower) in a valid run (see section II).
4. In a valid run, specimens with an analyte signal (glower signal) greater than the analyte cutoff are not invalidated even if the Internal Control signal is below the cutoff. Specimens with an Internal Control (IC) signal above 500,000 RLU are invalidated by the software and the reactive status cannot be assessed. Positive Calibrators with an IC signal above 500,000 RLU are invalidated by the software.

B. An assay run or an individual sample may be invalidated by an operator if specific technical/operator/instrument difficulties were observed and documented. If individual samples are invalidated by an operator, then the percent invalid rate must be manually calculated.

C. The PROCLEIX System Software will print an alert on the run report when more than 10% of the calibrators and specimens in a run are invalid (see the PROCLEIX® System QRG for details). Specimens that are invalid solely due to insufficient sample or wTCR are not included in the calculation of the 10% invalid rate.

D. For runs that exceed the 10% invalid rate, further evaluation is required. Review package insert procedures to identify operator errors. In addition, the run report should be reviewed using the criteria described below.

   1. If the invalid specimens are all from the same TTU, those specimens contributing to the 10% invalid rate may have been inadequately washed, or erroneous reagent addition may have occurred. All nonreactive and invalid specimens in the affected TTU should be retested.
   2. If the invalid specimens are randomly located throughout the run and no specific cause can be identified, the nonreactive and invalid specimens must be retested.
   3. If the invalid specimens are randomly located throughout the run, a specific cause that explains the invalid result can be identified, and the remaining valid results have consistent IC RLU values, only invalid specimens may be retested.

   Note: Specimens with an overall interpretation of Reactive, as determined by the software, must become the test of record. The specimens should be resolved according to the resolution algorithm for reactive specimens, as explained in the INTERPRETATION OF RESULTS section.

II. ACCEPTANCE CRITERIA FOR THE CALIBRATION AND CALCULATION OF CUTOFF FOR THE PROCLEIX® WNV ASSAY

Negative Calibrator Acceptance Criteria

The Negative Calibrator (NC) is run in triplicate in the PROCLEIX® WNV Assay. Each individual Negative Calibrator replicate must have an Internal Control (IC) value greater than or equal to 75,000 RLU and less than or equal to 400,000 RLU. Each individual Negative Calibrator replicate must also have an analyte value less than or equal to 40,000 RLU and greater than or equal to 0 RLU. If one of the Negative Calibrator replicate values is invalid due to an IC value or an analyte value outside of these limits, the Negative Calibrator mean (NC_x) will be recalculated based upon the two acceptable values. The run is invalid and must be repeated if two or more of the three Negative Calibrator replicate values have IC values or analyte values that are outside of these limits.

Determination of the mean of the Negative Calibrator values (NC_x) for Internal Control [NC_x (Internal Control)]

Example:

Negative Calibrator		Internal Control Relative Light Units
1		235,000
2		200,000
3		210,000
Total Internal Control RLU	=	645,000

$$NC_x\,(\text{Internal Control}) = \frac{\text{Total Internal Control RLU}}{3} = 215{,}000$$

Determination of the mean of the Negative Calibrator values (NC_x) for Analyte [NC_x (Analyte)]

Example:

Negative Calibrator		Analyte Relative Light Units
1		14,000
2		16,000
3		15,000
Total Analyte RLU	=	45,000

$$NC_x\,(\text{Analyte}) = \frac{\text{Total Analyte RLU}}{3} = 15{,}000$$

Positive Calibrator Acceptance Criteria

The Positive Calibrator (PC) is run in triplicate in the PROCLEIX WNV Assay. Individual Positive Calibrator analyte values must be less than or equal to 2,700,000 RLU and greater than or equal to 400,000 RLU. IC values may not exceed 500,000 RLU. If one of the Positive Calibrator values is outside these limits, the Positive Calibrator mean (PC_x) will be recalculated based upon the two acceptable Positive Calibrator values. The run is invalid and must be repeated if two or more of the three Positive Calibrator analyte values are outside of these limits.

Determination of the mean of the Positive Calibrator (PC_x) values for Analyte [PC_x (Analyte)]

Example:

Positive Calibrator		Analyte Relative Light Units
1		1,250,000
2		1,500,000
3		1,150,000
Total Analyte RLU	=	3,900,000

$$PC_x\,(\text{Analyte}) = \frac{\text{Total Analyte RLU}}{3} = 1{,}300{,}000$$

Calculation of the Internal Control Cutoff Value

Internal Control Cutoff Value = 0.5 X [NC_x (Internal Control)]

Using values given in the Negative Calibrator example above:

Internal Control Cutoff Value = 0.5 X (215,000)

Internal Control Cutoff Value = 107,500 RLU

Calculation of the WNV Analyte Cutoff Value

Analyte Cutoff Value = NC_x (Analyte) + [0.03 X PC_x (Analyte)]

Using values given in the Negative Calibrator and Positive Calibrator examples above:

Analyte Cutoff Value = 15,000 + (0.03 X 1,300,000)

Analyte Cutoff Value = 54,000 RLU

Summary of Acceptance Criteria for PROCLEIX® WNV Assay

Acceptance Criteria:	
Negative Calibrator	
Analyte	≥ 0 and ≤ 40,000 RLU
Internal Control	≥ 75,000 and ≤ 400,000 RLU
Positive Calibrator	
Analyte	≥ 400,000 and ≤ 2,700,000 RLU
Internal Control	≤ 500,000 RLU

Summary of Cutoff Calculations for PROCLEIX® WNV Assay

Analyte Cutoff =	NC Analyte Mean RLU + [0.03 X (PC Analyte Mean RLU)]
Internal Control Cutoff =	0.5 X (Negative Calibrator IC Mean RLU)

INTERPRETATION OF RESULTS

All calculations described above are performed by the luminometer software. Two cutoffs are determined for the PROCLEIX® WNV Assay: one for the Analyte Signal (glower signal) termed the Analyte Cutoff and one for the Internal Control Signal (flasher signal) termed the Internal Control Cutoff (IC Cutoff). The calculation of these cutoffs is shown above. For each sample, an Analyte Signal RLU value and Internal Control Signal RLU value is determined. Analyte Signal RLU divided by the Analyte Cutoff is abbreviated as the Analyte Signal/Cutoff (S/CO) on the report.

A specimen is Nonreactive if the Analyte Signal is less than the Analyte Cutoff (i.e., Analyte S/CO < 1.00) and the Internal Control (IC) Signal is greater than or equal to the Internal Control Cutoff (IC Cutoff) and less than or equal to 500,000 RLU. A specimen is Reactive if the Analyte Signal is greater than or equal to the Analyte Cutoff (i.e., Analyte S/CO ≥1.00) and the Internal Control Signal is less than or equal to 500,000 RLU. Reactive results will be designated by the software. A specimen is Invalid if the Analyte Signal is less than the Analyte Cutoff (i.e., Analyte S/CO < 1.00) and the Internal Control Signal is less than the Internal Control Cutoff. Any specimen with Internal Control values greater than 500,000 RLU is considered Invalid and the reactive status cannot be assessed.

Cadaveric blood specimens, when tested neat, may be invalid due to inhibitory substances within the specimen. These invalid specimens may be diluted as described in SPECIMEN COLLECTION, STORAGE AND HANDLING, Cadaveric Blood Specimens, and retested in singlet.

Summary of Specimen Interpretation:

Specimen Interpretation	Criteria
NonReactive	Analyte S/CO < 1.00 and IC ≥ IC Cutoff and IC ≤ 500,000 RLU
Reactive	Analyte S/CO ≥ 1.00 and IC ≤ 500,000 RLU
Invalid*	IC > 500,000 RLU or Analyte S/CO < 1.00 and IC < IC Cutoff

*For specimens with IC signal greater than 500,000 RLU, the specimen will be invalidated by the software and the reactive status cannot be assessed.

1. Any specimen, including cadaveric specimens, with an interpretation of Invalid in the PROCLEIX WNV Assay must be retested in singlet. Cadaveric specimens previously diluted 1:5 may be retested diluted 1:5.
2. If at any point in the testing algorithm there is insufficient volume to complete the testing then an alternate specimen from the index donation may be used as long as the storage criteria in the package insert are met.
3. Specimens with a valid Internal Control value and with an Analyte S/CO less than 1.00 in the PROCLEIX WNV Assay are considered Nonreactive for WNV RNA.
    a. IF THE NONREACTIVE SPECIMEN IS A POOL, then each of the individual specimens comprising the pool is considered Nonreactive and no further testing is required.
    b. IF THE NONREACTIVE SPECIMEN IS FROM AN INDIVIDUAL DONATION, then the individual specimen is considered Nonreactive for WNV and no further testing is required.
4. Specimens with an Analyte S/CO greater than or equal to 1.00 with IC signal less than or equal to 500,000 RLU are considered Reactive.
    a. IF THE REACTIVE SPECIMEN IS A POOL, then each of the individual specimens comprising the pool must be tested with the PROCLEIX WNV Assay.
        1. If an individual specimen tests Nonreactive with the PROCLEIX WNV Assay, then the specimen is considered Nonreactive for WNV and no further testing is required.
        2. If an individual specimen tests Reactive with the PROCLEIX WNV Assay, then the individual specimen is considered Reactive for WNV. Further clarification of the Reactive specimens for informational purposes may be obtained by testing an alternate specimen from the index donation with the PROCLEIX WNV Assay and/or by follow-up testing. Results of testing obtained for clarification do not replace test results for purposes of donor eligibility.
    b. IF THE REACTIVE SPECIMEN IS FROM AN INDIVIDUAL DONATION, then the individual specimen is considered Reactive for WNV. Further clarification of the Reactive specimens for informational purposes may be obtained by testing an alternate specimen from the index donation with the PROCLEIX WNV Assay and/or by follow-up testing. Results of testing obtained for clarification do not replace test results for purposes of donor eligibility.
5. Reactive specimens in an operator-invalidated run due to the 10% invalid rate are identified by the software as reactive and must become the test of record. Any reactive result should be resolved according to the resolution algorithm for reactive specimens, as explained in the INTERPRETATION OF RESULTS section, step 4.

PROCLEIX® TIGRIS® SYSTEM USERS

MATERIALS PROVIDED

PROCLEIX® WNV Assay **5000 Test Kit** P/N 301187

Internal Control Reagent
Target Capture Reagent
Amplification Reagent
Enzyme Reagent
Probe Reagent
Selection Reagent
PROCLEIX® WNV Negative Calibrator
PROCLEIX® WNV Positive Calibrator

MATERIALS REQUIRED BUT PROVIDED SEPARATELY

PROCLEIX® Assay Fluids P/N 301116

Wash Solution
Oil
Buffer for Deactivation Fluid

PROCLEIX® Auto Detect Reagents P/N 301120

Auto Detect 1
Auto Detect 2

PROCLEIX® System Fluid Preservative P/N 301175

PROCLEIX® WNV TIGRIS® Controls P/N 301185

PROCLEIX® WNV TIGRIS® Negative Control
PROCLEIX® WNV TIGRIS® Positive Control

Disposables

(Disposables are single use only, do not reuse. Use of other disposables is not recommended.)

Multi-Tube Units (MTUs) – case of 100 P/N 104772

Waste Bag Kit (MTU and Tiplet) – 30 of each P/N 900907

MTU Waste Cover P/N 105523

MTU Waste Deflector P/N 900931

Reagent Spare Caps
(TCR, Selection, Probe Reagent) P/N CL0039

Reagent Spare Caps
(Amplification Reagent) P/N CL0042

Reagent Spare Caps
(Enzyme Reagent) P/N 501619

PROCLEIX® TIGRIS® System Maintenance Bottle Kit P/N 105655

Equipment/Software

PROCLEIX® TIGRIS® System, PROCLEIX® TIGRIS® System Software, PROCLEIX® WNV Assay Software, and operator's manual

PROCLEIX® Reagent Preparation Incubator (RPI), independent temperature monitor (ITM), and operator's manual

Other

PROCLEIX® TIGRIS® System *Quick Reference Guide* (PROCLEIX® TIGRIS® System QRG)

Any applicable technical bulletins

OTHER MATERIALS AVAILABLE FROM CHIRON FOR USE WITH PROCLEIX® WNV ASSAY

PROCLEIX® WNV Assay Calibrators P/N 301186

PROCLEIX® WNV Negative Calibrator
PROCLEIX® WNV Positive Calibrator

PROCLEIX® Oil P/N 302441

General Equipment/Software

TECAN GENESIS RSP instrument (front end pipettor) for pooling only, PROCLEIX® CPT Pooling Software, operator's manual, and quick reference guide

For instrument specifics and ordering information, contact Chiron Customer Support.

MATERIALS REQUIRED BUT NOT PROVIDED

Disposable conductive filter tips (DiTis) in rack approved for use with equipment (required for pooling only)

Bleach

For use in final concentrations of 5% sodium hypochlorite and 0.5% sodium hypochlorite

Bleach alternative (optional)

Contact Chiron Technical Support for a list of bleach alternatives and instructions for use.

Alcohol (70% ethanol, 70% isopropyl alcohol solution, or 70% isopropyl alcohol wipes)

Water for the PROCLEIX TIGRIS System

For water specifications for the PROCLEIX TIGRIS System, see the *PROCLEIX®* TIGRIS® System *Operator's Manual.*

Disposable 1000 µL conductive filter tips in rack approved for use with the PROCLEIX® TIGRIS® System. Contact Chiron Technical Support for approved tips.

PRECAUTIONS

A. For *In Vitro* diagnostic use.

B. When performing testing with different PROCLEIX® Assays using shared instrumentation, ensure appropriate segregation is maintained to prevent mix-up of samples during processing. In addition, verify that the correct set of reagents is being used for the assay that is being run.

C. Specimens may be infectious. Use Universal Precautions when performing the assay[21]. Proper handling and disposal methods should be established according to local, state and federal regulations[22,23]. Only personnel qualified as proficient in the use of the PROCLEIX® WNV Assay and trained in handling infectious materials should perform this procedure.

D. Use routine laboratory precautions. Do not pipette by mouth. Do not eat, drink or smoke in designated work areas. Wear disposable gloves and laboratory coats when handling specimens and kit reagents. Wash hands thoroughly after handling specimens and kit reagents.

E. To reduce the risk of invalid results, carefully read the entire package insert for the PROCLEIX WNV Assay and the operator's manual for the PROCLEIX® TIGRIS® System prior to performing an assay run.

F. Material Safety Data Sheets are available upon request.

G. Avoid contact of Auto Detect Reagents 1 and 2 with skin, eyes and mucous membranes. Wash with water if contact with these reagents occurs. If spills of these reagents occur, dilute with water before wiping dry and follow appropriate site procedures.

H. Dispose of all materials that have come in contact with specimens and reagents according to local, state and federal regulations[22,23]. Thoroughly clean and disinfect all work surfaces.

I. Use only supplied or specified required disposables.

J. Do not use this kit after its expiration date. DO NOT interchange, mix, or combine reagents from kits with different master lot numbers.

K. Avoid microbial and ribonuclease contamination of reagents.

L. Store all assay reagents at specified temperatures. The performance of the assay may be affected by use of improperly stored assay reagents. See STORAGE INSTRUCTIONS and REAGENT PREPARATION.

M. Store all specimens at specified temperatures. The performance of the assay may be affected by use of improperly stored specimens. See SPECIMEN COLLECTION, STORAGE AND HANDLING for specific instructions.

N. Only combine assay reagents or fluids as instructed to by the PROCLEIX WNV Assay package insert. Do not top off reagents or fluids. The PROCLEIX TIGRIS System verifies reagent levels.

O. The PROCLEIX TIGRIS System groups a quadrant of reagents into a matched set the first time that it scans their barcodes during the inventory process and are required to be run as a set in all subsequent worklists. Bottles belonging to a matched set cannot be swapped with bottles in other kits of reagents. Refer to the PROCLEIX® TIGRIS® System QRG for more information.

P. When running a worklist or control bracket of 50 or fewer specimens, the software does not apply the 10% invalid rate. See PROCLEIX TIGRIS System Users, QUALITY CONTROL PROCEDURES.

Q. Resolution of pools is not performed by the PROCLEIX TIGRIS System. Follow laboratory procedures for resolving pools.

R. Refer to precautions in the appropriate PROCLEIX® Assay package inserts and the PROCLEIX TIGRIS System operator's manual and QRG.

REAGENT PREPARATION

A. Room temperature is defined as 15° to 30°C.

B. Choose a new or opened matched set of reagents that will be sufficient to complete testing of the number of samples in a worklist. Do not use reagents that have been used outside the PROCLEIX® TIGRIS® System or on another PROCLEIX TIGRIS System, as the instrument verifies reagent volumes.

C. Verify that the reagents have not exceeded the expiration date and/or storage stability times, including onboard stability.

1. The PROCLEIX TIGRIS System does not track the room temperature stability of reagents or fluids. However, it does track the number of hours each reagent and fluid is loaded onboard the analyzer. The PROCLEIX TIGRIS System will not allow an assay to be run using reagents that have expired or exceeded their onboard stability. Consult the following table for onboard stability information.

Reagent/Fluid	Onboard Stability*
wTCR, Probe Reagent, Enzyme Reagent, Amplification Reagent, Selection Reagent	60 hours**
Wash Solution, Oil, System Fluid, Deactivation Fluid, Auto Detect Reagents	14 days

* The onboard time must occur within the room temperature times listed in General Information, STORAGE INSTRUCTIONS.

** Worklists cannot be queued using reagents that have been onboard for more than 48 hours.

2. Print an Assay Reagent Status Report to check the stability remaining for unexpired reagent sets in the system's database.

D. Remove a bottle of Selection Reagent from room temperature storage.

1. The Selection Reagent must be at room temperature before use.
2. If Selection Reagent has been inadvertently stored at 2° to 8°C or the temperature of the laboratory falls between 2° and 15°C, use the RPI as described in the PROCLEIX® TIGRIS® System QRG, as precipitate may form.
3. If cloudiness or precipitate is present, use the RPI as described in the PROCLEIX TIGRIS System QRG. Do not use if precipitate or cloudiness persists.
4. If foam is present, carefully remove it with sterile swabs or sterile pipettes. Use a new swab or pipette for each vial.
5. Record the date that it was first opened (OPEN DATE) on the space provided on the label.

E. To prepare the following reagents using the RPI, refer to the PROCLEIX TIGRIS System QRG: TCR, Probe Reagent, Enzyme Reagent, and Amplification Reagent. Record the date of thaw (THAW DATE) for reagent on the space provided on the label.

F. Ensure that precipitates are dissolved. Do not use a reagent if gelling, precipitate or cloudiness is present.

G. Prepare working Target Capture Reagent (wTCR):

1. Remove TCR from 2° to 8°C storage. IMMEDIATELY upon removing from storage, mix vigorously (at least 10 inversions). DO NOT VORTEX.
2. Place TCR into the RPI, and refer to PROCLEIX TIGRIS System QRG for instructions.
3. Thaw one vial of Internal Control (IC) Reagent up to 24 hours at 2° to 8° C or up to 8 hours at room temperature. **Do not use the RPI to thaw Internal Control Reagent.**
4. Mix the Internal Control Reagent thoroughly by gentle inversion or vortexing.
5. After unloading TCR from the RPI and warming the Internal Control Reagent to room temperature, pour the entire vial of Internal Control Reagent into the TCR bottle. This is now the working Target Capture Reagent (wTCR). Mix thoroughly.
6. Use the space indicated on the TCR bottle to record the date Internal Control Reagent was added and lot number used (IC LOT). Record the expiration date of the wTCR in the space provided on the label.
7. Retain the Internal Control vial to scan the barcode label into the system.

H. Thaw calibrators at room temperature. **Do not use the RPI to thaw calibrators.**

1. These are single use vials which must be thawed prior to each run.
2. Mix calibrators gently by inversion to avoid foaming.
3. If foam is present, remove it with sterile swabs or sterile pipettes. Use a new swab or pipette for each vial.

I. Follow instructions provided in the PROCLEIX® WNV TIGRIS® Controls package insert for preparation of PROCLEIX WNV TIGRIS Controls. **Do not use the RPI to thaw PROCLEIX WNV TIGRIS Controls.**

1. Avoid reagent foaming.
2. If foam is present, carefully remove it with sterile swabs or sterile pipettes. Use a new swab or pipette for each vial.

J. Wash Solution is shipped at ambient temperature and stored at room temperature. Precipitates may form in the Wash Solution during shipment or during storage when temperatures fall to between 2° and 15ºC. Wash Solution may be warmed to facilitate dissolution of precipitate. **Do not use the RPI to warm the Wash Solution**. Temperature should not exceed 30ºC. Ensure that precipitates in the Wash Solution are dissolved prior to use. Do not use if precipitate or cloudiness is present.

K. For the Wash Solution, Oil, Auto Detect 1, and Auto Detect 2, record the date the fluid was first opened and loaded onto the PROCLEIX TIGRIS System (OPEN DATE) in the space provided on the label.

L. To prepare Deactivation Fluid, combine Buffer for Deactivation Fluid with 5% sodium hypochlorite in the Deactivation Fluid bottle.

1. Fill the Deactivation Fluid bottle with 5% sodium hypochlorite to between the liquid fill lines.
2. Pour entire contents of one bottle of Buffer for Deactivation Fluid into the Deactivation Fluid bottle.
3. Place the barcode label from the Buffer for Deactivation Fluid bottle on the top of the Deactivation Fluid bottle. This barcode is required to be scanned into the system during Fluid Inventory.
4. Record the date the Deactivation Fluid was prepared on the Buffer for Deactivation Fluid label.

M. To prepare System Fluid, combine PROCLEIX® System Fluid Preservative with water for the PROCLEIX TIGRIS System in the System Fluid Bottle. For water specifications for the PROCLEIX TIGRIS System, see the *PROCLEIX® TIGRIS® System Operator's Manual.*

1. Fill the System Fluid Bottle to the liquid fill line with water for the PROCLEIX TIGRIS System.
2. Pour entire contents of one bottle of PROCLEIX System Fluid Preservative into the System Fluid bottle.
3. Invert System Fluid Bottle to mix completely.
4. Place the barcode label from the PROCLEIX System Fluid Preservative on the top of the System Fluid bottle. This barcode is required to be scanned into the system during Fluid Inventory.
5. Record the date the System Fluid was prepared on the System Fluid Preservative label.

N. Load Fluids on the PROCLEIX TIGRIS System according to instructions provided in the PROCLEIX TIGRIS System QRG.

PROCEDURAL NOTES

Note: Refer to the PROCLEIX® TIGRIS® System QRG for maintenance procedures and information about software operation.

A. To reduce the risk of invalid results, carefully read the entire package insert for the PROCLEIX® WNV Assay prior to performing an assay run. This package insert must be used with the PROCLEIX TIGRIS System QRG and any applicable technical bulletins.

B. RUN SIZE

1. Kit size is based on an average run size of 55 tests. Smaller run sizes will result in a lower number of tests performed per kit.
2. Each run (also identified as a worklist) may contain up to 500 tests.

C. EQUIPMENT PREPARATION

See the PROCLEIX TIGRIS System QRG.

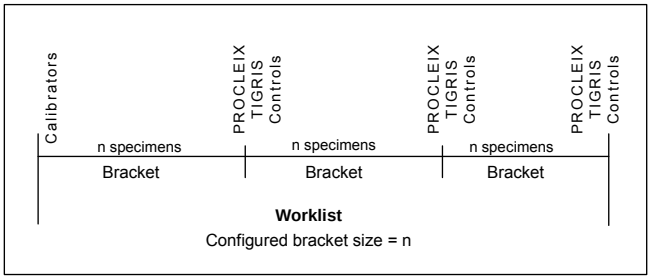
D. RUN CONFIGURATION

1. Each run (also identified as a worklist) must have a set of PROCLEIX® WNV Assay Calibrators at the beginning and a set of PROCLEIX® WNV TIGRIS® Controls at the end.

   a. A set of calibrators consists of one vial each of PROCLEIX® WNV Negative Calibrator and PROCLEIX® WNV Positive Calibrator. Each calibrator is run in triplicate.

   b. A set of PROCLEIX WNV TIGRIS Controls consists of one vial each of PROCLEIX® WNV TIGRIS® Negative Control and PROCLEIX® WNV TIGRIS® Positive Control. Each PROCLEIX WNV TIGRIS Control is run in singlet.

2. Using additional sets of PROCLEIX WNV TIGRIS Controls, each run (worklist) can be divided into smaller subsets called control brackets. A control bracket is defined as a group of specimens within a worklist that has a set of PROCLEIX WNV TIGRIS Controls at each end. The results of each bracket are reported based on the validity criteria of each control set (see PROCLEIX TIGRIS System Users, QUALITY CONTROL PROCEDURES for more details). The default bracket size is 172, but this feature is configurable in the PROCLEIX® TIGRIS® System Software and can be changed to any value between 1 and 492. In the first bracket of a worklist, PROCLEIX WNV TIGRIS Controls are not required at the beginning of the bracket.



3. A printed worklist report may assist operators in locating the rack and tube position where calibrators and controls are to be placed in a worklist. Refer to the PROCLEIX TIGRIS System QRG for instructions on how to view/print a worklist report.
4. Calibrator and PROCLEIX WNV TIGRIS Control tube placement is automatically read and verified by the PROCLEIX TIGRIS System. The PROCLEIX® TIGRIS® System will not allow assay processing if a calibrator or PROCLEIX WNV TIGRIS Control is placed in an incorrect tube position in a worklist or has an unreadable or missing barcode.
5. Test results from completed brackets of in-process run (worklist) can be viewed or printed by the operator before processing of the entire run is finished. Refer to the PROCLEIX TIGRIS System QRG for instruction on how to view/print test results.

E. WORK FLOW

1. Perform reagent preparation in a clean (amplicon- and template-free) area.
2. The sample loading area must be amplicon free.

F. ENVIRONMENTAL CONDITIONS

1. The operational conditions of the room in which the PROCLEIX TIGRIS System runs must be within a temperature of 15° to 25°C and humidity of 20 to 85%.
2. Refer to instrument and software operator's manuals for additional environmental conditions requirements.

G. DECONTAMINATION

1. The extremely sensitive nature of the test makes it imperative to take all possible precautions to avoid contamination. Laboratory bench surfaces must be decontaminated daily with 0.5% sodium hypochlorite in water (diluted bleach). Allow bleach to contact

surfaces for at least 15 minutes, then follow with a water rinse. Chlorine solutions may pit equipment and metal. Thoroughly rinse bleached equipment to avoid pitting.

2. A bleach alternative may be used in the sample preparation/ RPI areas only. **Do not use bleach alternatives on the PROCLEIX TIGRIS System.**
3. The PROCLEIX TIGRIS System automates the decontamination step by adding Deactivation Fluid to MTUs prior to disposal.
4. Follow instructions provided in the PROCLEIX TIGRIS System QRG for instrument decontamination and maintenance procedures.

H. WATER FOR THE PROCLEIX TIGRIS SYSTEM

Water for the PROCLEIX TIGRIS System is required. For water specifications for the PROCLEIX TIGRIS System, see the *PROCLEIX*® TIGRIS® System *Operator's Manual.* Excursions up to 100 cfu/mL do not adversely affect assay results. Refer to manufacturer instructions for maintaining the water system.

ASSAY PROCEDURE

All specimens (individual donations or pooled specimens) should be run in singlet in the PROCLEIX® WNV Assay.

PROCLEIX® WNV Assay Calibrators are to be used with the corresponding master lot of the PROCLEIX WNV Assay. The operator must check to ensure that the PROCLEIX WNV Assay Calibrators are used with the corresponding master lot of kit reagents as indicated on the PROCLEIX WNV Assay master lot sheet in use. The software will generate an error if calibrators from a different master lot are used.

Specimens from other living donors (except whole blood or blood components) and from cadaveric donors must be tested neat using the individual donor testing method only. If the initial test result from a cadaveric blood specimen is invalid, the specimen may be diluted to overcome potential inhibitory substances as described in SPECIMEN COLLECTION, STORAGE AND HANDLING, Cadaveric Blood Specimens, and retested in singlet.

For equipment preparation, rack setup, and assay procedure information, see instructions in the PROCLEIX® TIGRIS® System QRG.

QUALITY CONTROL PROCEDURES

I. ACCEPTANCE CRITERIA FOR THE PROCLEIX® WNV ASSAY

A. Run validity:
A run (also identified as a worklist) is valid if the minimum numbers of calibrators meet their acceptance criteria and are valid (see section II below).

1. In a PROCLEIX® WNV Assay run, at least four of the six calibrator replicates must be valid. At least two of the three Negative Calibrator replicates and two of the three Positive Calibrator replicates must be valid.
2. Calibrator acceptance criteria are automatically verified by the PROCLEIX® TIGRIS® System Software. If less than the minimum number of calibrator replicates is valid, the PROCLEIX TIGRIS System Software will automatically invalidate the run.
3. In a valid run, cutoff values will be automatically calculated for Internal Control (flasher) and analyte (glower).
4. If a run is invalid, sample results are reported as Invalid and all specimens must be retested.

B. Sample validity:

1. In a valid run, a sample result is valid if the IC signal is equal to or above the IC cutoff, with the following exceptions:
   a. Specimens with an analyte signal (glower signal) greater than the analyte cutoff are not invalidated even if the Internal Control (IC) signal is below the cutoff.
   b. Specimens with an IC signal above 750,000 RLU are invalidated by the software and their reactive status cannot be assessed. The software also automatically invalidates positive Calibrators and Positive PROCLEIX® WNV TIGRIS® Controls with an IC signal above 750,000 RLU.
2. A sample may also be invalidated due to instrument and results processing errors. Refer to the QRG for details.
3. All individual specimen results that are Invalid in a valid run or control bracket must be retested.

C. Control bracket validity:

1. A valid control bracket requires valid PROCLEIX® WNV TIGRIS® Control sets at the beginning and end of the bracket (excluding the first bracket which has calibrators at the beginning and PROCLEIX WNV TIGRIS Controls at the end). A set of PROCLEIX WNV TIGRIS Controls consists of one vial each of PROCLEIX® WNV TIGRIS® Negative Control and PROCLEIX® WNV TIGRIS® Positive Control. Each PROCLEIX WNV TIGRIS Control is run in singlet. A valid control set requires that all PROCLEIX WNV TIGRIS Controls in the set be valid. Controls acceptance criteria are automatically verified by the PROCLEIX TIGRIS System Software. Instructions for handling specimens in brackets with invalid PROCLEIX WNV TIGRIS Control sets are described in item E below.
2. In addition, a valid bracket requires that no more than 10% of the specimens in the bracket are invalid. For the purpose of calculating the 10% invalid rate, a specimen can be any kind of specimen tested, excluding PROCLEIX® WNV Assay Calibrators or PROCLEIX WNV TIGRIS Controls. Specimens include, but are not limited to donor samples, proficiency panels, and external quality controls. If control bracketing is not being used, the 10% invalid rate is determined from all the specimens in the run found between the PROCLEIX WNV Assay Calibrators at the beginning and the PROCLEIX WNV TIGRIS Control set at the end of the run. For runs or brackets of more than 50 specimens, the PROCLEIX TIGRIS System Software automatically applies the 10% invalid rate and nonreactive specimens are labeled as "Suspect" (see item D3 below). For runs or brackets of 50 or fewer specimens, the PROCLEIX TIGRIS System Software does not automatically apply the 10% invalid rate. The invalid rate must be manually calculated by the operator (see instructions in section F.2 and the PROCLEIX® TIGRIS® System QRG). If individual specimens are invalidated by an operator outside the PROCLEIX TIGRIS System Software, then the 10% invalid rate must be manually recalculated. Instructions for handling Suspect specimens due to greater than 10% invalid results are described in item F below.

D. Specimen results interpretation when bracket acceptance criteria are not met:

1. Specimens with an analyte S/CO <1.00 and IC RLU less than the IC cutoff will be marked as Invalid by the PROCLEIX TIGRIS System Software.
2. Specimens with an analyte S/CO greater than or equal to 1.00 and with IC signal between 0 and 750,000 RLU will be marked as Reactive by the PROCLEIX TIGRIS System Software and are the test of record.
3. Specimens with an analyte S/CO <1.00 and IC RLU greater than or equal to the IC cutoff will be flagged as Suspect by the PROCLEIX TIGRIS System Software. For the PROCLEIX® TIGRIS® System, the term "Suspect" refers to nonreactive specimens that are not automatically invalid, but must be further evaluated and resolved (see sections E and F).

E. Resolution of Suspect specimens due to invalid PROCLEIX WNV TIGRIS Control sets:

1. Suspect specimens that result from invalid PROCLEIX WNV TIGRIS Control sets are flagged with error code ”x” on the Assay Results Run Report. PROCLEIX WNV TIGRIS Controls may be invalid for one of two reasons (see the PROCLEIX® TIGRIS® System QRG for definitions):
   a. Instrument processing errors (error codes in UPPER CASE letters)
   b. Results processing errors (error codes in lower case letters)
2. If PROCLEIX WNV TIGRIS Control sets are invalidated due to instrument processing errors, results from all Suspect specimens should be considered valid non-reactive if the next set of PROCLEIX WNV TIGRIS Controls is valid. If no valid PROCLEIX WNV TIGRIS Control results are available in the subsequent bracket(s), all Suspect specimens should be considered invalid and be retested.
3. If PROCLEIX WNV TIGRIS Control results are invalidated due to results processing errors, all Suspect specimens should be considered invalid and be retested regardless of the status of subsequent PROCLEIX WNV TIGRIS Controls.

F. Resolution of Suspect specimens due to >10% invalid results:

1. In a PROCLEIX WNV Assay bracket or run of more than 50 specimens, when more than 10% of the specimens (all specimens tested, excluding PROCLEIX WNV Assay Calibrators or PROCLEIX WNV TIGRIS Controls) in the bracket are invalid, those specimens with an analyte S/CO less than 1.00 and IC RLU greater than or equal to the IC cutoff will be marked Suspect and flagged with error code “v” in the Run Report. All Suspect specimens in such runs or brackets must be retested.
2. For runs or brackets of 50 or fewer specimens, the operator must manually calculate the invalid rate. If more than 10% of the specimens (all specimens tested, excluding PROCLEIX WNV Assay Calibrators or PROCLEIX WNV TIGRIS Controls) in a run or bracket of 50 or fewer specimens are invalid due to multiple (two or more) occurrences of the same error, the bracket(s) should be invalidated and any specimens that the software has not identified as reactive should be retested.

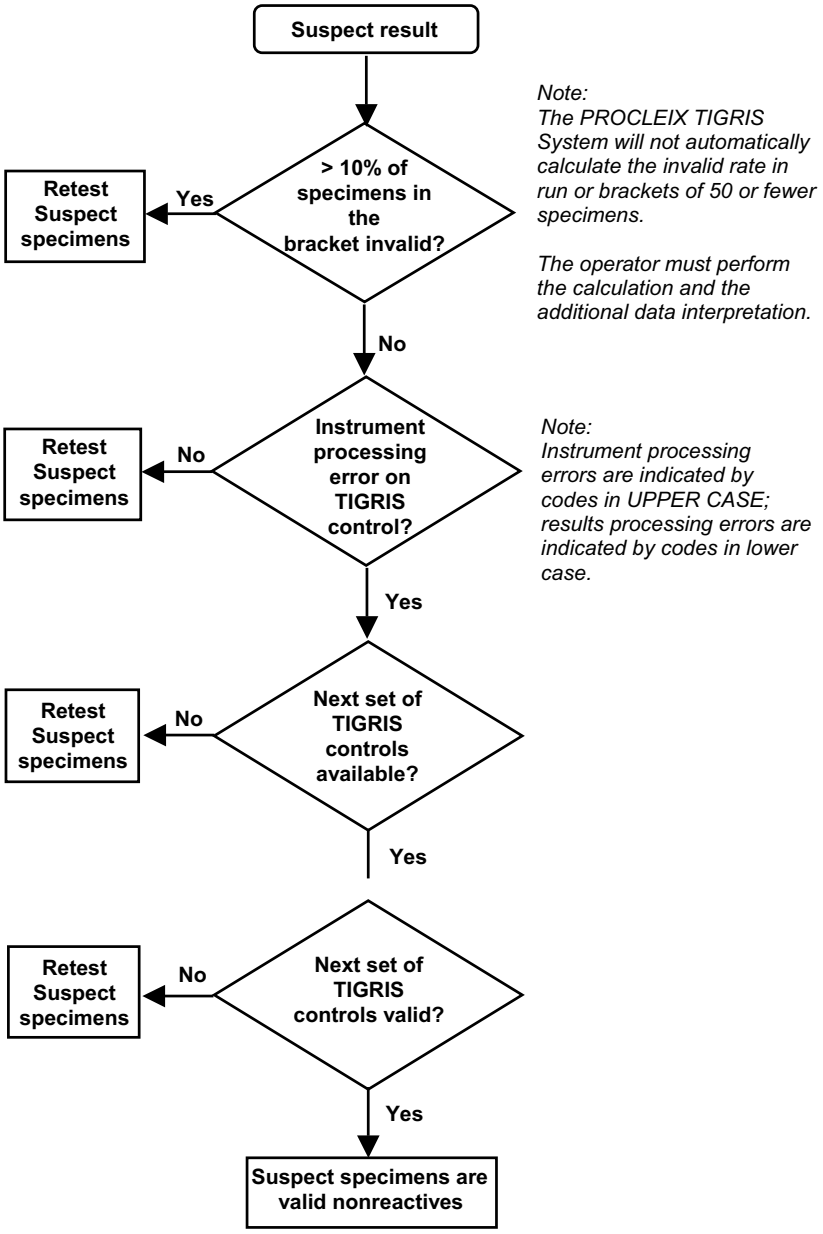
Note: Some errors are not included in calculating the invalid rate—see the PROCLEIX® TIGRIS® System QRG for a complete list and description of all error codes.

G. Summary of Specimen Result Interpretation
The following table and flow chart summarize results interpretation on the PROCLEIX TIGRIS System:

Interpretation assigned by PROCLEIX TIGRIS System Software on run report	Status of PROCLEIX WNV TIGRIS Controls for the bracket	Percent invalid specimens per bracket	Analyte S/CO	IC result	User Action Required
Reactive (test of record)	Valid or Invalid	NA	≥1.00	0 to 750,000 RLU	None
Valid, Non-reactive	Valid	≤10%	<1.00	≥IC C/O, ≤ 750,000 RLU	None
Valid, Non-reactive (for brackets with 50 or fewer specimens)*	Valid	>10% (user calculated)	<1.00	≥ IC C/O, ≤750,000 RLU	Follow instructions in section F, step 2.
Suspect (marked with error code “v”)	Valid	>10%	<1.00	≥IC C/O, ≤750,000 RLU	Retest (see section F and flow chart below for Suspect results).
Suspect (marked with error code “x”)	Invalid	≤10%	<1.00	≥IC C/O, ≤750,000 RLU	Follow instructions in section E and flow chart below for Suspect results.
Invalid	NA	NA	NA	NA	Retest

* User must calculate the percent invalid for brackets with 50 or fewer specimens.
NA = Not applicable.

If Suspect results are observed in the Run Report, consult the following chart for direction:



Note: Specimens with an overall interpretation of Reactive, as determined by the software, must become the test of record. The specimens should be resolved according to the resolution algorithm for the reactive specimens, as explained in the PROCLEIX TIGRIS System USERS, INTERPRETATION OF RESULTS section.

Note: A run or an individual sample may also be invalidated by an operator if package insert instructions for specimen or reagent handling were not followed.

II. ACCEPTANCE CRITERIA FOR CALIBRATION AND CALCULATION OF CUTOFF

Negative Calibrator Acceptance Criteria

The Negative Calibrator (NC) is run in triplicate in the PROCLEIX® WNV Assay. Each individual Negative Calibrator replicate must have an Internal Control (IC) value greater than or equal to 75,000 RLU and less than or equal to 400,000 RLU. Each individual Negative Calibrator replicate must also have an analyte value less than or equal to 40,000 RLU and greater than or equal to 0 RLU. If one of the Negative Calibrator replicate values is invalid due to an IC value or an analyte value outside of these limits, the Negative Calibrator mean (NC_x) will be recalculated based upon the two acceptable values. The run is invalid and must be repeated if two or more of the three Negative Calibrator replicate values have IC values or analyte values that are outside of these limits.

Determination of the mean of the Negative Calibrator values (NC_x) for Internal Control [NC_x (Internal Control)]

Example:

Negative Calibrator		Internal Control Relative Light Units
1		235,000
2		200,000
3		210,000
Total Internal Control RLU	=	645,000

$$NC_x\,(\text{Internal Control}) = \frac{\text{Total Internal Control RLU}}{3} = 215{,}000$$

Determination of the mean of the Negative Calibrator values (NC_x) for Analyte [NC_x (Analyte)]

Example:

Negative Calibrator		Analyte Relative Light Units
1		14,000
2		16,000
3		15,000
Total Analyte RLU	=	45,000

$$NC_x\,(\text{Analyte}) = \frac{\text{Total Analyte RLU}}{3} = 15{,}000$$

Positive Calibrator Acceptance Criteria

The Positive Calibrator is run in triplicate in the PROCLEIX® WNV Assay. Individual Positive Calibrator (PC) analyte values must be less than or equal to 2,700,000 RLU and greater than or equal to 400,000 RLU. IC values may not exceed 750,000 RLU. If one of the Positive Calibrator replicate values is outside these limits, the Positive Calibrator mean (PC_x) will be recalculated based upon the two acceptable Positive Calibrator replicate values. The run is invalid and must be repeated if two or more of the three Positive Calibrator analyte values are outside of these limits.

Determination of the mean of the Positive Calibrator (PC_x) values for Analyte [PC_x (Analyte)]

Example:

Positive Calibrator		Analyte Relative Light Units
1		1,250,000
2		1,500,000
3		1,150,000
Total Analyte RLU	=	3,900,000

$$PC_x\,(\text{Analyte}) = \frac{\text{Total Analyte RLU}}{3} = 1{,}300{,}000$$

Calculation of the Internal Control Cutoff Value

Internal Control Cutoff Value = 0.5 X [NC_x (Internal Control)]

Using values given in the Negative Calibrator example above:

Internal Control Cutoff Value = 0.5 X (215,000)

Internal Control Cutoff Value = 107,500 RLU

Calculation of the WNV Analyte Cutoff Value

Analyte Cutoff Value = NC_x (Analyte) + [0.03 X PC_x (Analyte)]

Using values given in the Negative Calibrator and Positive Calibrator examples above:

Analyte Cutoff Value = 15,000 + (0.03 X 1,300,000)

Analyte Cutoff Value = 54,000 RLU

Summary of Acceptance Criteria for PROCLEIX® WNV Assay

Acceptance Criteria:	
Negative Calibrator	
Analyte	≥ 0 and ≤ 40,000 RLU
Internal Control	≥ 75,000 and ≤ 400,000 RLU
Positive Calibrator	
Analyte	≥ 400,000 and ≤ 2,700,000 RLU
Internal Control	≤ 750,000 RLU

Summary of Cutoff Calculations for PROCLEIX® WNV Assay

Analyte Cutoff =	NC Analyte Mean RLU + [0.03 X (PC Analyte Mean RLU)]
Internal Control Cutoff =	0.5 X (Negative Calibrator IC Mean RLU)

III. ACCEPTANCE CRITERIA FOR PROCLEIX® WNV TIGRIS® CONTROLS IN THE PROCLEIX® WNV ASSAY

In the PROCLEIX® WNV Assay, a valid set of controls is required at the beginning and end of a bracket (excluding the first bracket, which only has controls at the end) for the results for that bracket to be valid. The PROCLEIX® WNV TIGRIS® Negative Control must have an S/CO less than 1.00 (nonreactive) to be accepted. The PROCLEIX® WNV TIGRIS® Positive Control must have an S/CO greater than or equal to 1.00 (reactive) and less than 100.00 to be accepted.

Acceptance Criteria:			
Negative Control			
Analyte	≥ 0	and	≤ 150,000 RLU
Analyte S/CO		< 1.00	
Internal Control	≥ 75,000	and	≤ 400,000 RLU
Internal Control S/CO		≥ 1.00	
Positive Control			
Analyte	≥ 0	and	≤ 2,700,000 RLU
Analyte S/CO	≥ 1.00	and	< 100.00
Internal Control		≤ 750,000 RLU	

INTERPRETATION OF RESULTS

All calculations described above are performed by the assay software of the PROCLEIX® TIGRIS® System**.** Two cutoffs are determined for each assay: one for the Analyte Signal (glower signal) termed the Analyte Cutoff and one for the Internal Control Signal (flasher signal) termed the Internal Control Cutoff. The calculation of these cutoffs is shown above. For each sample, an Analyte Signal RLU value and Internal Control Signal RLU value are determined. Analyte Signal RLU divided by the Analyte Cutoff is abbreviated as the Analyte Signal/Cutoff (S/CO) on the report.

A specimen is Nonreactive if the Analyte Signal is less than the Analyte Cutoff (i.e., Analyte S/CO <1.00) and the Internal Control (IC) Signal is greater than or equal to the Internal Control Cutoff (IC Cutoff) and less than or equal to 750,000 RLU. A specimen is Reactive if the Analyte Signal is greater than or equal to the Analyte Cutoff (i.e., Analyte S/CO ≥ 1.00) and the IC Signal is less than or equal to 750,000 RLU. Reactive results will be designated by the software. A specimen is invalid if the Analyte Signal is less than the Analyte Cutoff (i.e., Analyte S/CO <1.00) and the Internal Control Signal is less than the Internal Control Cutoff. Any specimen with Internal Control values greater than 750,000 RLU is considered Invalid and the reactive status cannot be assessed.

Cadaveric blood specimens, when tested neat, may be invalid due to inhibitory substances within the specimen. These invalid specimens may be diluted as in SPECIMEN COLLECTION, STORAGE AND HANDLING, Cadaveric Blood Specimens, and retested in singlet.

Summary of Specimen Interpretation:

Specimen Interpretation	Criteria
NonReactive	Analyte S/CO < 1.00 and IC ≥ IC Cutoff and IC ≤ 750,000 RLU
Reactive	Analyte S/CO ≥ 1.00 and IC ≤ 750,000 RLU
Invalid*	IC > 750,000 RLU or Analyte S/CO < 1.00 and IC < Cutoff

*For specimens with IC signal greater than 750,000 RLU, the specimen will be invalidated by the software and the reactive status cannot be assessed.

1. Any specimen, including cadaveric specimens, with an interpretation of Invalid in the PROCLEIX WNV Assay must be retested in singlet. Cadaveric specimens previously diluted 1:5 may be retested diluted 1:5.
2. If at any point in the testing algorithm there is insufficient volume to complete the testing then an alternate specimen from the index donation may be used as long as the storage criteria in the package insert are met.
3. Specimens with a valid Internal Control value and with an Analyte S/CO less than 1.00 in the PROCLEIX WNV Assay are considered Nonreactive for WNV RNA.
    a. IF THE NONREACTIVE SPECIMEN IS A POOL, then each of the individual specimens comprising the pool is considered Nonreactive and no further testing is required.
    b. IF THE NONREACTIVE SPECIMEN IS FROM AN INDIVIDUAL DONATION, then the individual specimen is considered Nonreactive for WNV and no further testing is required.
4. Specimens with an Analyte S/CO greater than or equal to 1.00 with IC Signal less than or equal to 750,000 RLU are considered Reactive.
    a. IF THE REACTIVE SPECIMEN IS A POOL, then each of the individual specimens comprising the pool must be tested with the PROCLEIX WNV Assay.
        1. If an individual specimen tests Nonreactive with the PROCLEIX WNV Assay, then the specimen is considered Nonreactive for WNV and no further testing is required.
        2. If an individual specimen tests Reactive with the PROCLEIX WNV Assay, then the individual specimen is considered Reactive for WNV. Further clarification of the Reactive specimens for informational purposes may be obtained by testing an alternate specimen from the index donation with the PROCLEIX WNV Assay and/or by follow-up testing. Results of testing obtained for clarification do not replace test results for purposes of donor eligibility.
    b. IF THE REACTIVE SPECIMEN IS FROM AN INDIVIDUAL DONATION, then the individual specimen is considered Reactive for WNV. Further clarification of the Reactive specimens for informational purposes may be obtained by testing an alternate specimen from the index donation with the PROCLEIX WNV Assay and/or by follow-up testing. Results of testing obtained for clarification do not replace test results for purposes of donor eligibility.
5. Reactive specimens in an operator-invalidated run due to the 10% invalid rate (see QUALITY CONTROL PROCEDURES for PROCLEIX® TIGRIS® SYSTEM USERS, step F) are identified by the software as reactive and must become the test of record. Any reactive result should be resolved according to the resolution algorithm for reactive specimens, as explained in the INTERPRETATION OF RESULTS section, step 4 above.

GENERAL INFORMATION

LIMITATIONS OF THE PROCEDURE

This assay has been evaluated with the PROCLEIX® System and PROCLEIX® TIGRIS® System only.

The clinical sensitivity for the PROCLEIX® WNV Assay has been demonstrated for specimens with WNV viral concentrations equal to or greater than 100 copies/mL. Samples with less than 100 copies/mL may not yield reproducible results.

Assay performance characteristics for use in testing plasma specimens from paid source plasma donors have not been determined.

Assays must be performed, and results interpreted according to the procedures provided.

Deviations from these procedures, adverse shipping and/or storage conditions, or use of outdated calibrators and/or reagents may produce unreliable results.

PERFORMANCE CHARACTERISTICS

REPRODUCIBILITY

PROCLEIX® System

Reproducibility of the PROCLEIX® WNV Assay was evaluated at three blood testing laboratories. For determination of reproducibility, a ten-member panel comprised of tissue culture-derived WNV was procured from Boston Biomedica (BBI, West Bridgewater, MA) (Table 1). Seven panel members were positive for WNV (50, 50, 100, 100, 300, 1,000, and 10,000 copies/mL) and three panel members were WNV negative.

The reproducibility panel members were tested by six operators (two from each testing site) with three different clinical reagent kit lots over multiple days, using automated (TECAN GENESIS RSP instrument) or manual pipetting methods. Twenty-four runs were tested at each site across three clinical lots, with each panel member tested in triplicate per run and each operator performing testing for at least six days.

Of the 79 runs generated on the PROCLEIX® System, 6 (7.6%) were invalid. Of these invalidated runs, 4 were invalidated by the instrument due to an insufficient number of valid calibrators. The remaining 2 of 6 runs were invalidated by the operator: 1 was due to operator error and 1 was invalidated because the run contained greater than 10% invalid test results that were due to Internal Control (IC) failures. From the valid assay runs, 2,162 test results were generated. Of these, 17 (0.8%) were invalid due to IC failures.

In Table 1, assay signal values were expressed as Analyte Signal to Cutoff (S/CO) ratios for panel members containing target and as IC S/CO ratios for negative panel members. Signals were expressed as analyte Relative Light Units (RLU) for the Positive Calibrator and as IC RLU for the Negative Calibrator in the PROCLEIX WNV Assay. Signal variability of the assays was calculated for intra- and inter-run, inter-lot, and inter-site in terms of standard deviation (SD) and percent coefficient of variation (%CV). Data were also analyzed as percent agreement with expected outcome and mean S/CO ratio or RLU. Since no significant difference in assay reproducibility was observed between automated and manual pipetting, results from the two pipetting methods were combined and shown in Table 1.

The overall percent agreement of test results with expected outcomes was 100% for negative panels and greater than or equal to 99.8% for positive panel members. With regard to variability, intra-run (or random error) and inter-run factors were the largest and second largest contributors to total variance (according to SD values) in the PROCLEIX WNV Assay. While these factors were responsible for the majority of the variance in the assay, the %CV of each of these components by itself did not exceed 13.7% for any positive or negative samples. The inter-site %CVs were 6.1% or less and the inter-lot %CVs were less than 4%, indicating that these factors had little impact on assay performance. Therefore, the reproducibility of the assay is robust and much of the variation that is observed can be attributed to random error.

Table 1. Reproducibility of the PROCLEIX® WNV Assay*

BBI Panel	n	Concentration Copies/mL	Number of replicates	% Agreement	Mean S/CO	Intra-Run		Inter-Run		Inter-Lot		Inter-Site	
						SD	%CV	SD	%CV	SD	%CV	SD	%CV
Negative**	3	0	641	100	1.96	0.11	5.8	0.09	4.7	0.03	1.6	0***	0
WNV	2	50	426	99.8	26.70	3.44	12.9	2.29	8.6	0***	0	0.77	2.9
WNV	2	100	431	99.8	26.64	3.65	13.7	2.37	8.9	0.95	3.6	1.25	4.7
WNV	1	300	215	100	28.62	1.38	4.8	2.13	7.4	0.48	1.7	1.55	5.4
WNV	1	1,000	216	100	28.94	1.16	4.0	1.85	6.4	0***	0	1.74	6.0
WNV	1	10,000	216	100	29.69	1.62	5.5	1.85	6.2	0.52	1.8	1.82	6.1
Sample			**Number of replicates**	**% Agreement**	**Mean RLU**	**Intra-Run**		**Inter-Run**		**Inter-Lot**		**Inter-Site**	
						SD	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**
Negative Calibrator**			215	NA	179,345	9,364	5.2	10,248	5.7	4,462	2.5	9,531	5.3
WNV Positive Calibrator			214	NA	1,256,371	45,949	3.7	38,338	3.1	3,559	0.3	35,437	2.8

n = Number of panel members combined for this analysis
* Analysis of analyte signals, unless otherwise noted
** Analysis of internal control signal
*** Per CLSI guidelines (EP5-A, page 7), numbers <0 are recorded as 0.

PROCLEIX® TIGRIS® System

Reproducibility of the PROCLEIX® WNV Assay was evaluated at three blood testing laboratories. For determination of the reproducibility of each assay, 10 members from a reproducibility panel were tested as individual samples (Table 2). The panel, comprised of tissue culture-derived WNV, was procured from Boston Biomedica (BBI, West Bridgewater, MA). Seven panel members were positive for WNV (50, 50, 100, 100, 300, 1,000, and 10,000 copies/mL) and three panel members were WNV negative.

The reproducibility panel members were tested by a total of six operators (two from each testing site) with three different clinical reagent kit lots over multiple days using three PROCLEIX® TIGRIS® System instruments. Each operator performed three worklists (i.e., runs) per PROCLEIX WNV Assay clinical reagent kit lot on one of the three PROCLEIX TIGRIS System instruments. Nine worklists were completed by each operator for a total of 54 worklists overall. The worklists were repeated three times, totaling 162 results per panel member.

Of the 62 runs generated on the PROCLEIX TIGRIS System, 7 (11.3%) were invalid. Of these invalid runs, 6 were due to one incident of a hardware error in 1 run, which was invalidated by the operator: the error caused the instrument to shut down and the 5 subsequent runs in the queue were invalidated by the instrument. The remaining 1 of 7 invalidated runs was invalidated by the operator because the run contained greater than 10% invalid test results that were due to an instrument communication failure. From the valid assay runs, 1,620 test results were generated. Of these, 1 (0.1%) was invalid due to an assay processing error.

In Table 2, assay signal values were expressed as analyte signal to cutoff (S/CO) ratios for panel members containing target and as internal control (IC) S/CO ratios for negative panel members. Signals were expressed as analyte Relative Light Units (RLU) for the Positive Calibrator and as IC RLU for the Negative Calibrator in the PROCLEIX WNV Assay. Signal variability of the runs was calculated for intra- and inter-run, inter-lot, and inter-site in terms of standard deviation (SD) and percent coefficient of variation (%CV). Data were also analyzed as percent agreement to expected outcome and mean S/CO ratio or RLU.

The overall percent agreement of test results with expected outcomes was 99.8% for negative panels and greater than or equal to 99.7% for positive panel members. With regard to variability, inter-run and intra-run (or random error) factors were the largest and second largest contributors, respectively, to total variance (according to SD values) in the PROCLEIX WNV Assay. While these factors were responsible for the majority of the variance in the assay, the %CV of each of these components by itself did not exceed 11.2% for any positive or negative samples. The inter-lot %CVs were 4.1% or less and the inter-instrument %CVs were 9.9% or less, indicating that these factors had less impact on assay performance. Therefore, the reproducibility of the assay is robust.

Table 2. PROCLEIX® TIGRIS® System - Reproducibility of the PROCLEIX® WNV Assay*

BBI Panel	n	Concentration Copies/mL	Number of replicates	% Agreement	Mean S/CO	Intra-Run		Inter-Run		Inter-Lot		Inter-Instrument	
						SD	%CV	SD	%CV	SD	%CV	SD	%CV
Negative**	3	0	486	99.8	2.1	0.1	5.9	0.1	2.8	0.0***	0	0.0***	0
WNV	2	50	324	99.7	28.6	3.2	11.2	2.8	9.8	1.2	4.1	2.2	7.8
WNV	2	100	323	99.7	29.1	3.0	10.2	2.8	9.6	0.7	2.4	2.0	6.9
WNV	1	300	162	100	28.9	1.2	4.1	2.5	8.8	1.1	3.8	2.9	9.9
WNV	1	1,000	162	100	28.8	1.2	4.1	2.5	8.8	1.1	3.9	2.8	9.7
WNV	1	10,000	162	100	30.1	1.4	4.6	2.5	8.4	0.8	2.6	2.0	6.7

Sample	Number of replicates	% Agreement	Mean RLU	Intra-Run		Inter- Run		Inter-Lot		Inter-Site	
				SD	%CV	SD	%CV	SD	%CV	SD	%CV
Negative Calibrator**	156	N/A	148,970.3	9,051.6	6.1	3,888.5	2.6	3,741.8	2.5	11,715.6	7.9
WNV Positive Calibrator	161	N/A	1,514,280.8	68,309.2	4.5	0.0***	0.0	12,630.8	0.8	20,206.2	1.3

n = Number of panel members combined for this analysis
*Analysis of analyte signals, unless otherwise noted
**Analysis of internal control signal
*** Per CLSI guidelines (EP5-A, page 7), numbers <0 are recorded as 0.

SPECIFICITY IN NORMAL BLOOD DONORS

Specificity of the PROCLEIX® WNV Assay

The clinical specificity of the PROCLEIX® WNV Assay was determined in prospectively collected samples tested linked as 16-sample pools and as individual plasma samples from voluntary blood or blood component donors. Nine hundred four (904) runs were generated from testing of the pools and individual donor samples (IDS) on the PROCLEIX System. Of these, 14 (1.5%) runs were invalid. Ten of the 14 runs were invalidated by the instrument due to an insufficient number of valid calibrators. The remaining 4 of 14 runs were invalidated by the operator: 3 contained greater than 10% invalid test results due to Internal Control failures, and 1 was due to operator error. From the valid assay runs, 16,885 and 43,503 test results were generated from pools and IDS, respectively; none were invalid.

Specificity of the PROCLEIX WNV Assay was calculated from 16,885 16-sample pools and 43,503 IDS from whole blood donations. For calculations of clinical specificity, reactive results from the PROCLEIX WNV Assay were compared to results from a commercial WNV IgM assay and/or validated WNV Alternate NAT. The overall clinical specificity results are summarized in Table 3. Donors whose samples were initially reactive in the PROCLEIX WNV Assay were pursued for enrollment into a follow-up study for additional testing.

The study was conducted at four blood testing laboratories using samples from donors representing geographically diverse regions of the United States. During this study, all testing was performed linked using three clinical lots of PROCLEIX WNV Assay reagent kits. All 16 member samples from a PROCLEIX WNV Assay reactive pool were tested individually in the PROCLEIX WNV Assay. Reactive samples, whether identified from pool testing or individual donor testing, were retested with the PROCLEIX WNV Assay and also tested with a validated WNV nucleic acid test (Alternate NAT) and a commercial immunoglobulin M (IgM) assay.

Specificity of the PROCLEIX® WNV Assay in 16-Sample Pools

A total of 16,885 pools were tested in the PROCLEIX® WNV Assay at two blood testing sites. Of these, 16,855 tested nonreactive and were considered true negative. Thirty pools were reactive in the PROCLEIX WNV Assay. Of these, 21 pools contained at least one reactive sample when the constituents of the pool were tested individually. The 21 reactive pools were determined to be true positive pools as the PROCLEIX WNV Assay reactive results were confirmed by reactive Alternate NAT and/or positive IgM antibody results. Nine reactive pools were considered false positive as all individual samples of the pool tested nonreactive in the PROCLEIX WNV Assay. The overall specificity of 16-sample pools from whole blood donations in these studies was 16,876/16,885=99.95% (95%CI: 99.90-99.98%).

Specificity of the PROCLEIX® WNV Assay in Individual Donor Samples

For the evaluation of individual donor samples (IDS) specificity of the PROCLEIX® WNV Assay, a total of 43,503 IDS were tested at four blood testing laboratories. There were 43,427 IDS that tested nonreactive and were considered true negative. There were 76 IDS that tested reactive in the PROCLEIX WNV Assay. Of these, 30 reactive IDS results were confirmed by Alternate NAT and/or IgM immunoassay results and were considered true positive and the remaining 46 IDS were considered false positive. The overall specificity of IDS from whole blood donations in these studies was 43,457/43,503=99.89% (95%CI: 99.86-99.92%).

Combining the results from 16-sample pools and individual donor testing, the overall specificity of the PROCLEIX WNV Assay in these studies was 60,333/60,388=99.91% (95% CI: 99.86-99.96%).

Table 3. PROCLEIX® System - Clinical Specificity of the PROCLEIX® WNV Assay in Pools and IDS from Whole Blood Donations

Sample	n	TN	TP	FP	Specificity (%)	95% CI
16-Sample Pools	16,885	16,855	21	9	99.95	99.90-99.98
IDS	43,503	43,427	30	46	99.89	99.86-99.92
Overall	60,388	60,282	51	55	99.91	99.86-99.96

n = Number of Samples
TN = True Negative
TP = True Positive
FP = False Positive
CI = Confidence Interval

Comparison of PROCLEIX® WNV Assay with IgM Serology and Alternate NAT

Results generated from pooled and individual donation testing for the clinical specificity study allow comparison of the PROCLEIX® WNV Assay results with WNV serology and Alternate NAT results (Table 4). Of the 97 individual donor samples that were reactive in the PROCLEIX WNV Assay, 50 (51.5%) were Alternate NAT reactive and/or IgM positive at index. Of these, 8 samples were both IgM positive and Alternate NAT reactive, 11 samples were IgM positive only, and 31 samples were Alternate NAT reactive only. One additional sample, which tested nonreactive in Alternate NAT and negative for WNV IgM at index, demonstrated seroconversion at follow up. These 51 reactive results were classified as true positive. For the 31 donors with IgM-negative results at index, follow-up sample results were IgM positive. Thus, seroconversion was observed for all 51 donors with true positive PROCLEIX WNV Assay results.

Forty-six (46) samples, which tested initially reactive in the PROCLEIX WNV Assay, had nonreactive PROCLEIX WNV Assay results upon retest. These samples were IgM negative and Alternate NAT nonreactive at index and were considered false positive. Follow-up samples were obtained from 38 of the 46 donors with false positive PROCLEIX WNV Assay results; all samples were PROCLEIX WNV Assay and Alternate NAT nonreactive and were IgM negative.

Of the 51 samples with true positive PROCLEIX WNV Assay results, eight samples were both IgM positive and Alternate NAT reactive at index. This pattern is consistent with individuals infected with WNV in the early stage of immune response. Thirty-one samples were Alternate NAT reactive and IgM negative, consistent with individuals in the viremic phase of infection with little to no antibody production. Eleven samples were IgM positive, but were nonreactive in the Alternate NAT at index. Five of these 11 samples were repeat reactive in the PROCLEIX WNV Assay and nine of the 11 samples were reactive in the PROCLEIX WNV Assay at follow-up. The variability between PROCLEIX WNV Assay and Alternate NAT results in this set of samples is consistent with low levels of WNV RNA during a later stage of infection.

Table 4. PROCLEIX® System- Clinical Specificity Study: Comparison with WNV Serology and Alternate NAT

Outcome	Test Results for Index Donation		n	%
TP	Alternate NAT+	IgM +	8	8.2
TP	Alternate NAT+	IgM-	31	32.0
TP	Alternate NAT -	IgM+	11	11.3
TP	Alternate NAT -	IgM-	1*	1.0
Subtotal			51	52.6
FP	Alternate NAT -	IgM-	46	47.4
Total			**97**	**100**

* Sample was IgM positive at follow up
TP = True Positive
FP = False Positive
IgM+ = positive for WNV Immunoglobulin M antibody
IgM- = negative for WNV Immunoglobulin M antibody
n = number of samples

NON-SPECIFICITY STUDIES

SPECIFICITY AND SENSITIVITY OF THE PROCLEIX® WNV ASSAY IN THE PRESENCE OF DONOR AND DONATION FACTORS

PROCLEIX® System

To test for cross-reactivity, specimens with various donor and donation factors were tested with the PROCLEIX® WNV Assay. To test for interference, detection (sensitivity) of the PROCLEIX WNV Assay was evaluated by spiking the various donor and donation specimens to a final concentration of 150 copies/mL of WNV.

When tested with the PROCLEIX WNV Assay, no cross-reactivity or interference was observed for naturally occurring hemolyzed, icteric or lipemic specimens or plasma containing the following substances: serum albumin (up to 6 g/dL), hemoglobin (up to 500 mg/dL) and lipids (up to 3,000 mg/dL), and plasma containing bilirubin up to 20 mg/dL.

No cross-reactivity or interference was observed in specimens from patients with autoimmune diseases or with liver diseases not caused by hepatitis C virus or hepatitis B virus infection. Multiple specimens from each group of patients with the following autoimmune conditions were evaluated: rheumatoid arthritis (n=10), rheumatoid factor (n=10), antinuclear antibody (n=10), multiple sclerosis (n=6), lupus (n=10), and multiple myeloma (n=10). Also tested were samples from patients with hyperglobulinemia, with elevated ALT (n=10) and from patients with alcoholic liver cirrhosis (n=10).

No cross-reactivity or interference was observed in bacterially contaminated plasma or in specimens from patients infected with other blood borne pathogens. Multiple specimens from each group of patients with the following viral infections were evaluated: herpes simplex virus 1/2 (n=10), human T-cell lymphotropic virus type I/II (n=10), hepatitis A virus (n=10), hepatitis B virus (n=10), hepatitis C virus (n=10), hepatitis G virus (n=10), cytomegalovirus (n=10), Epstein-Barr virus (n=10), rubella virus (n=10), parvovirus B-19 (n=4) and human immunodeficiency virus type 1 (n=10) and type 2 (n=10). Also tested were donor samples from influenza virus (n=10) and HBV vaccinees (n=10), and samples spiked with tissue culture-derived viruses related to members of the Japanese encephalitis virus (JEV) sero-complex, including dengue virus (n=4), Saint Louis encephalitis virus (n=1), Murray Valley encephalitis virus (n=1), and yellow fever virus (n=1) with no cross-reactivity or interference. The PROCLEIX WNV Assay detected Kunjin virus (n=1), a variant of WNV.

PROCLEIX® TIGRIS® System

To test for cross-reactivity, specimens with various donor and donation factors were tested with the PROCLEIX® WNV Assay. To test for interference, detection (sensitivity) of the PROCLEIX WNV Assay was evaluated by spiking the various donor and donation specimens to a final concentration of 150 copies/mL of WNV.

When tested with the PROCLEIX WNV Assay, no cross-reactivity or interference was observed for naturally occurring hemolyzed, icteric or lipemic specimens or plasma containing the following substances: serum albumin (up to 6 g/dL), hemoglobin (up to 500 mg/dL) and lipids (up to 3,000 mg/dL), and plasma containing bilirubin up to 20 mg/dL.

No cross-reactivity or interference was observed in specimens from patients with autoimmune diseases or with liver diseases not caused by hepatitis C virus or hepatitis B virus infection. Multiple specimens from each group of patients with the following autoimmune conditions were evaluated: rheumatoid arthritis (n=10), multiple sclerosis (n=6), rheumatoid factor (n=10), antinuclear antibody (n=10), lupus (n=10) and multiple myeloma (n=10). Also tested were samples from patients with elevated hyperglobulinemia, with elevated ALT (n=10) and from patients with alcoholic liver cirrhosis (n=10).

No cross-reactivity or interference was observed in bacterially contaminated plasma. No cross-reactivity was observed in specimens from patients infected with other blood borne pathogens. Multiple specimens from each group of patients with the following viral infections were evaluated: herpes simplex virus 1 (n=9) and 2 (n=10), human T-cell lymphotropic virus type I/II (n=10), hepatitis A virus (n=10), hepatitis B virus (n=10), hepatitis C virus (n=10), hepatitis G virus (n=4), cytomegalovirus (n=11), Epstein-Barr virus (n=10), rubella virus (n=10), and human immunodeficiency virus type 1 (n=11). Donor samples from influenza virus vaccinees (n=10) were also tested with no cross-reactivity or interference.

CLINICAL SENSITIVITY

Testing of Known-Positive Samples

Two hundred and three (203) WNV known-positive samples were procured from a blood bank repository. These samples were determined to be positive for WNV RNA by testing with two validated NAT methods. In addition to NAT, the samples were tested for the presence of IgM antibodies to WNV. The clinical sensitivity study was performed at two blood testing laboratories using three clinical reagent kit lots of the PROCLEIX® WNV Assay. The positive samples were tested neat (i.e., undiluted; n=202) and in a 1:16 dilution (n=203) in the PROCLEIX WNV Assay. Negative plasma samples were also tested in the PROCLEIX WNV Assay at each clinical site as a control for potential study bias. For determination of clinical sensitivity, neat and diluted sample test results from the PROCLEIX WNV Assay were compared to the known viral status of each sample when tested neat (Table 5).

Of the 15 runs generated on the PROCLEIX System for the clinical sensitivity study, none were invalid. Of the 202 and 203 test results generated from neat and 1:16 diluted samples, respectively, none were invalid.

For the sensitivity study, neat samples had known WNV RNA concentrations equal to or greater than 100 copies/mL. Known-positive samples with WNV RNA copy levels below the sensitivity claim of 100 copies/mL after the 1:16 dilution were included in the clinical sensitivity analyses.

The sensitivity of the PROCLEIX WNV Assay in neat (undiluted) WNV known-positive samples in this study was 100% (95% CI: 98.2-100%). The sensitivity of the PROCLEIX WNV Assay in diluted (1:16) WNV known-positive samples in this study was 91.6% (95% CI: 86.9-95.0%). All of the 17 diluted samples with false negative results were derived from neat samples that had low WNV viral loads. The sensitivity of the PROCLEIX WNV Assay in diluted samples with copy levels greater than or equal to the sensitivity claim of 100 copies/mL in this study was 100%.

Table 5. PROCLEIX® System - Clinical Sensitivity of the PROCLEIX® WNV Assay in Known-Positive Samples

Assay	n	TP	FN	Sensitivity (%)	95% CI
Neat	202*	202	0	100	98.2-100
Diluted 1:16	203	186	17	91.6	86.9-95.0

* One neat sample not tested
n = Number of samples
TP = True Positive
FN = False Negative
CI = Confidence Interval

Testing of Known-Positive 16-Sample Pools

The clinical sensitivity of the PROCLEIX® WNV Assay in pooled samples was determined by testing 98 sixteen-sample pools composed of 1 to 3 WNV positive samples and 13 to 15 negative samples. The 98 positive samples from different blood donors were procured from a blood bank repository. These specimens were determined to be positive for WNV RNA by testing with two validated NAT methods. In addition to NAT, the samples were tested for the presence of IgM antibodies to WNV. Two clinical sites participated in the study using three clinical reagent kit lots. Pools contained known-positive samples with neat viral concentrations ranging from 200 to 430,000 copies/mL. Six of the 98 pools contained less than 100 copies/mL after pooling.

Known-positive pools were tested in the same runs with the neat and 1:16 diluted known-positive samples described above; all runs were valid. Of the 98 test results generated from pooled samples tested, none were invalid. The sensitivity of the PROCLEIX WNV Assay in 98 known-positive pools in this study was 100% (95% CI: 96.3-100%) (Table 6).

Table 6. PROCLEIX® System - Clinical Sensitivity of the PROCLEIX® WNV Assay in 16-Sample Pools Containing Known-Positive Samples

n	TP	FN	Sensitivity (%)	95% CI
98*	98	0	100	96.3 - 100

* Included 61 pools with 1 positive sample, 25 pools with 2 positive samples, and 12 pools with 3 positive samples
n = Number of samples
TP = True Positive
FN = False Negative
CI = Confidence Interval

ANALYTICAL SENSITIVITY

Determination of Analytical Sensitivity Using a Dilutional Sensitivity Panel Made From the Health Canada WNV Reference Standard

An analytical sensitivity panel comprised of serially diluted WNV provided by Health Canada was used to evaluate assay sensitivity. The WNV panel was prepared by serial dilution of heat-treated tissue culture-derived viral stock (1,000 copies/mL). Three operators tested 30 replicates of each copy level with three clinical lots using the PROCLEIX® System for a total of 90 replicates. The 95% confidence intervals (CI) of the reactive rates were based on the exact binomial distribution. Estimations of 50% and 95% detection rates by probit analysis are provided.

In this study, WNV RNA detection with the PROCLEIX® WNV Assay was 100% at 100 copies/mL and at 30 copies/mL for both the PROCLEIX® System and the PROCLEIX® TIGRIS® System. Reactivity at 10 copies/mL was 97% and 91% for the PROCLEIX System and the PROCLEIX TIGRIS System, respectively. At 3 copies/mL, the detection rates were 53% and 58% for the PROCLEIX System and the PROCLEIX TIGRIS System, respectively (Tables 7a and 7b).

Table 7a. PROCLEIX® System - Detection of WNV RNA in Health Canada Analytical Sensitivity Panel

WNV RNA copies/mL	Number reactive/ tested*	% Reactive	95% CI		Average S/CO**	%CV
			Lower	Upper		
100	89/89	100	97	100	30.05	9
30	90/90	100	97	100	29.46	10
10	87/90	97	91	99	27.16	25
3	47/89	53	42	63	23.43	35
1	26/89	29	20	40	21.10	49
0	0/89	0	0	3	0.06	120

*Only valid reactions were included
**Average of reactive replicates unless all tests were nonreactive, in which case the average analyte S/CO of nonreactive replicates is shown
CI = Confidence Interval

Table 7b. PROCLEIX® TIGRIS® System - Detection of WNV RNA in Health Canada Analytical Sensitivity Panel

WNV RNA copies/mL	Number of reactive/ tested*	% Reactive	95% CI		Average S/CO**	%CV
			Lower	Upper		
100	77/77	100	96	100	30.22	14
30	74/74	100	96	100	29.21	18
10	82/90	91	83	96	26.52	27
3	52/90	58	47	68	24.16	36
1	19/90	21	13	31	17.03	61
0	0/90	0	0	3	0.11	101

*Samples were QNS for 30 replicates with one of the three clinical lots
**Average of reactive replicates unless all tests were nonreactive, in which case the average analyte S/CO of nonreactive replicates is shown
CI = Confidence Interval

Probit Analysis

The predicted 50% and 95% detection rates, in copies/mL, were determined by probit analysis of the analytical sensitivity results. The predicted 95% detection level for WNV RNA in this study was 8.2 copies/mL for the PROCLEIX® System and 9.8 copies/mL for the PROCLEIX® TIGRIS® System with the Health Canada Sensitivity Panel (Table 8).

Table 8. Detection Probabilities of WNV RNA using a Sensitivity Panel from Health Canada Reference Standard

Assay System	Detection Probabilities (copies/mL)	
	50% (95% CI)	95% (95% CI)
PROCLEIX® System	3.4 (1.8 – 7.2)	8.2 (5.5 – 21.5)
PROCLEIX® TIGRIS® System	4.0 (1.7 – 8.8)	9.8 (6.5 – 27.3)

CI = Confidence Interval

Determination of Analytical Sensitivity Using an FDA WNV Reference Panel

An analytical sensitivity panel provided by the Center for Biologics Evaluation and Research (CBER) and manufactured by Boston Biomedica (BBI, West Bridgewater, MA) was used to evaluate assay sensitivity. Performance of the assay was evaluated by testing four replicates of each copy level with three clinical lots using the PROCLEIX® System for a total of 12 replicates. The same panel was tested in ten replicates using the PROCLEIX® TIGRIS® System with one instrument and one lot of reagents. Detection of all panel members with a WNV RNA titer of 100 copies/mL or greater was 100% with both the PROCLEIX System and the PROCLEIX TIGRIS System (Tables 9a and 9b).

Table 9a. PROCLEIX® System - Detection of Lineage 1 WNV in an FDA WNV Reference Panel

Panel I.D.	WNV Strain	Copy Level (copies/ mL)	Number reactive/ tested	% Reactive	Average S/CO*	%CV
1	NY99	100	12/12	100	31.53	4
2	NY99	10	12/12	100	29.24	8
3	Hu2002	0	0/12	0	0.10	85
4	Hu2002	50	12/12	100	31.62	4
5	NY99	0	0/12	0	0.05	70
6	NY99	1000	12/12	100	32.48	3
7	Hu2002	100	12/12	100	32.34	6
8	Hu2002	1000	12/12	100	31.74	9
9	Hu2002	5	12/12	100	25.07	43
10	NY99	5	11/12	92	23.64	44
11	NY99	500	12/12	100	32.22	4
12	Hu2002	10	12/12	100	28.48	26
13	NY99	50	12/12	100	31.61	6
14	Hu2002	500	12/12	100	32.19	5

*Average of reactive replicates unless all tests were nonreactive, in which case the average analyte S/CO of nonreactive replicates is shown.

Table 9b. PROCLEIX® TIGRIS® System - Detection of Lineage 1 WNV in an FDA WNV Reference Panel

Panel I.D.	WNV Strain	Copy Level (copies/ mL)	Number reactive/ tested*	% Reactive	Average S/CO**	%CV
1	NY99	100	10/10	100	31.88	4
2	NY99	10	8/10	80	28.82	24
3	Hu2002	0	0/9	0	0.03	131
4	Hu2002	50	9/10	90	31.15	3
5	NY99	0	0/9	0	0.04	115
6	NY99	1000	10/10	100	31.43	4
7	Hu2002	100	10/10	100	30.64	8
8	Hu2002	1000	9/9	100	29.73	6
9	Hu2002	5	10/10	100	29.67	12
10	NY99	5	7/10	70	20.80	50
11	NY99	500	10/10	100	30.66	4
12	Hu2002	10	10/10	100	29.40	11
13	NY99	50	9/10	90	31.14	4
14	Hu2002	500	10/10	100	31.30	6

*Only valid reactions were included
**Average of reactive replicates unless all tests were nonreactive, in which case the average analyte S/CO of nonreactive replicates is shown

Determination of Analytical Sensitivity Using a Dilutional Sensitivity Panel made from Lineage 2 WNV from Boston Biomedica (BBI)

An analytical sensitivity panel comprised of serially diluted WNV provided by BBI was used to evaluate assay sensitivity. Heat-inactivated, lineage 2 virus from the Qualification Panel QWN701 (10,000 copies/mL) was used to make a serially diluted analytical sensitivity panel. Three operators each tested 10 to 20 replicates of each copy level with each of the three clinical lots using the PROCLEIX® System for a total of 100 replicates. The same panel was tested using the PROCLEIX TIGRIS System. Three instruments were each used to test 10 replicates of each copy level with each of the three clinical lots for a total of 90 replicates. The 95% confidence intervals of the reactive rates were based on the exact binomial distribution.

WNV RNA detection with the PROCLEIX® WNV Assay was 98% and 100% at 100 copies/mL for the PROCLEIX System and the PROCLEIX® TIGRIS® System, respectively. Reactivity at 30 copies/mL was 99% for the PROCLEIX System and 97% for the PROCLEIX TIGRIS System. At 10 copies/mL, the detection rates were 89% for the PROCLEIX System and 82% for the PROCLEIX TIGRIS System (Tables 10a and 10b).

Table 10a. PROCLEIX® System - Detection of Lineage 2 WNV in BBI Analytical Sensitivity Panel

WNV RNA copies/mL	Number reactive/ tested*	% Reactive	95% CI		Average S/CO**	%CV
			Lower	Upper		
100	98/100	98	93	100	10.73	18
30	99/100	99	95	100	6.10	45
10	89/100	89	81	94	2.92	69
3	30/100	30	21	40	2.09	54
1	4/99	4	1	10	1.95	39
0	0/100	0	0	3	0.08	82

*Invalid reactions were not included
**Average of reactive replicates unless all tests were nonreactive, in which case the average analyte S/CO of nonreactive replicates is shown
CI = Confidence Interval

Table 10b. PROCLEIX® TIGRIS® System - Detection of Lineage 2 WNV in BBI Analytical Sensitivity Panel

WNV RNA copies/mL	Number reactive/ tested*	% Reactive	95% CI		Average S/CO**	%CV
			Lower	Upper		
100	90/90	100	97	100	10.72	19
30	85/88	97	90	99	5.78	45
10	73/89	82	72	89	2.60	66
3	15/88	17	10	27	1.64	45
1	1/90	1	0	6	1.09	n/a
0	0/90	0	0	3	0.08	113

*Only valid reactions included
**Average of reactive replicates unless all tests were nonreactive, in which case the average analyte S/CO of nonreactive replicates is shown
CI = Confidence Interval

PERFORMANCE OF THE PROCLEIX® WNV ASSAY IN CADAVERIC BLOOD SPECIMENS FROM TISSUE DONORS

REPRODUCIBILITY

The inter-assay reproducibility of the PROCLEIX® WNV Assay with cadaveric blood specimens was assessed by determining the %CVs obtained when each of 20 cadaveric and 20 control specimens spiked with 150 copies/mL WNV were tested with 3 clinical reagent kit lots: one lot was tested on only the PROCLEIX® System, a second lot was tested on both the PROCLEIX System and the PROCLEIX® TIGRIS® System, and a third lot was tested on only the PROCLEIX TIGRIS System. The reactive rates, S/COs, and %CVs are shown in Table 11. For the WNV spiked specimens tested with the PROCLEIX® System, the %CVs for the cadaveric and control specimens were 18% and 14%, respectively. For the WNV spiked specimens tested with the PROCLEIX TIGRIS System, the cadaveric and controls specimen %CVs were 8% and 7%, respectively. The percent reactive rate for cadaveric specimens and control specimens in this study was 100% for both the PROCLEIX System and the PROCLEIX TIGRIS System.

Table 11. PROCLEIX® WNV Assay with Cadaveric and Control Specimens Spiked with 150 copies/mL of WNV

	Sample	Number of donors	Number of replicates	% Reactive (95% CI)	Mean Analyte S/CO	%CV
PROCLEIX® System	Cadaveric	20	120	100% (97.5-100)	27.46	18
	Control	20	120	100% (97.5-100)	28.30	14
PROCLEIX® TIGRIS® System	Cadaveric	20	120	100% (97.5-100)	28.56	8
	Control	20	120	100% (97.5-100)	29.03	7

CI = Confidence Interval

SPECIFICITY

WNV-negative cadaveric serum specimens were tested to determine the specificity of the PROCLEIX® WNV Assay. Forty-five cadaveric specimens and 45 normal blood donor specimens were tested on the PROCLEIX® System and 51 cadaveric specimens and 51 normal blood donor specimens were tested on the PROCLEIX® TIGRIS® System. The cadaveric and control specimens were tested using three clinical lots. The specificity of the PROCLEIX WNV Assay for the cadaveric specimens in this study was 100% (95% CI: 94%-100%) for both the PROCLEIX System and the PROCLEIX TIGRIS System (Tables 12a and 12b). No invalid results were observed with the cadaveric specimens.

Table 12a. PROCLEIX® System - Specificity of PROCLEIX® WNV Assay with Cadaveric Blood Specimens

	Control	Cadaveric
n	44*	45
Mean IC S/CO	2.13	2.07
Analyte S/CO	0.12	0.15
Percent Specificity	100	100
95% CI	94-100	94-100

*45 samples were tested. One sample was invalid and was not used in the results analysis.
n = Number of samples
CI = Confidence Interval

Table 12b. PROCLEIX® TIGRIS® System - Specificity of PROCLEIX® WNV Assay with Cadaveric Blood Specimens

	Control	Cadaveric
n	51	51
Mean IC S/CO	2.05	2.16
Analyte S/CO	0.20	0.16
Percent Specificity	100	100
95% CI	94-100	94-100

n = Number of samples
CI = Confidence Interval

SENSITIVITY

WNV-negative cadaveric serum specimens spiked with a low level of WNV (approximately 150 copies/mL) were tested within 6 hours of spiking to determine the sensitivity of the PROCLEIX® WNV Assay. Forty-five cadaveric specimens and 45 normal blood donor specimens were tested on the PROCLEIX® System and 51 cadaveric specimens and 51 normal blood donor specimens were tested on the PROCLEIX® TIGRIS® System. The spiked cadaveric and control samples were tested using three clinical lots. The reactive rate of the PROCLEIX WNV Assay for the cadaveric specimens in this study was 100% (95% CI: 94%-100%) for both the PROCLEIX System and the PROCLEIX TIGRIS System (Tables 13a and 13b). No invalid results were observed with the cadaveric samples.

Table 13a. PROCLEIX® System - Sensitivity of the PROCLEIX® WNV Assay with Cadaveric Blood Specimens

	Control	Cadaveric
n	45	45
Analyte S/CO	33.16	29.55
Percent Sensitivity	100	100
95% CI	94-100	94-100

n = Number of samples
CI = Confidence Interval

Table 13b. PROCLEIX® TIGRIS® System - Sensitivity of the PROCLEIX® WNV Assay in Cadaveric Blood Specimens

	Control	Cadaveric
n	50*	51
Analyte S/CO	23.81	26.55
Percent Sensitivity	100	100
95% CI	94-100	94-100

*51 samples were tested. One sample was invalid and was not used in the results analysis.
n = Number of samples
CI = Confidence Interval

COMPARABILITY OF THE PROCLEIX® TIGRIS® SYSTEM AND THE PROCLEIX® SYSTEM

The comparability of the PROCLEIX® TIGRIS® System and the PROCLEIX® System was evaluated in panels composed of WNV positive and negative samples. The panels tested in the PROCLEIX® WNV Assay (n=510) contained positive members that were IgM-positive with RNA copy levels greater than 300 copies/mL, IgM-negative with RNA copy levels greater than 300 copies/mL, IgM-positive with RNA copy levels less than or equal to 300 copies/mL, IgM-negative with RNA copy levels less than or equal to 300 copies/mL, and negative members with various anticoagulants, interfering substances, and blood-borne pathogens. Three replicates of each panel were tested on the PROCLEIX TIGRIS System at three sites and on the PROCLEIX System at one site. The contents of the panels were masked during testing to control for bias. Testing was performed using one PROCLEIX WNV Assay clinical lot.

Of the 20 runs generated on the PROCLEIX System, 2 (10.0%) were invalidated by the operator because of operator error. From the valid assay runs, 2 of 1,526 (0.1%) test results were invalid on the PROCLEIX System; both were due to Internal Control failures. For the PROCLEIX TIGRIS System, 2 of 26 (7.7%) runs were invalid; both runs were invalidated by the operator because they contained more than 10% invalid test results. From the valid assay runs, 16 of 4,570 (0.4%) test results were invalid on the PROCLEIX TIGRIS System. Of the 16 invalid test results, 1 was due to Internal Control failure, 7 were due to instrument failures, 7 were due to clots in the samples, and 1 was due to insufficient sample volume.

To demonstrate equivalent performance in the samples with valid test results, the accuracy was calculated for each system using the PROCLEIX WNV Assay. The accuracies of the two systems were compared for all positive samples, the subcategories of the positive samples, all negative samples, and all samples combined. In addition, analysis of the S/CO values (IC for negative samples and analyte for positive samples) was performed for each system using the PROCLEIX WNV Assay. The S/CO values of the two systems were compared for all positive samples, the subcategories of the positive samples, and all negative samples.

Performance of the PROCLEIX WNV Assay on the PROCLEIX TIGRIS System was equivalent to that of the PROCLEIX System. The accuracy for all sample types was 99.6% (95% CI: 99.1%-99.9%) for the PROCLEIX System and 99.9% (95% CI: 99.8%-100%) for the PROCLEIX TIGRIS System (Table 14a). The accuracies were also similar between the two systems when using the PROCLEIX WNV Assay for the positive and negative samples. The mean analyte S/CO values for the positive samples generated from the PROCLEIX WNV Assay were 29.32 for the PROCLEIX System and 30.35 for the PROCLEIX TIGRIS System (Table 14b). The mean analyte S/CO values were also similar between the two systems for the various positive sample subcategories. The mean IC S/CO values for the negative samples were 1.96 for the PROCLEIX System and 2.11 for the PROCLEIX TIGRIS System.

Table 14a. Comparison of PROCLEIX® WNV Assay Performance with the PROCLEIX® TIGRIS® System and the PROCLEIX® System - Analysis of Accuracy

Sample Type	PROCLEIX® System			PROCLEIX® TIGRIS® System		
	Correct	Total	Accuracy (%) (95% CI)	Correct	Total	Accuracy (%) (95% CI)
All Samples	1518	1524	99.6 (99.1, 99.9)	4551	4554	99.9 (99.8, 100)
Positive Samples	297	299	99.3 (97.6, 99.9)	887	887	100 (99.6, 100)
> 300 copies/mL, IgM +	9	9	100 (66.4, 100)	27	27	100 (87.2, 100)
> 300 copies/mL, IgM -	146	146	100 (97.5, 100)	436	436	100 (99.2, 100)
≤ 300 copies/mL, IgM +	25	27	92.6 (75.7, 99.1)	80	80	100 (95.5, 100)
≤ 300 copies/mL, IgM -	117	117	100 (96.9, 100)	344	344	100 (98.9, 100)
Negative Samples	1221	1225	99.7 (99.2, 99.9)	3664	3667	99.9 (99.8, 100)

IgM+ = IgM positive
IgM- = IgM negative
CI = Confidence Interval
N/A = not applicable

Table 14b. Comparison of the PROCLEIX® WNV Assay Signal to Cutoff Values for the PROCLEIX® TIGRIS® System and the PROCLEIX® System

Sample Type	n		Mean S/CO		SD		%CV	
	PROCLEIX® System	PROCLEIX® TIGRIS® System	PROCLEIX® System	PROCLEIX® TIGRIS® System	PROCLEIX® System	PROCLEIX® TIGRIS® System	PROCLEIX® System	PROCLEIX® TIGRIS® System
Positive Samples	297	887	29.32	30.35	4.26	3.60	14.52	11.87
> 300 copies/mL, IgM +	9	27	31.31	30.32	4.58	3.40	14.62	11.21
> 300 copies/mL, IgM -	146	436	29.80	30.66	3.76	3.41	12.62	11.14
≤ 300 copies/mL, IgM +	25	80	27.46	27.86	5.89	6.20	21.44	22.27
≤ 300 copies/mL, IgM -	117	344	28.97	30.55	4.30	2.72	14.85	8.89
Negative Samples*	1221	3664	1.96	2.11	0.15	0.15	7.68	7.15

n = Number of samples
S/CO = Signal to cutoff ratio
SD = Standard deviation
CV = Coefficient of variation
* Analysis of internal control signal

An additional migration study sensitivity panel in which approximately 50% of the specimens were below 100 copies/mL was tested on the PROCLEIX System and the PROCLEIX TIGRIS System at 1 site. An analysis of accuracy of both systems for detection of the 414 replicates tested (3 replicates of 138 unique WNV-positive clinical specimens) is shown in Table 15a. With samples at or above 100 copies/mL, 178/180 (98.9%) of the replicates tested were detected on the PROCLEIX System, compared to 179/180 (99.4%) on the PROCLEIX TIGRIS System. With samples below 100 copies/mL, 183/234 replicates (78.2%) were detected on the PROCLEIX System and 170/234 (72.6%) on the PROCLEIX TIGRIS System. Although small differences were seen in the overall results (e.g. greater detection with the PROCLEIX TIGRIS System at or above 100 copies/mL and greater detection with the PROCLEIX System below 100 copies/mL) there were no statistically significant differences between the performances of the two platforms, as the 95% confidence intervals for the percent differences in each case included 0. In addition to the WNV-positive samples tested, 20 unique WNV-negative specimens were tested in 3 replicates each (60 replicates on each platform), yielding all non-reactive results on both the PROCLEIX System and PROCLEIX TIGRIS System platforms (Table 15a).

The mean analyte S/CO values for the positive samples generated from the PROCLEIX WNV Assay were 25.71 for the PROCLEIX System and 23.92 for the PROCLEIX TIGRIS System (Table 15b). The mean analyte S/CO values for the positive samples with less 100 copies/mL WNV were 19.90 for the PROCLEIX System and 18.51 for the PROCLEIX TIGRIS System. The mean analyte S/CO values for samples with greater than or equal to 100 copies/mL WNV were 33.27 for the PROCLEIX System and 30.95 for the PROCLEIX TIGRIS System. The mean IC S/CO values for the negative samples were 1.98 for the PROCLEIX System and 2.05 for the PROCELIX TIGRIS System.

Table 15a. Additional Migration Study: Accuracy of the PROCLEIX® WNV Assay on the PROCLEIX® TIGRIS® System Compared to the PROCLEIX® System

Sample Type	PROCLEIX® System			PROCLEIX® TIGRIS® System			
	Correct	Total	Accuracy (%) (95% CI)	Correct	Total	Accuracy (%) (95% CI)	Difference (%) (95% CI)
All Positive Sample Replicates	361	414	87.2 (82.8, 91.6)	349	414	84.3 (79.5, 89.1)	2.90 (–0.48, 6.28)
< 100 copies/mL	183	234	78.2 (71.1, 85.2)	170	234	72.6 (65.2, 80.1)	5.56 (–0.05, 11.16)
≥ 100 copies/mL	178	180	98.9 (96.3, 100)	179	180	99.4 (98.4, 100)	–0.56 (–2.99, 1.88)
Negative Samples	60	60	100 (95.1, 100)	60	60	100 (95.1, 100)	N/A

CI = Confidence Interval
N/A = not applicable

Table 15b. Additional Migration Study: Comparison of the PROCLEIX® WNV Assay Signal to Cutoff Values for the PROCLEIX® TIGRIS® System and the PROCLEIX® System

Sample Type	n		Mean S/CO		SD		%CV	
	PROCLEIX® System	PROCLEIX® TIGRIS® System	PROCLEIX® System	PROCLEIX® TIGRIS® System	PROCLEIX® System	PROCLEIX® TIGRIS® System	PROCLEIX® System	PROCLEIX® TIGRIS® System
All Positive Sample Replicates	414	414	25.71	23.92	12.85	12.09	49.96	50.56
< 100 copies/mL	234	234	19.90	18.51	12.87	13.51	64.65	72.99
≥ 100 copies/mL	180	180	33.27	30.95	7.98	3.45	23.99	11.15
Negative Samples*	60	60	1.98	2.05	0.11	0.11	5.63	5.34

n = number of samples
S/CO = Signal to cutoff ratio
SD = Standard deviation
CV = Coefficient of variation
* Analysis of internal control signal

BIBLIOGRAPHY

1. **Campbell G. L., A. A. Marfin, R. S. Lanciotti, and D. J. Gubler.** 2002. West Nile Virus. Lancet Infect Dis. **2**:519-529.
2. **Petersen L. R., and A. A. Marfin.** 2002. West Nile Virus: A Primer for the Clinician. Ann Intern Med. **137**:173-179.
3. **Centers for Disease Control.** January 24, 2002. West Nile Virus (WNV) infection: information for clinicians. Clinical features. Website posting.
4. **Centers for Disease Control.** September 29, 2004. West Nile Virus: Background Information for Clinicians. Website posting.
5. **Centers for Disease Control.** January 8, 2003. West Nile Virus: Virus History and Distribution. Website posting.
6. **Center for Biologics Evaluation and Research (CBER).** May 2003. Guidance for Industry: Revised Recommendations for the Assessment of Donor Suitability and Blood and Blood Product Safety in Cases of Known or Suspected West Nile Virus Infection, Final Guidance.
7. **Centers for Disease Control.** December 23, 2002. West Nile Virus: Questions and Answers. Website posting.
8. **Centers for Disease Control.** Statistics, Surveillance, and Control: Case Count 2002 (posted September 17, 2003), 2003 (posted May 21, 2004), and 2004 (posted November 30, 2004). Website postings.
9. **Centers for Disease Control.** March 13, 2003. Update on Investigations of West Nile Virus Transfusion Transmitted Cases. Presentation at the Blood Products Advisory Board.
10. **Centers for Disease Control.** September 13, 2002. Public Health Dispatch: Investigation of Blood Transfusion Recipients with West Nile Virus Infections. MMWR. **51**:823.
11. **Centers for Disease Control.** 2002. CDC Public Health Dispatch: Possible West Nile Virus Transmission to an Infant through Breast-Feeding. MMWR. **51**:877-878.
12. **Centers for Disease Control.** February 27, 2004. Interim Guidelines for the Evaluation of Infants Born to Mothers Infected with West Nile Virus During Pregnancy. MMWR. **53**:154-157.
13. **Centers for Disease Control.** August 20, 2004. Possible Dialysis-Related West Nile Virus Transmission. MMWR. **53**:738-739.
14. **Centers for Disease Control.** April 9, 2004. Update: West Nile Virus Screening of Blood Donations and Transfusion-Associated Transmission. MMWR. **53**:281-284.
15. **Centers for Disease Control.** September 17, 2004. Transfusion-Associated Transmission of West Nile Virus. MMWR. **53**:842-844.
16. **Giachetti, C., J. M. Linnen, D. P. Kolk, J. Dockter, et al.** 2002. Highly Sensitive Multiplex Assay for Detection of Human Immunodeficiency Virus Type 1 and Hepatitis C Virus RNA. J Clin Microbiol. **40**:2408-2419.
17. **Kacian, D. L. and T. J. Fultz.** 1995. Nucleic acid sequence amplification methods. U.S. Patent 5,399,491.
18. **Arnold, L. J., P. W. Hammond, W. A. Wiese, and N. C. Nelson.** 1989. Assay formats involving acridinium-ester-labeled DNA probes. Clin Chem **35**:1588-1594.
19. **Nelson, N. C., A. Cheikh, E. Matsuda and M. Becker.** 1996. Simultaneous detection of multiple nucleic acid targets in a homogeneous format. Biochem. **35**:8429-8438.
20. **Title 42, Code of Federal Regulations**, Part 72, 2004.
21. **29 CFR Part 1910.1030.** Occupational exposure to bloodborne pathogens; Final Rule, Federal Register/Vol. **56**, No. 235/ December 6, 1991.
22. **National Committee for Clinical Laboratory Standards.** 1986. Clinical laboratory hazardous waste; proposed guidelines. NCCLS Document GP5-P. Villanova, PA.
23. **U. S. Environmental Protection Agency.** EPA guide for infectious waste management. Washington, DC: U.S. Environmental Protection Agency, Publication No. EPA/530-SW-86-014, 1986.

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FOCUS Diagnostics	**Standard Operating Procedure** **TECHNICAL** TITLE: **West Nile Virus IgM Enzyme–Linked Immunosorbent Assay (ELISA)** AUTHOR: **Tiffany Phan, CLS**	Doc. #: **TSOP.127.144** Revision: **G2** Page 1 of 9

1.0 BACKGROUND AND PRINCIPLE

Most medically important arthropod-borne viruses (arboviruses) belong to three virus families: togaviridae, flaviviridae, and bunyaviridae. West Nile virus is a flavivirus within the Japanese encephalitis antigenic complex, which includes 4 related viruses that cause CNS infection: St. Louis encephalitis (SLE), Japanese encephalitis (JE), Murray Valley encephalitis, and West Nile virus (WNV).

WNV was first isolated from a febrile human in the West Nile district of Uganda in 1937. It was soon recognized as one of the most widely distributed flaviviruses. West Nile genome is a single-stranded RNA that encodes 3 structural proteins (capsid, premembrane, and envelope) and 7 nonstructural proteins. The mature virion is a nucleocapsid enveloped in a lipid bilayer with projecting envelope proteins that mediate cellular attachment and membrane fusion and appear to be important virulence factors.

West Nile fever is a mosquito-borne flavivirus infection transmitted to vertebrate primarily by various species of Culex mosquitoes. Birds (especially crows) usually become infected from the bite of an infected mosquito. Infected ticks have also been reported and direct bird-to-bird transmission has been experimentally demonstrated in crows sharing common cages. After female mosquito ingest blood from infected birds, virus replicates in the mosquito gut and salivary glands and is transmitted in salivary fluid during subsequent bites. After the bite of an infected mosquito, humans and horses can develop viremia, but it is not known what role humans and other mammals contribute to virus amplification and transmission. No data suggest that human infections result from direct contact with infected humans or vertebrates or from infectious aerosols in natural settings.

Clinically, WN fever is an acute self-limited febrile illness, ranging from nonspecific viral syndrome to fatal encephalitis. Viral incubation period ranges 3–15 days. Mild illness may be accompanied by headache, rash, lymphadenopathy, polyartropathy, myalgia and anorexia. Rarely acute hepatitis or pancreatitis has been reported. CNS infection presumably occurs when virus crosses the blood-brain barrier by endothelial replication or axonal transport through neurons. Factors that enhance progression of CNS infections among the elderly may include those that disrupt the blood-brain barrier (e.g. hypertension) or increase the duration and level of viremia (e.g., immune suppression). Physicians should include WNV infection in the differential diagnosis of summertime febrile illnesses and unexpected encephalitis or aseptic meningitis. Currently, no human or veterinary vaccine is available to prevent WNV infection, and mosquito control is the only practical strategy to combat the spread of the disease.

FOCUS Diagnostics	**Standard Operating Procedure** **TECHNICAL** TITLE: **West Nile Virus IgM Enzyme–Linked Immunosorbent Assay (ELISA)**	Doc. #: **TSOP.127.144** Revision: **G2** Page 2 of 9

The Focus Diagnostics WNV IgM ELISA is an antibody-capture assay. Specimens are added to IgM capture plates coated with goat anti human IgM. After the incubation period, the unbound materials are washed away and antigen solution, containing recombinant WNV antigen is added which binds to specific anti WNV IgM captured on the plate. This complex is reacted with peroxidase-conjugated mouse monoclonal anti flavivirus antibody (MAB), followed by chromogen. The OD is read at 450 nm and is directly proportional to the amount of anti-WNV IgM present in the original serum. OD's are transformed into an index value as calculated against a reference calibrator serum included with each assay run.

2.0 SPECIMEN

2.1 Serum, plasma and CSF are acceptable sources for West Nile virus capture ELISA.

2.2 Qualified personnel should collect blood samples using approved venipuncture techniques. Transfer serum / plasma aseptically to a tightly closing sterile container. Store specimens at 2–8°C. If testing is to be delayed longer than 14 days, freezing the sample at –20°C ± 10°C is recommended. Hyperlipemic, grossly hemolyzed and contaminated specimens may give erroneous test results and should be avoided.

3.0 REAGENTS—SPECIAL SUPPLIES AND EQUIPMENT

3.1 Test Components: Kits and kit reagents are stable through the end of the month indicated by the label expiration date when stored at 2–8°C.

3.1.1 IgM Capture Wells, 96 wells

3.1.2 Anti-Flavivirus Conjugate, 16 mL

3.1.3 10X Wash Buffer, 100 mL – Prepare 1X was buffer solution before use.

3.1.4 Positive Control, 0.3 mL

3.1.5 Negative Control, 0.3 mL

3.1.6 Cut-Off Calibrator, 0.3 mL

FOCUS Diagnostics	**Standard Operating Procedure** **TECHNICAL** TITLE: **West Nile Virus IgM Enzyme–Linked Immunosorbent Assay (ELISA)**	Doc. #: **TSOP.127.144** Revision: **G2** Page 3 of 9

3.1.7 Sample Diluent, 12 mL

3.1.8 Substrate Reagent, 16 mL

3.1.9 Stop Reagent, 16 mL

3.1.10 Sealing Tape

3.2 **Preparation of Reagents**

3.2.1 Working wash buffer:

3.2.1.1 To 900 mL distilled water, add 100 mL concentrated (10X) wash buffer.

3.2.1.2 Mix completely. Store working wash buffer at 2–8°C for up to one month.

3.2.2 WNV antigen solution

3.2.2.1 Lyophilized antigen aliquots (lyospheres) are stored at 2–8°C.

3.2.2.2 Stock solution: prepare by adding 8 mL Focus Diagnostics IgG diluent to each vial containing 40 microliters of lyophilized VPA antigen (final dilution, 1:200). Mix by inversion. Store at 2–8°C for up to 30 days.

3.2.2.3 Working solution: remove sufficient volume from cold stock to allow 100 µL per well. Transfer to 15 cc or 50 cc centrifuge tube(s). This aliquot of working antigen solution may be warmed to room temperature or used cold. Do not re-use warmed working antigen solution.

3.3 Equipment

3.3.1 Vortex mixer

3.3.2 Wash bottle or automated plate washer

3.3.3 Glass tubes

3.3.4 Marsh tubes or mini tubes

3.3.5 Hamilton / Multiprobe diluter

3.3.6 Serological pipettes

3.3.7 20 µL, 200 µL and 1000 µL pipettors with disposable tips

3.3.8 Timer

3.3.9 Paper towels

3.3.10 Plate reader and printer

4.0 QUALITY CONTROL

4.1 Each assay run must include the Negative control, Cutoff calibrator, and positive control.

4.2 Controls

4.2.1 The Negative Control index values should be less than 0.8.

4.2.2 The Positive Control index should be between 1.5 and 3.5.

4.2.3 The mean value for the Cut-Off Calibrator OD's must be within 0.100 to 0.700 OD units.

4.2.4 For acceptance criteria, refer to GSOP.110.014, "Immunology QC/QI Program".

4.3 **Parallel Testing of new lots of Reagent Packs**

For evaluation guidelines, acceptability, and documentation refer to PROC.105.010, "Parallel Testing".

FOCUS Diagnostics	**Standard Operating Procedure** **TECHNICAL** TITLE: **West Nile Virus IgM Enzyme–Linked Immunosorbent Assay (ELISA)**	Doc. #: **TSOP.127.144** Revision: **G2** Page 5 of 9

5.0 PROCEDURE—STEPWISE

To prepare all reagents using for the assay, refer to Preparations of Reagent Section.

5.1 **West Nile IgM Screen**: Bring Sample diluent, controls, working wash buffer and IgM capture plates to (20–25°C) room temperature. Place required number of strips in strip holder. Return the remaining strips to the foil pouch, seal tightly and keep refrigerated.

5.2 Dilute Cutoff calibrator, Positive and Negative control, and sera 1:101 with Sample diluent. Dilute CSF specimens 1:2 with Sample diluent. All dilutions may be prepared manually or by automated diluters (Hamilton/Multiprobe).

Note: *Short CSF samples may be conserved by pipetting 50 µL of IgG sample diluent directly to the test well following by the addition of 50 µL CSF sample (final dilution: 1:2).*

5.3 Run Cutoff calibrator in triplicate, Negative and Positive control in duplicate, and samples in singlicate. Assign well A1 as a Blank well.

5.4 Add 100 µL of each diluted control and specimen to assigned wells; and 100 µL Sample diluent to the Blank well.

5.5 Cover plate and incubate for 1.0 hour at 20–25°C (room temp).

5.6 Wash 3 times with wash buffer manually or by plate washer. Remove all residual wash buffer by blotting on absorbent paper.

5.7 Add 100 µL of working Antigen solution to each well; cover and incubate the plate for 2 hours at 20–25°C (room temp).

5.8 Wash the plate as described in step 5.6.

5.9 Add 100 µL conjugate to each well. Cover and incubate for 30 minutes at 20–25°C (room temp).

5.10 Wash the plate as step 5.6.

5.11 Add 100 µL of TMB Substrate to each well and incubate for 10 minutes at 20–25°C (room temp).

5.12 Add 100 µL of Stop reagent to each well.

5.13 Read plate within 30 minutes at 450 nm using WNV-M Protocol of Bio-Tek Reader.

5.14 **West Nile IgM Confirmation**: Use the same IgM capture plates and reagents used for WNV IgM Test. For reagent preparation refer to WNV IgM procedure (3.2.2).

5.15 Dilute Positive control, Negative control, Cutoff calibrator, serum, plasma, and CSF specimens with sample diluent as described for WNV IgM Assay.

5.16 Add 100 µL of diluted Controls and samples to the corresponding wells according to the following template: (Leave a dummy strip for position No.2; this strip will not be used for the assay).

	1		3	4	5	6	7	8	9	10	11	12
A	**BLANK**		**1**	1	**9**	9	**17**	17	**25**	25	**33**	33
B	**NC**		**2**	2	**10**	10	**18**	18	**26**	26	**34**	34
C	**NC**		**3**	3	**11**	11	**19**	19	**27**	27	**35**	35
D	**CO**		**4**	4	**12**	12	**20**	20	**28**	28	**36**	36
E	**CO**		**5**	5	**13**	13	**21**	21	**29**	29	**37**	37
F	**CO**		**6**	6	**14**	14	**22**	22	**30**	30	**38**	38
G	**HPC**		**7**	7	**15**	15	**23**	23	**31**	31	**39**	39
H	**HPC**		**8**	8	**16**	16	**24**	24	**32**	32	**40**	40

5.17 Cover the plate and incubate at (20–25°C) room temperature for 1.0 hours.

5.18 Wash 3 times with wash buffer manually or by plate washer. Remove all residual wash buffers by blotting on absorbent paper.

5.19 Add 100 µL of Antigen solution to the following strips: **1, 3, 5, 7, 9, 11**

5.20 Add 100 µL of Sample diluent to the following strips: **4, 6, 8,10, 12**

5.21 Incubate the plate for 2 hours at 20–25°C (room temp.).

5.22 Wash the plate as step 5.17.

5.23 Add 100 µL conjugate to each well. Cover and incubate for 30 minutes 20–25°C (room temp.)

5.24 Wash the plate as step 5.17.

5.25 Add 100 µL of TMB Substrate to each well and incubate for 10 minutes at room temp.

5.26 Add 100 µL of Stop reagent to each well.

5.27 Read plate within 30 minutes at 450 nm using WNV-M Confirm Protocol of Bio-Tek Reader.

5.28 For controls, the index value is calculated by dividing the OD of each control by the mean OD of the Cut off calibrator.

5.29 For each patient, first the OD of the diluent-well is subtracted from the OD of the corresponding antigen-well to attain the corrected OD.

5.30 To calculate the index value, the corrected OD of each patient is divided by the mean OD of the Cut-off calibrator. All calculations are done by Bio-Tek reader software.

6.0 REPORTING RESULTS

6.1 Calculations

West Nile IgM Screen: Calculations are done by Bio-Tek reader software. The blank OD is subtracted from each well's OD to calculate the corrected OD. Then the Index Value is calculated by dividing the corrected OD of each control and specimen by the mean corrected OD of the Cutoff calibrator.
Sample well with OD less than the Blank's well should be repeated.

6.2 Report indexes rounded to the second decimal point. For example, a generated index of 1.477 would be reported as 1.48.

6.3 Report IgM index values < 0.90 as "< 0.90", rather than the actual index value.

FOCUS Diagnostics	**Standard Operating Procedure** **TECHNICAL** TITLE: **West Nile Virus IgM Enzyme–Linked Immunosorbent Assay (ELISA)**	Doc. #: **TSOP.127.144** Revision: **G2** Page 8 of 9

6.4 Serum results for IgM are interpreted based upon the IgG results obtained from the same sample:

IgG Index	IgM Index	Interpretation
< 1.30	< 0.90	Antibody not detected
< 1.30	0.90–1.10	Equivocal
< 1.30	> 1.10	Recent/current infection
1.30–1.50	< 0.90	Equivocal
1.30–1.50	0.90–1.10	Equivocal
1.30–1.50	> 1.10	Recent/current infection
> 1.50	< 0.90	Past infection
> 1.50	0.90–1.10	Recent/current infection
> 1.50	> 1.10	Recent/current infection

6.5 All of the serum/plasma specimens with IgM screen index value of ≥ 0.90 must be confirmed by WNV IgM Confirmatory Test (regardless of IgG result).

6.6 CSF results for IgM are interpreted based upon the IgG results obtained from the same sample:

IgG Index	IgM Index	Interpretation
< 1.30	< 0.90	Antibody not detected
< 1.30	0.90 – 1.10	Equivocal
< 1.30	> 1.10	Antibody detected
1.30 – 1.50	< 0.90	Equivocal
1.30 – 1.50	0.90 – 1.10	Equivocal
1.30 – 1.50	> 1.10	Antibody detected
> 1.50	< 0.90	Antibody detected
> 1.50	0.90 – 1.10	Antibody detected
> 1.50	> 1.10	Antibody detected

6.7 All of the CSF specimens with IgM index value of ≥ 0.90 must be confirmed by WNV IgM Confirmatory Test (regardless of IgG result).

6.8 **West Nile IgM Confirmation:** For serum/plasma/CSF specimen, if the index value of confirmatory test is ≥ 0.90, report the actual index value of confirmatory test with 2 decimal places.

FOCUS Diagnostics	**Standard Operating Procedure** **TECHNICAL** TITLE: **West Nile Virus IgM Enzyme–Linked Immunosorbent Assay (ELISA)**	Doc. #: **TSOP.127.144** Revision: **G2** Page 9 of 9

6.9 For serum/plasma/CSF specimen, if the index value of confirmatory test is < 0.90, report the WNV IgM result as "< 0.90".

6.10 Client services will contact the clients and send specimens positive for WNV IgM to the appropriate State Public Health laboratory for confirmatory testing.

7.0 LIMITATION OF THE PROCEDURE

Strong cross reactivity is seen with other flaviviruses, thus a positive result may be caused by infection with another flavivirus. Traditionally, only the plaque reduction neutralization test (PRNT) or virus isolation provides unambiguous identification of West Nile virus infection.

8.0 REFERENCES

WEST NILE Virus IgM Capture ELISA, Focus Diagnostics Cypress, CA. P.I. EL0300M, REV. I1 Date written: 27-Dec-2007

END OF DOCUMENT

FOCUS Diagnostics	**Standard Operating Procedure** **TECHNICAL** TITLE: **West Nile Virus IgG Antibody, ELISA** AUTHOR: **Tiffany Phan, CLS**	Doc. #: **TSOP.127.002** Revision: **G1** Page 1 of 6

1.0 BACKGROUND AND PRINCIPLE

Most medically important arthropod-borne viruses (arboviruses) belong to three virus families: togaviridae, flaviviridae, and bunyaviridae. West Nile virus is a flavivirus within the Japanese encephalitis antigenic complex, which includes 4 related viruses that cause CNS infection: St. Louis encephalitis (SLE), Japanese encephalitis (JE), Murray Valley encephalitis, and West Nile virus (WNV).

WNV was first isolated from a febrile human in the West Nile district of Uganda in 1937. It was soon recognized as one of the most widely distributed flaviviruses. West Nile genome is a single-stranded RNA that encodes 3 structural proteins (capsid, premembrane, and envelope) and 7 nonstructural proteins. The mature virion is a nucleocapsid enveloped in a lipid bilayer with projecting envelope proteins that mediate cellular attachment and membrane fusion and appear to be important virulence factors.

West Nile fever is a mosquito-borne flavivirus infection transmitted to vertebrate primarily by various species of Culex mosquitoes. Birds (especially crows) usually become infected from the bite of an infected mosquito. Infected ticks have also been reported and direct bird-to-bird transmission has been experimentally demonstrated in crows sharing common cages. After female mosquito ingest blood from infected birds, virus replicates in the mosquito gut and salivary glands and is transmitted in salivary fluid during subsequent bites. After the bite of an infected mosquito, humans and horses can develop viremia, but it is not known what role humans and other mammals contribute to virus amplification and transmission. No data suggest that human infections result from direct contact with infected humans or vertebrates or from infectious aerosols in natural settings.

Clinically, WN fever is an acute self-limited febrile illness, ranging from nonspecific viral syndrome to fatal encephalitis. Viral incubation period ranges 3–15 days. Mild illness may be accompanied by headache, rash, lymphadenopathy, polyartropathy, myalgia and anorexia. Rarely acute hepatitis or pancreatitis has been reported. CNS infection presumably occurs when virus crosses the blood-brain barrier by endothelial replication or axonal transport through neurons. Factors that enhance progression of CNS infections among the elderly may include those that distrupt the blood-brain barrier (e.g. hypertension) or increase the duration and level of viremia (e.g., immune suppression). Physicians should include WNV infection in the differential diagnosis of summertime febrile illnesses and unexpected encephalitis or aseptic meningitis. Currently, no human or veterinary vaccine is available to prevent WNV infection, and mosquito control is the only practical strategy to combat the spread of the disease.

FOCUS Diagnostics	**Standard Operating Procedure** **TECHNICAL** TITLE: **West Nile Virus IgG Antibody, ELISA**	Doc. #: **TSOP.127.002** Revision: **G1** Page 2 of 6

The Focus Diagnostics WNV IgG assay is an indirect ELISA. Each well is coated with recombinant VPA antigen. Diluted specimen is added, and serum IgG reacts with the antigen-coated wells. The complexes are reacted with peroxidase-conjugated goat anti-human IgG followed by chromogen. The OD is read at 450 nm and is directly proportional to the amount of anti-WNV IgG present in serum. OD's are transformed into an index value as calculated against a reference calibrator serum included with each assay run.

2.0 SPECIMEN

2.1 Serum, plasma and CSF are acceptable sources for West Nile IgG ELISA.

2.2 Blood samples should be collected using approved venipuncture techniques by qualified personnel. Allow sample to clot and separate serum by centrifugation. Transfer serum aseptically to a tightly closing sterile container. Store at 2–8°C. If testing is to be delayed longer than 14 days, freezing the sample at –20°C (± 10°C) is recommended. Hyperlipemic and contaminated serum specimens may give erroneous test results and should be avoided.

3.0 REAGENTS—SPECIAL SUPPLIES AND EQUIPMENT

3.1 Test components: Kits and kit reagents are stable through the end of the month indicated by the label expiration date when stored at 2–8°C.

3.1.1 IgG Antigen Wells, 96 wells

3.1.2 IgG Sample Diluent, 100 mL

3.1.3 Positive Control, 0.3 mL

3.1.4 Cut-off Calibrator, 0.3 mL

3.1.5 Negative Control, 0.3 mL

3.1.6 IgG Conjugate, 16 mL

3.1.7 10X Wash Buffer, 100 mL

3.1.8 Substrate Reagent, 16 mL

3.1.9 Stop Reagent, 16 mL

3.2 Equipment

3.2.1 Vortex Mixer

3.2.2 Wash Bottle Or Automated Plate Washer

3.2.3 1 Liter Graduated Cylinder

3.2.4 Glass Tubes Or Mini-Tubes

3.2.5 Serological Pipettes

3.2.6 20 µL And 200 µL Pipettors With Disposable Tips

3.2.7 Timer

3.2.8 Paper Towels

3.2.9 Plate Reader And Printer

3.3 Preparation of Reagents

Working wash buffer:

3.3.1 To 900 mL distilled water, add 100 mL concentrated (10X) wash buffer.

3.3.2 Mix completely. Store working wash buffer at 2–8°C for up to one month.

4.0 QUALITY CONTROL

4.1 Each assay run must include Negative Control, Cutoff calibrator (C/O), and Positive control.

4.2 Controls

4.2.1 The Negative Control index values should be less than 0.8

4.2.2 The Positive Control index values should be between 1.5 and 3.5.

4.2.3 The mean value for the Cut-off Calibrator OD's must be within 0.100 to 0.700 OD units.

4.2.4 For acceptance criteria, refer to GSOP.110.014, "Immunology QC/QI Program".

4.3 Parallel Testing of new component lots

For parallel testing guidelines and acceptance criteria, refer to PROC.105.010, "Parallel Testing".

5.0 PROCEDURE—STEPWISE

5.1 Calibrator is run in triplicate while the Negative Control and Positive controls are run in duplicate. Patient samples are run in singlicate. A minimum of one blank well should also be included.

5.2 Bring reagents to room temperature (20–25°C).

5.3 For each serum specimen or control, prepare a 1:101 dilution in the sample diluent provided. For example, pipet 5 microliters of serum into 500 microliters sample diluent.

5.3.1 Dilute CSF specimens 1:2 with Sample diluent.

5.3.2 All dilutions may be prepared manually or by automated diluters (Hamilton/Multiprobe).

Note: *Short CSF samples may be conserved by pipetting 50 µL of IgG sample diluent directly to the test well following by the addition of 50 µL CSF sample (final dilution: 1:2).*

5.4 Add 100 µL of each diluted specimen and control to assigned wells; be sure to include a blank consisting of sample diluent only.

5.5 Cover plate and incubate for 1 hour at room temperature (20–25°C).

5.6 Remove samples from wells. Wash 3 times with wash buffer.

5.7 Remove all residual buffers.

5.8 Add 100 µL of conjugate to each well; incubate the plate for 30 minutes at room temperature (20–25°C).

5.9 Wash the plates as in step 5.6–5.7.

5.10 Add 100 µL of TMB substrate to each well and incubate for 10 minutes at room temperature (20–25°C).

5.11 Add 100 µL of Stop Reagent to each well.

5.12 Read plate at 450 nm using WNV-G protocol of Bio-Tek reader.

6.0 CALCULATIONS (See Quality Control for Limitations)

6.1 Subtract the blank OD from each specimen, control and calibrator to calculate the net OD for each specimen.

6.2 Calculate the mean (corrected) Calibrator Value.

6.3 Calculate the Index Value by dividing the corrected OD for each specimen and control by the corrected mean cutoff calibrator value.

6.4 All calculations may be done using plate reader's software.

7.0 REPORTING RESULTS

7.1 Report indexes rounded to the second decimal point. For example, a generated index of 1.477 would be reported as 1.48.

7.2 Report IgG index values < 1.30 as "< 1.30", rather than the actual index value.

7.3 All index values ≥ 1.30 are repeated in next run.

7.4 Sample well with OD less than the Blank's well should be repeated.

7.5 Serum results for IgG are interpreted based upon the IgM results obtained from the same sample:

IgG Index	IgM Index	Interpretation
< 1.30	< 0.90	Antibody not detected
< 1.30	0.90–1.10	Equivocal
< 1.30	> 1.10	Recent/current infection
1.30–1.50	< 0.90	Equivocal
1.30–1.50	0.90–1.10	Equivocal
1.30–1.50	> 1.10	Recent/current infection
> 1.50	< 0.90	Past infection
> 1.50	0.90–1.10	Recent/current infection
> 1.50	> 1.10	Recent/current infection

FOCUS Diagnostics	**Standard Operating Procedure** **TECHNICAL** TITLE: **West Nile Virus IgG Antibody, ELISA**	Doc. #: **TSOP.127.002** Revision: **G1** Page 6 of 6

7.6 CSF results for IgG are interpreted based upon the IgM results obtained from the same sample:

IgG Index	IgM Index	Interpretation
< 1.30	< 0.90	Antibody not detected
< 1.30	0.90–1.10	Equivocal
< 1.30	> 1.10	Antibody detected
1.30–1.50	< 0.90	Equivocal
1.30–1.50	0.90–1.10	Equivocal
1.30–1.50	> 1.10	Antibody detected
> 1.50	< 0.90	Antibody detected
> 1.50	0.90–1.10	Antibody detected
> 1.50	> 1.10	Antibody detected

8.0 PROCEDURE NOTES

West Nile Virus is a flavivirus recently associated with an outbreak of encephalitis in the Eastern United States. West Nile Virus IgM is usually detectable by the time symptoms appear, but IgG may not be detectable until day 4 or day 5 of illness. Antibodies induced by West Nile Virus infection show extensive crossreactivity with other flaviviruses, including Dengue Fever Virus, Japanese Encephalitis, and St. Louis Encephalitis Virus.

9.0 LIMITATIONS OF PROCEDURE

9.1 Reactivity with individual West Nile Virus types cannot be determined.

9.2 Strong cross-reactivity is seen with other flaviruses, thus a positive result may be caused by infection with another flavivirus.

10.0 REFERENCES

WEST NILE IgG Focus Diagnostics, Cypress, CA 10/15/2004 Rev.G

END OF DOCUMENT

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Title: LabScan Luminex Reader Maintenance			Page 1 of 3		
Doc#	Imm003	**Revision:**		**Effective Date:**	12/10/09

1	Purpose		To provide instructions on how to maintain the Luminex reader
2	Scope	2.1	
3	Responsibilities	3.1	It is the responsibility of the supervisor to ensure that the Core Immunology Laboratory personnel have been trained in accordance with using the Labscan Luminex reader.
		3.2	It is the responsibility of the supervisor to ensure that the personnel have been trained in properly handling human specimens and wearing PPE.
4	Materials Required	4.1	LabScan Luminex 100 IS Reader
		4.2	Computer
		4.3	Bio-Rad MCV Plate
		4.4	Bio-Plex Manager Software
		4.5	Deionizer Water
		4.6	70% Isopropanol
		4.7	10% Bleach
		4.8	20 mL syringe
		4.9	3/32 inch hex wrench
		4.10	Sonicator
5	Procedure	**5.1**	**Daily Start-up**
		5.1.1	Make sure that the shield fluid cube contains sufficient volume for assays
		5.1.2	Turn power on the Luminex 100 analyzer, Luminex XYP platform and the Luminex Sheath Fluid Delivery System
		5.1.3	Start the BioPlex Manager software
		5.1.4	Software will connect with the reader and automatically start a 30 minute timer to warm up the laser, during which the prime and start-up program may be run
		5.1.5	Select *Prime* from the drop down menu
		5.1.6	Reader will perform a 2 minute prime of the machine, no plate is needed
		5.1.7	Add deionized water and 70% Isopropanol to the appropriate wells in the MCV plate

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		5.1.8	Click on the *Start-up* icon on the software toolbar
		5.1.9	Click on open tray and place the MCV plate on the tray platform
		5.1.10	Close tray and click on start
		5.1.11	Remove MCV plate after program is finished
		5.2	**Daily Shut Down**
		5.2.1	Add 10% bleach to the appropriate well in the MCV plate
		5.2.2	Click on the *Shut Down* icon the software toolbar
		5.2.3	Click on open tray and place the MCV plate on the tray platform
		5.2.4	Close tray and click on start
		5.2.5	Shut down program takes 10 minutes
		5.2.6	Remove MCV plate after program is finished
		5.2.7	Turn off the power on the Luminex 100 analyzer, Luminex XYP platform and the Luminex Sheath Fluid Delivery System
		5.2.8	Close the BioPlex Manager Software program
		5.3	**Clean the sample probe**
		5.3.1	Remove the clear plastic housing that covers the sample probe area
		5.3.2	Unsnap the light housing located above the probe
		5.3.3	Unscrew the fitting on top of the probe completely
		5.3.4	Gently move the probe up out of the housing
		5.3.5	Clean the probe by placing the narrow tip into the sonicator for 2 to 5 minutes
		5.3.6	Using a 20 ml syringe, back flush the probe with distilled water from the narrow end out through the larger end
		5.3.7	Replace the probe
		5.3.8	Run 3 Backflushes, 2 Alcohol Flushes, and 3 Washes with deionized water
		5.4	**Adjust the sample probe height**
		5.4.1	Remove the clear plastic housing that covers the sample probe area
		5.4.2	Using an old Millipore plate, add three small round metal alignment discs into position H12

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		5.4.3	Select the maintenance tab, then click Eject/Retract to eject the plate platform
		5.4.4	Place the Millipore plate onto the plate platform
		5.4.5	Click Eject/Retract to retract the plate
		5.4.6	Use the 3/32 inch hex wrench to loosen the height adjustment locking screw
		5.4.7	Click sample probe down
		5.4.8	Using the thumb wheel, lower the probe until it just touches the top of the alignment discs
		5.4.9	Use the 3/32 hex wrench to tighten the height adjustment locking screw
		5.4.10	Click Sample Probe Up to raise the sample probe
		5.4.11	Check the alignment by clicking Sample Probe Down and watching the sample probe as it touches the top of the alignment discs
		5.4.12	There should be a very slight downward movement of the Millipore plate as the sample probe touches the alignment discs
		5.4.13	Readjust if necessary
		5.4.14	Replace the plastic shield that covers the probe area

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Title: Milliplex High Sensitivity Human Cytokine / Chemokine Kit			Page 1 of 4		
Doc#	Imm002	**Revision:**		**Effective Date:**	4/22/10

1	Purpose		To provide instruction on performing the Milliplex Cytokine Kit assay
2	Scope	2.1	To measure high sensitivity cytokines in plasma or serum.
3	Responsibilities	3.1	The Antibody-Immobilized Beads are light sensitive and must be protected from light.
		3.2	Cover the assay plate containing beads with opaque plate lid or aluminum foil duringall incubation steps.
		3.3	Reagents must be at room temperature before use.
		3.4	The bottom of the Microtiter Filter Plate must not be in direct contact with any surface during assay setup or incubation times. Use the plate stand at all times.
		3.5	Blot the bottom of the Microtiter Filter Plate after each wash with a paper towel.
		3.6	Keep vacuum settings at lowest possible level.
		3.7	After hydration, all Standards and Controls must be transferred to polypropylene tubes.
		3.8	Standards prepared by serial dilution must be used within one hour.
		3.9	Plate shaker should be set between 500 to 800 RPM.
		3.10	Frozen plasma or serum must be completely thawed, vortexed and centrifuged before addition to plate.
		3.11	Vortex all reagents well before adding to plate.
4	Materials Required	4.1	LabScan Luminex 100 IS Reader
		4.2	Computer
		4.3	Bio-Plex Manager Software
		4.4	Plate Shaker
		4.5	4° C Refrigerator
		4.6	Pipetman P1000 and P200 with tips
		4.7	Rainin Multichannel Pipette 20 ul – 200 ul

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		4.8	Polypropylene Microfuge Tubes
		4.9	Laboratory Vortex Mixer
		4.10	Vacuum Filtration Unit
		4.11	Vacuum Pump
		4.12	Reagent Reservoirs
		4.13	Plate Stand
		4.14	Plate Map Form
5	Procedure	5.1	Preparation of Reagents
		5.1.1	Preparation of Antibody-Immobilized Beads
		5.1.2	If premixed beads are used, sonicate bottle for 30 seconds then vortex for 1 minute before use.
		5.1.3	For individual beads, sonicate each vial for 30 seconds then vortex for 1 minute.
		5.1.4	Add 60 µl from each bead vial into the mixing bottle and then bring the final volume up to 3.0 mL with Bead Diluent. Vortex the mixed beads well.
		5.1.5	Quality Controls
		5.1.6	Reconstitute QC 1and QC 2 vials with 250 µL deionized water.
		5.1.7	Invert vial several times to mix and vortex.
		5.1.8	Let stand 5 – 10 minutes and transfer to polypropylene tubes.
		5.1.9	Wash buffer
		5.1.10	Dilute 30 mL of 10X Wash Buffer with 270 mL deionized water.
		5.1.11	Store at 2-8°C for up to one month.
		5.2	Preparation of Serum Matrix
		5.2.1	Add 1.0 mL deionized water to the lyophilized Serum Matrix. Mix well.
		5.2.2	Allow at least 10 minutes for complete reconstitution.
		5.3	Preparation of Human Cytokine Standards
		5.3.1	Reconstitute Human Cytokine Standard with 250 µL deionized water.
		5.3.2	Invert vial several times to mix and vortex.
		5.3.3	Let stand 5 – 10 minutes and transfer to polypropylene tube.
		5.3.4	This will be used as the 2,000 pg/mL standard.
		5.3.5	Label five polypropylene microfuge tubes 400, 80,

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			16, 3.2, 0.64 and 0.13 pg/mL.
		5.3.6	Add 200 µL Assay Buffer to each tube.

Prepare serial dilution:

Standard Concentration	Volume of Assay Buffer	Volume of Standard to add
2000	200 µL	50 µL of 10,000 pg/mL
400	200 µL	50 µL of 2000 pg/mL
80	200 µL	50 µL of 400 pg/mL
16	200 µL	50 µL of 80 pg/mL
3.2	200 µL	50 µL of 16 pg/mL

		5.4	Immunoassay Procedure
		5.4.1	Fill in the Milliplex High Sensitivity Human Cytokine Plate Map with the tech ID, lot number, date performed, and expiration date of the kit.
		5.4.2	Indicate the location of all the samples to be run on the Plate Map.
		5.4.3	Prewet the plate with 200 µL of Wash Buffer into each well.
		5.4.4	Seal and mix on the plate shaker for 10 minutes.
		5.4.5	Remove Wash Buffer by vacuum. Blot bottom of plate with a paper towel.
		5.4.6	Vortex the Bead Bottle and add 25 µL to each well.
		5.4.7	Remove liquid from plate by vacuum. Blot bottom of plate with a paper towel.
		5.4.8	Add 50 µL of each Standard and Control into the appropriate wells.
		5.4.9	Add 50 µL of Assay Buffer to the sample wells.
		5.4.10	Add 50 µL of serum matrix to the background, standards, and control wells.
		5.4.11	Add 50 µL of sample into the appropriate wells.
		5.4.12	Seal the plate with a plate sealer and cover it with a lid or aluminum foil.
		5.4.13	Place on the Shaker at 4° C for 16 to 18 hours.
		5.4.14	Remove fluid by vacuum
		5.4.15	Wash plate 2 times with 200 µL/well Wash Buffer,

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			removing Wash Buffer by vacuum filtration between each wash.
		5.4.16	Blot plate with paper towel.
		5.4.17	Add 50 µL of Detection Antibodies into each well.
		5.4.18	Seal, cover plate with lid, and incubate one hour at room temperature on the shaker.
		5.4.19	Do Not Vacuum After Incubation
		5.4.20	Add 50 µL Streptavidin-Phycoerythin to each well.
		5.4.21	Seal, cover plate with lid, and incubate 30 minutes at room temperature on the shaker.
		5.4.22	Remove fluid by vacuum.
		5.4.23	Wash plate 2 times with 200 µL/well Wash Buffer, removing Wash Buffer by vacuum filtration between each wash.
		5.4.24	Blot plate with paper towel.
		5.4.25	Add 100 µL of Sheath Fluid to all wells.
		5.4.26	Resuspend the beads on the plate shaker for 5 minutes.
		5.4.27	Run plate on the Luminex 100. See settings below for reader setup
		5.4.28	Save file to the G drive after completion.

MILLIPORE™

MILLIPLEX® MAP

HIGH SENSITIVITY HUMAN CYTOKINE KIT PROTOCOL
96 Well Plate Assay

HSCYTO-60SK or
HSCYTO-60SPMX13 (premixed)

TABLE OF CONTENTS **PAGE**

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

INTRODUCTION

Low levels of inflammation are involved in many clinical and sub-clinical disease states, such as cardiovascular disease, diabetes, and cancer. Measuring picogram levels of cytokines is critical for understanding the pathogenesis of these diseases. Millipore is proud to announce that the former LINCO*plex* High Sensitivity Human Cytokine Panel now has the MILLIPLEX MAP optimized format. Combining the advantages of the Luminex® xMAP® technology, MILLIPLEX MAP High Sensitivity Human Cytokine Multiplex Panel offers high sensitivities, broad dynamic range and robust assay performance, including accuracy, precision and reproducibility. While you will instantly recognize the quality and reproducibility that you have always trusted, you will also enjoy the enhancements that we have built into MILLIPLEX MAP.

Millipore's MILLIPLEX MAP High Sensitivity Human Cytokine Panel is to be used for the simultaneous quantification of GM-CSF, IFNγ, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12 (p70), IL-13 and TNFα in human plasma, serum, and cell/tissue culture supernatant samples. This panel provides biomedical researchers quality tools for the study of low level inflammatory disease.

This kit is for research purposes only.

Please read entire protocol before use.

It is important to use same assay incubation conditions throughout your study.

PRINCIPLE

MILLIPLEX® MAP is based on the Luminex® xMAP® technology — one of the fastest growing and most respected multiplex technologies offering applications throughout the life sciences and capable of performing a variety of bioassays including immunoassays on the surface of fluorescent-coded beads known as microspheres.

- Luminex® uses proprietary techniques to internally color-code microspheres with two fluorescent dyes. Through precise concentrations of these dyes, 100 distinctly colored bead sets can be created, each of which is coated with a specific capture antibody.
- After an analyte from a test sample is captured by the bead, a biotinylated detection antibody is introduced.
- The reaction mixture is then incubated with Streptavidin-PE conjugate, the reporter molecule, to complete the reaction on the surface of each microsphere.
- The microspheres are allowed to pass rapidly through a laser which excites the internal dyes marking the microsphere set. A second laser excites PE, the fluorescent dye on the reporter molecule.
- Finally, high-speed digital-signal processors identify each individual microsphere and quantify the result of its bioassay based on fluorescent reporter signals.

The capability of adding multiple conjugated beads to each sample results in the ability to obtain multiple results from each sample. Open-architecture xMAP® technology enables multiplexing of many types of bioassays reducing time, labor and costs over traditional methods.

STORAGE CONDITIONS UPON RECEIPT

- Recommended storage for kit components is 2 - 8°C.
- Once the standards and controls have been reconstituted, immediately transfer contents into polypropylene vials. **DO NOT STORE RECONSTITUTED STANDARDS OR CONTROLS IN GLASS VIALS**. For long-term storage, freeze reconstituted standards and controls at ≤ -20°C. Avoid multiple (>2) freeze/thaw cycles.
- **DO NOT FREEZE Antibody-Immobilized Beads, Detection Antibodies, and Streptavidin-Phycoerythrin**.

REAGENTS SUPPLIED

Note: Store all reagents at 2 – 8°C

REAGENTS SUPPLIED	CATALOG NUMBER	VOLUME	QUANTITY
High Sensitivity Human Cytokine Standard	L-8060SEN	lyophilized	2 vials
High Sensitivity Human Cytokine Quality Controls 1 and 2	L-6060SEN	lyophilized	2 sets
Serum Matrix Note: Contains 0.08% Sodium Azide	LHHS-SM	lyophilized	1 vial (required for serum and plasma samples only)
Set of one 96-Well Filter Plate with 2 Sealers	MX-PLATE	-----	1 plate 2 sealers
Assay Buffer	L-ABIR	15 mL	1 bottle
10X Wash Buffer Note: Contains 0.05% Proclin	L-WB	30 mL	1 bottle
High Sensitivity Human Cytokine Detection Antibodies	L-1060SEN	5.5 mL	1 bottle
Streptavidin-Phycoerythrin	L-SAPE7	5.5 mL	1 bottle
Mixing Bottle (not provided with premixed panel)	----------	----------	1 bottle

High Sensitivity Human Cytokine Antibody-Immobilized Premixed Beads:

Premixed 13-plex Beads	HSHCB-PMX13	3.5 mL	1 bottle

Included High Sensitivity Human Cytokine Antibody-Immobilized Beads are dependent on customizable selection of analytes within the panel (see following table page 4).

High Sensitivity Human Cytokine Antibody-Immobilized Beads:

Bead/Analyte Name	Luminex Bead Region	Customizable 13 Analytes (20X concentration, 200µL) Available	Cat. #	13-Plex Premixed Beads
Anti-Human IL-1β Bead	1	✓	HSIL-1B	✓
Anti-Human IL-2 Beads	3	✓	HSIL-2	✓
Anti-Human IL-4 Beads	9	✓	HSIL-4	✓
Anti-Human IL-5 Beads	10	✓	HSIL-5	✓
Anti-Human IL-6 Beads	12	✓	HSIL-6	✓
Anti-Human IL-7 Beads	13	✓	HSIL-7	✓
Anti-Human IL-8 Beads	20	✓	HSIL-8	✓
Anti-Human IL-10 Beads	23	✓	HSIL-10	✓
Anti-Human IL-12p70 Beads	25	✓	HSIL-12	✓
Anti-Human IL-13 Beads	26	✓	HSIL-13	✓
Anti-Human IFNγ Beads	35	✓	HSIFN-G	✓
Anti-Human GM-CSF Beads	39	✓	HSGM-CSF	✓
Anti-Human TNFα Beads	40	✓	HSTNF-A	✓

MATERIALS REQUIRED BUT NOT PROVIDED

Reagents

1. Luminex Sheath Fluid (Luminex Catalogue #40-50000)

Instrumentation / Materials

1. Adjustable Pipettes with Tips capable of delivering 25 µL to 1000 µL
2. Multichannel Pipettes capable of delivering 5 µL to 50 µL or 25 µL to 200 µL
3. Reagent Reservoirs
4. Polypropylene Microfuge Tubes
5. Rubber Bands
6. Absorbent Pads
7. Laboratory Vortex Mixer
8. Sonicator (Branson Ultrasonic Cleaner Model #B200 or equivalent)
9. Titer Plate Shaker (Lab-Line Instruments Model #4625 or equivalent)
10. Vacuum Filtration Unit (Millipore Vacuum Manifold Catalog #MSVMHTS00 or equivalent with Millipore Vacuum Pump Catalog #WP6111560 or equivalent)
11. Luminex 100™ IS, 200™, or HTS by Luminex Corporation
12. Plate Stand (Millipore Catalog # MX-STAND)

SAFETY PRECAUTIONS

- All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions as established by the Centers for Disease Control and Prevention and by the Occupational Safety and Health Administration when handling and disposing of infectious agents.
- Sodium azide or Proclin has been added to some reagents as a preservative. Although the concentrations are low, sodium azide and Proclin may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build up.

TECHNICAL GUIDELINES

To obtain reliable and reproducible results, the operator should carefully read this entire manual and fully understand all aspects of each assay step before running the assay. The following notes should be reviewed and understood before the assay is set up.

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- Do not use beyond the expiration date on the label.
- Do not mix or substitute reagents with those from other lots or sources.
- The Antibody-Immobilized Beads are light sensitive and must be protected from light at all times. Cover the assay plate containing beads with opaque plate lid or aluminum foil during all incubation steps.
- It is important to allow all reagents to warm to room temperature (20-25°C) before use in the assay.
- The bottom of the Microtiter Filter Plate should not be in direct contact with any surface during assay set-up or incubation times. The plate can be set on a plate stand or on the non-flat side of the plate cover or any other plate holder to raise the plate from the surface. A plate stand can be purchased separately from Millipore (Millipore Catalog #MX-STAND).
- Incomplete washing can adversely affect the assay outcome. All washing must be performed with the Wash Buffer provided.
- After the wash steps, keep the bottom of the Microtiter Filter Plate clean by blotting on paper towels or absorbent pads to prevent any leakage due to capillary action.
- Keep the vacuum suction on the plate as low as possible. It is recommended to have a vacuum setting that will remove 200 µL of buffer in $\geq$ 5 seconds (equivalent to < 100 mmHg).
- After hydration, all Standards and Controls must be transferred to polypropylene tubes.
- The Standards prepared by serial dilution must be used within 1 hour of preparation. Discard any unused standards except the standard stock which may be stored at $\leq$ -20°C for 1 month and at $\leq$ -80°C for greater than one month.

- If samples fall outside the dynamic range of the assay, further dilute the samples with the appropriate diluent and repeat the assay.
- Any unused mixed Antibody-Immobilized Beads may be stored in the Mixing Bottle at 2-8°C for up to one month.
- During the preparation of the standard curve, make certain to mix the higher concentration well before making the next dilution. Use a new tip with each dilution.
- The plate should be read immediately after the assay is finished. If, however, the plate cannot be read immediately, seal the plate, cover with aluminum foil or an opaque lid, and store the plate at 2-8°C for up to 24 hours. Prior to reading, agitate the plate on the plate shaker at room temperature for 10 minutes. Delay in reading a plate may result in decreased sensitivity for some cytokines and chemokines.
- The titer plate shaker should be set at a speed to provide maximum orbital mixing without splashing of liquid outside the wells. For the recommended plate shaker, this would be a setting of 5-7 which is approximately 500-800 rpm.
- Ensure that the needle probe is clean. This may be achieved by sonication and/or alcohol flushes. Adjust probe height according to the protocols recommended by Luminex to the kit filter plate using 3 alignment discs prior to reading an assay.
- For cell culture supernatants or tissue extraction, use the culture or extraction medium as the matrix solution in background, standard curve and control wells. If samples are diluted in Assay Buffer, use the Assay Buffer as matrix.
- For serum/plasma samples, use the Serum Matrix provided in the kit.
- For cell/tissue homogenate, the final cell or tissue homogenate should be prepared in a buffer that has a neutral pH, contains minimal detergents or strong denaturing detergents, and has an ionic strength close to physiological concentration. Avoid debris, lipids, and cell/tissue aggregates. Centrifuge samples before use.
- Vortex all reagents well before adding to plate.

SAMPLE COLLECTION AND STORAGE

A. Preparation of Serum Samples:

- Allow the blood to clot for at least 30 minutes before centrifugation for 10 minutes at 1000xg. Remove serum and assay immediately or aliquot and store samples at ≤ -20°C.
- Avoid multiple (>2) freeze/thaw cycles.
- When using frozen samples, it is recommended to thaw the samples completely, mix well by vortexing and centrifuge prior to use in the assay to remove particulates.
- If dilution is required for serum samples, use Serum Matrix provided in the kit as the sample diluent.

B. Preparation of Plasma Samples:

- Plasma collection using EDTA as an anti-coagulant is recommended. Centrifuge for 10 minutes at 1000xg within 30 minutes of blood collection. Remove plasma and assay immediately or aliquot and store samples at ≤ -20°C.
- Avoid multiple (>2) freeze/thaw cycles.
- When using frozen samples, it is recommended to thaw the samples completely, mix well by vortexing and centrifuge prior to use in the assay to remove particulates.
- If dilution is required for plasma samples, use Serum Matrix provided in the kit as the sample diluent.

C. Preparation of Tissue Culture Supernatant:

- Centrifuge the sample to remove debris and assay immediately or aliquot and store samples at ≤ -20°C.
- Avoid multiple (>2) freeze/thaw cycles.
- Tissue culture supernatant may require a dilution with an appropriate control medium (consistent with samples in terms of composition, ionic strength and pH) prior to assay.

NOTE:

- A maximum of 50 µL per well of serum or plasma can be used. Tissue culture or other media may also be used.
- All samples must be stored in polypropylene tubes. **DO NOT STORE SAMPLES IN GLASS.**
- Avoid using samples with gross hemolysis or lipemia.
- All samples should be free of particles, excess lipids and precipitates. Particles in the samples may clog the sample probe. It is a good practice to centrifuge samples before addition to sample wells.

PREPARATION OF REAGENTS FOR IMMUNOASSAY

A. Preparation of Antibody-Immobilized Beads

If premixed beads are used, sonicate the premixed bead bottle 30 seconds and then vortex for 1 minute before use.

For individual vials of beads, sonicate each antibody-bead vial for 30 seconds; vortex for 1 minute. Add 150 µL from each antibody bead vial to the Mixing Bottle and bring final volume to 3.0 mL with Assay Buffer. Vortex the mixed beads well. Unused portion may be stored at 2-8°C for up to one month.

Example 1: When using all 13 cytokine antibody-immobilized beads, add 150 µL from each of the 13 bead sets to the Mixing Bottle. Then add 1.05 mL Assay Buffer.

Example 2: When using 3 cytokine antibody-immobilized beads, add 150 µL from each of the 3 bead sets to the Mixing Bottle. Then add 2.55 mL Assay Buffer.

B. Preparation of Quality Controls

Before use, reconstitute Quality Control 1 and Quality Control 2 with 250 µL deionized water. Invert the vial several times to mix and vortex. Allow the vial to sit for 5-10 minutes and then transfer the controls to appropriately labeled polypropylene microfuge tubes. Unused portion should be discarded.

C. Preparation of Wash Buffer

Bring the 10X Wash Buffer to room temperature and mix to bring all salts into solution. Dilute 30 mL of 10X Wash Buffer with 270 mL deionized water. Store unused portion at 2-8°C for up to one month.

D. Preparation of Serum Matrix

This step is required for serum or plasma samples only.

Add 5.0 mL deionized water to the bottle containing lyophilized Serum Matrix. Mix well. Allow at least 10 minutes for complete reconstitution. Leftover reconstituted Serum Matrix should be stored at ≤ -20°C for up to one month.

E. Preparation of High Sensitivity Human Cytokine Standard

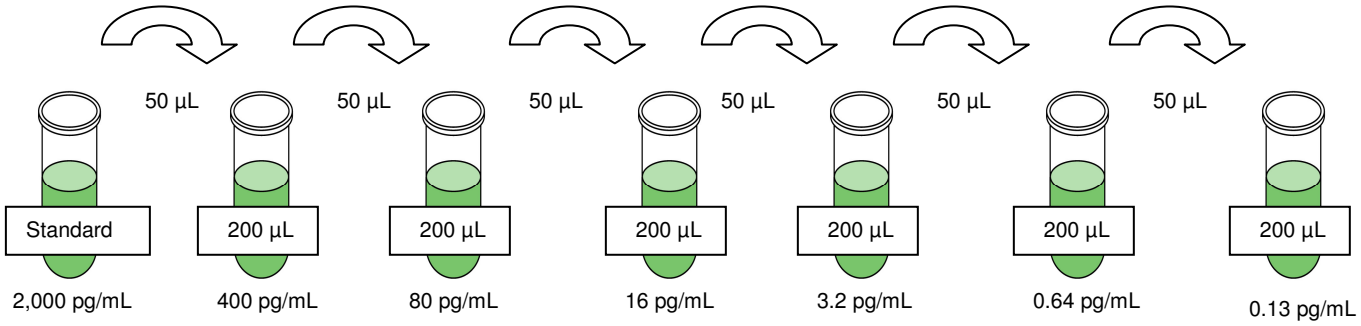
1.) Prior to use, reconstitute the High Sensitivity Human Cytokine Standard with 250 µL deionized water to give a 2,000 pg/mL concentration of standard for all analytes. Invert the vial several times to mix. Vortex the vial for 10 seconds. Allow the vial to sit for 5-10 minutes and then transfer the standard to an appropriately labeled polypropylene microfuge tube. This will be used as the 2,000 pg/mL standard; the unused portion should be discarded.

2.) Preparation of Working Standards

The following description and the Plate Map illustrate 1:5 dilution of standards. However, the end user has an option to select 1:4 or 1:3 dilution of standards with lowest concentration point at ~0.1 pg/ml. Label six polypropylene microfuge tubes 400, 80, 16, 3.2, 0.64, and 0.13 pg/ml. Add 200 µL of Assay Buffer to each of the six tubes. Prepare serial dilutions by adding 50 µL of the 2,000 pg/mL reconstituted standard to the 400 pg/mL tube, mix well and transfer 50 µL of the 400 pg/mL standard to the 80 pg/mL tube, mix well and transfer 50 µL of the 80 pg/mL standard to the 16 pg/mL tube, mix well and transfer 50 µL of the 16 pg/mL standard to 3.2 pg/mL tube, mix well and transfer 50 µL of the 3.2 pg/mL standard to the 0.64 pg/mL tube, mix well and transfer 50 µL of the 0.64 pg/mL standard to the 0.13 pg/mL tube and mix well. The 0 pg/mL standard (Background) will be Assay Buffer.

Standard Concentration (pg/mL)	Volume of Deionized Water to Add	Volume of Standard to Add
2,000	250 µL	0

Standard Concentration (pg/mL)	Volume of Assay Buffer to Add	Volume of Standard to Add
400	200 µL	50 µL of 2000 pg/mL
80	200 µL	50 µL of 400 pg/mL
16	200 µL	50 µL of 80 pg/mL
3.2	200 µL	50 µL of 16 pg/mL
0.64	200 µL	50 µL of 3.2 pg/mL
0.13	200 µL	50 µL of 0.64 pg/mL



IMMUNOASSAY PROCEDURE

- Prior to beginning this assay, it is imperative to read this protocol completely and to thoroughly understand the Technical Guidelines.
- Allow all reagents to warm to room temperature (20-25°C) before use in the assay.
- Diagram the placement of Standards [0 (Background), 0.13, 0.64, 3.2, 16, 80, 400, and 2000 pg/mL], Controls 1 and 2, and Samples on Well Map Worksheet in a vertical configuration. (Note: Most instruments will only read the 96-well plate vertically by default.) It is recommended to run the assay in duplicate.
- Set the filter plate on a plate holder at all times during reagent dispensing and incubation steps so that the bottom of the plate does not touch any surface.

1. Prewet the filter plate by pipetting 200 µL of 1X Wash Buffer into each well of the Microtiter Filter Plate. Seal and mix on a plate shaker for 10 minutes at room temperature (20-25°C).
2. Remove Wash Buffer by vacuum. **(NOTE: DO NOT INVERT PLATE.)** Blot excess Wash Buffer from the bottom of the plate with an absorbent pad or paper towels.
3. Sonicate bead bottle for 30 seconds and then vortex for minute. Add 25 µL of the Mixed or Premixed Beads to each well. (Note: During addition of Beads, shake bead bottle intermittently to avoid settling.)
4. Remove liquid from the wells by vacuum. **(Note: DO NOT INVERT PLATE.)** Blot excess liquid from the bottom the plate by with an absorbent pad or paper towels.
5. Add 50 µL of each Standard or Control into the appropriate wells. Assay Buffer should be used for the 0 pg/mL standard (Background).
6. Add 50 µL of Assay Buffer to sample wells.
7. Add 50 µL of appropriate matrix solution to the background, standards, and control wells. When assaying serum or plasma, use the Serum Matrix provided in the kit. When assaying tissue culture supernatant samples, use proper control culture medium as the matrix solution.
8. Add 50 µL of Sample into the appropriate wells. Before addition to wells, the samples should be centrifuged to remove any precipitates or denatured proteins that occurred during storage and handling.

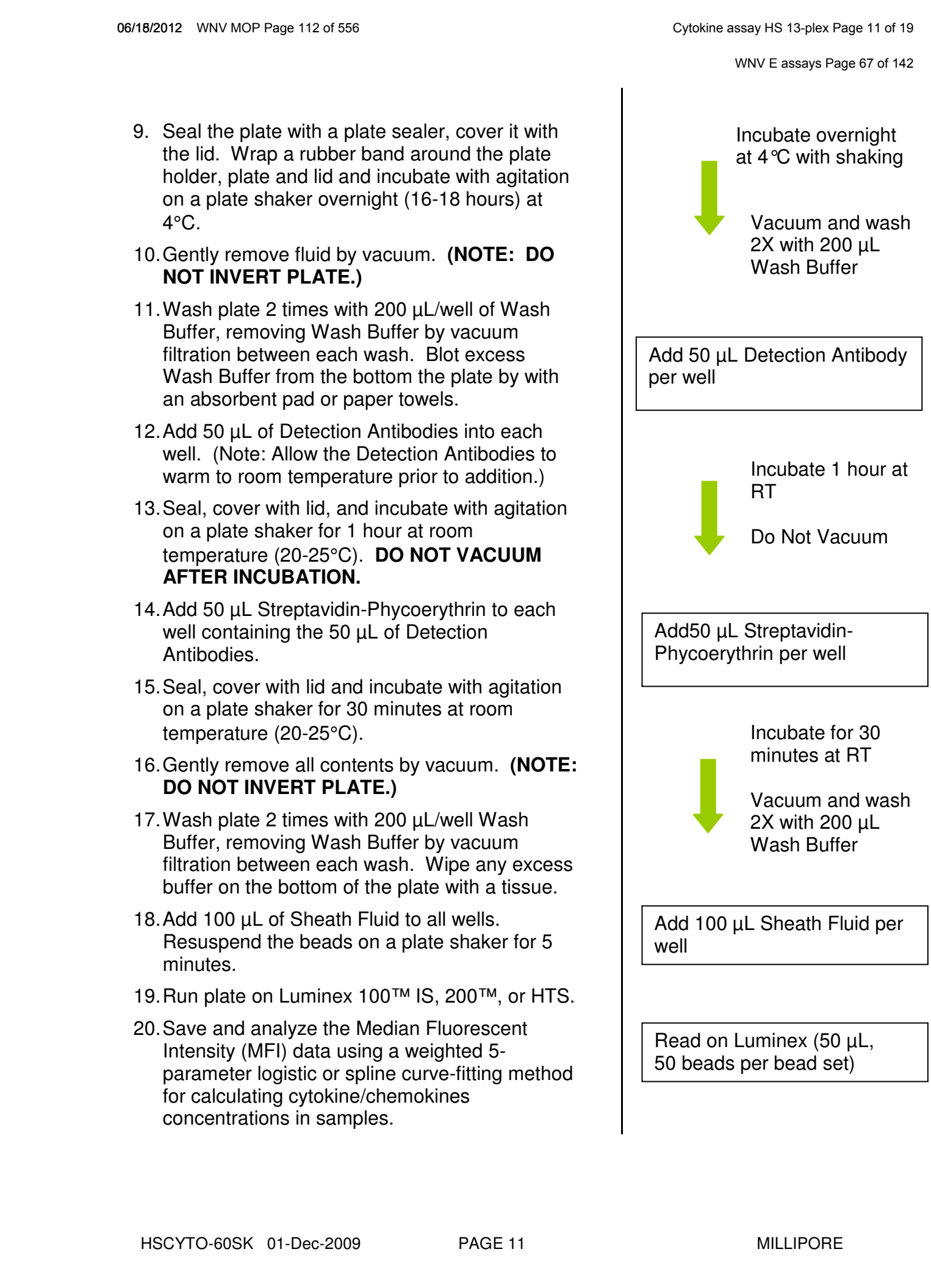
Add 200 µL 1X Wash Buffer per well

Shake 10 min, RT

Vacuum

- Add 25 µL Beads to each well then vacuum
- Add 50 µL Standard or Control to appropriate wells
- Add 50 µL Assay Buffer to background and sample wells
- Add 50 µL Matrix to background, standards and control wells
- Add 50 µL Samples to sample wells

9. Seal the plate with a plate sealer, cover it with the lid. Wrap a rubber band around the plate holder, plate and lid and incubate with agitation on a plate shaker overnight (16-18 hours) at 4°C.
10. Gently remove fluid by vacuum. **(NOTE: DO NOT INVERT PLATE.)**
11. Wash plate 2 times with 200 µL/well of Wash Buffer, removing Wash Buffer by vacuum filtration between each wash. Blot excess Wash Buffer from the bottom the plate by with an absorbent pad or paper towels.
12. Add 50 µL of Detection Antibodies into each well. (Note: Allow the Detection Antibodies to warm to room temperature prior to addition.)
13. Seal, cover with lid, and incubate with agitation on a plate shaker for 1 hour at room temperature (20-25°C). **DO NOT VACUUM AFTER INCUBATION.**
14. Add 50 µL Streptavidin-Phycoerythrin to each well containing the 50 µL of Detection Antibodies.
15. Seal, cover with lid and incubate with agitation on a plate shaker for 30 minutes at room temperature (20-25°C).
16. Gently remove all contents by vacuum. **(NOTE: DO NOT INVERT PLATE.)**
17. Wash plate 2 times with 200 µL/well Wash Buffer, removing Wash Buffer by vacuum filtration between each wash. Wipe any excess buffer on the bottom of the plate with a tissue.
18. Add 100 µL of Sheath Fluid to all wells. Resuspend the beads on a plate shaker for 5 minutes.
19. Run plate on Luminex 100™ IS, 200™, or HTS.
20. Save and analyze the Median Fluorescent Intensity (MFI) data using a weighted 5-parameter logistic or spline curve-fitting method for calculating cytokine/chemokines concentrations in samples.



EQUIPMENT SETTINGS

These specifications are for the Luminex 100™ IS v.1.7 or Luminex 100™ IS v2.1/2.2, Luminex 200™ v2.3, xPONENT, and Luminex HTS. Luminex instruments with other software (e.g. MasterPlex, StarStation, LiquiChip, Bio-Plex, LABScan100) would need to follow instrument instructions for gate settings and additional specifications from the vendors.

Events:	50, per bead		50, per bead	
Sample Size:	50 µL		50 µL	
Gate Settings	8,000 to 15,000			
Reporter Gain	Default (low PMT)			
Time Out	60 seconds			
Bead Set:	13-Plex Premix Beads		Customizable 13-Plex Beads	
	IL-1β	1	IL-1β	1
	IL-2	3	IL-2	3
	IL-4	9	IL-4	9
	IL-5	10	IL-5	10
	IL-6	12	IL-6	12
	IL-7	13	IL-7	13
	IL-8	20	IL-8	20
	IL-10	23	IL-10	23
	IL-12(p70)	25	IL-12(p70)	25
	IL-13	26	IL-13	26
	IFNγ	35	IFNγ	35
	GM-CSF	39	GM-CSF	39
	TNFα	40	TNFα	40

QUALITY CONTROLS

The ranges for each analyte in Quality Control 1 and 2 are provided on the card insert or can be located at the MILLIPORE website www.millipore.com/techlibrary/index.do using the catalog number as the keyword.

ASSAY CHARACTERISTICS

Assay Sensitivities (minimum detectable concentrations, pg/mL)

MinDC: Minimum Detectable Concentration is calculated by the StatLIA® Immunoassay Analysis Software from Brendan Technologies. It measures the true limits of detection for an assay by mathematically determining what the empirical MinDC would be if an infinite number of standard concentrations were run for the assay under the same conditions.

Cytokine	MinDC
IL-1β	0.06
IL-2	0.16
IL-4	0.13
IL-5	0.01
IL-6	0.10
IL-7	0.12
IL-8	0.11
IL-10	0.15
IL-12(p70)	0.11
IL-13	0.48
IFNγ	0.29
GM-CSF	0.46
TNFα	0.05

Precision

Intra-assay precision is generated from the mean of the %CV's from 8 reportable results across two different concentration of cytokines in one experiment. Inter-assay precision is generated from the mean of the %CV's from two reportable results each for two different concentrations of cytokine across 8 different experiments.

Cytokine	Intra-assay (%CV)	Inter-assay (%CV)
IL-1β	3.11	2.16
IL-2	4.27	7.48
IL-4	4.16	9.12
IL-5	4.50	14.27
IL-6	3.51	4.48
IL-7	4.75	6.24
IL-8	3.26	6.48
IL-10	3.31	11.84
IL-12(p70)	4.31	5.08
IL-13	5.86	11.99
IFNγ	4.88	7.79
GM-CSF	4.14	5.03
TNFα	3.49	3.78

Accuracy

Spike Recovery: The data represent mean percent recovery of 7 levels of spiked standards ranging from 3.13 to 400 pg/mL in serum matrix in 7 independent experiments.

Cytokine	%Recovery
IL-1β	102.9
IL-2	98.8
IL-4	98.9
IL-5	93.0
IL-6	100.7
IL-7	112.0
IL-8	103.1
IL-10	93.1
IL-12(p70)	100.2
IL-13	93.2
IFNγ	98.4
GM-CSF	100.4
TNFα	103.6

Cross-Reactivity

There was no or negligible cross-reactivity between the antibodies and any of the other analytes in this panel.

TROUBLESHOOTING GUIDE

Problem	Probable Cause	Solution
Filter plate will not vacuum	Vacuum pressure is insufficient	Increase vacuum pressure such that 0.2mL buffer can be suctioned in 3-5 seconds.
	Samples have insoluble particles	Centrifuge samples just prior to assay set-up and use supernatant. If high lipid concentration, after centrifugation, remove lipid layer and use supernatant.
	Sample too viscous	May need to dilute sample.
Insufficient bead count	Vacuum pressure too high	Adjust vacuum pressure such that 0.2mL buffer can be suctioned in 3-5 seconds.
	Bead mix prepared incorrectly	Sonicate bead vials and vortex just prior to adding to bead mix bottle according to protocol. Agitate bead mix intermittently in reservoir while pipetting into the plate.
	Samples cause interference due to particulate matter or viscosity	See above. Also sample probe may need to be cleaned with alcohol flush, backflush and washes; or, if needed, probe should be removed and sonicated.
	Probe height not adjusted correctly	Adjust probe to 3 alignment discs in well H6.
Plate leaked	Vacuum pressure too high	Adjust vacuum pressure such that 0.2mL buffer can be suctioned in 3-5 seconds. May need to transfer contents to a new (prewetted) plate and continue.
	Plate set directly on table or absorbent towels during incubations or reagent additions	Set plate on plate stand or raised edge so bottom of filter is not touching any surface.
	Insufficient blotting of filter plate bottom causing wicking	Blot the bottom of the filter plate well with absorbent towels after each wash step.
	Pipette touching plate filter during additions	Pipette to the side of well.
	Probe height not adjusted correctly	Adjust probe to 3 alignment discs in well H6.
Background is too high	Background wells were contaminated	Avoid cross-well contamination by using sealer appropriately and by pipetting with multichannel pipets without touching reagent in plate.
	Matrix used has endogenous analyte or interference	Check matrix ingredients for crossreacting components (e.g. interleukin modified tissue culture medium).
	Insufficient washes	Increase number of washes.

Beads not in region or gate	Luminex not calibrated correctly or recently	Calibrate Luminex based on instrument manufacturer's instructions at least once a week or if temperature has changed by >3°C.
	Gate settings not adjusted correctly	Some Luminex instruments (e.g. Bio-Plex) require different gate settings than those described in the kit protocol. Use instrument default settings.
	Wrong bead regions in protocol template	Check kit protocol for correct bead regions or analyte selection.
	Incorrect sample type used	Samples containing organic solvents or if highly viscous should be diluted or dialyzed as required.
	Instrument not washed or primed	Prime the Luminex 4 times to eliminate air bubbles. Wash 4 times with sheath fluid or water if there is any remnant alcohol or sanitizing liquid.
	Beads were exposed to light	Keep plate and bead mix covered with dark lid or aluminum foil during all incubation steps.
Signal for whole plate is same as background	Incorrect or no Detection Antibody was added	Add appropriate Detection Antibody and continue.
	Streptavidin-Phycoerythrin was not added	Add Streptavidin-Phycoerythrin according to protocol. If Detection Antibody has already been vacuumed out, sensitivity may be low.
Low signal for standard curve	Detection Antibody may have been vacuumed out prior to adding Streptavidin Phycoerythrin	May need to repeat assay if desired sensitivity not achieved.
	Incubations done at incorrect temperatures, timings or agitation	Assay conditions need to be checked.
Signals too high, standard curves are saturated	Calibration target value set too high	With some Luminex instruments (e.g. Bio-Plex) default target setting for RP1 calibrator is set at High PMT. Use low target value for calibration and reanalyze plate.
	Plate incubation was too long with standard curve and samples	Use shorter incubation time.
Sample readings are out of range	Samples contain no or below detectable levels of analyte	If below detectable levels, it may be possible to use higher sample volume. Check with tech support for appropriate protocol modifications.
	Samples contain analyte concentrations higher than highest standard point	Samples may require dilution and reanalysis for that particular analyte.
	Standard curve was saturated at higher end of curve	See above.

High variation in samples and/or standards	Multichannel pipet may not be calibrated	Calibrate pipets.
	Plate washing was not uniform	Confirm all reagents are vacuumed out completely in all wash steps.
	Samples may have high particulate matter or other interfering substances	See above.
	Plate agitation was insufficient	Plate should be agitated during all incubation steps using a vertical plate shaker at a speed where beads are in constant motion without splashing.
	Cross-well contamination	Check when reusing plate sealer that no reagent has touched sealer. Care should be taken when using same pipet tips that are used for reagent additions and that pipet tip does not touch reagent in plate.

REPLACEMENT REAGENTS	**Catalog #**
High Sensitivity Human Cytokine Standard	L-8060SEN
High Sensitivity Human Cytokine Quality Controls	L-6060SEN
Serum Matrix	LHHS-SM (optional)
High Sensitivity Human Cytokine Detection Antibodies	L-1060SEN
Streptavidin-Phycoerythrin	L-SAPE7
Assay Buffer	L-ABIR
Set of two 96-Well Filter Plates with Sealers	MX-PLATE
10X Wash Buffer	L-WB

Antibody-Immobilized Beads

Cytokine	Bead #	Cat. #
IL-1β	1	HSIL-1B
IL-2	3	HSIL-2
IL-4	9	HSIL-4
IL-5	10	HSIL-5
IL-6	12	HSIL-6
IL-7	13	HSIL-7
IL-8	20	HSIL-8
IL-10	23	HSIL-10
IL-12p70	25	HSIL-12
IL-13	26	HSIL-13
IFNγ	35	HSIFN-G
GM-CSF	39	HSGM-CSF
TNFα	40	HSTNF-A
Premixed 13-plex Beads		HSHCB-PMX13

ORDERING INFORMATION

To place an order:

To assure the clarity of your custom cytokine kit order, please FAX the following information to our customer service department:

- Your name, telephone and/or fax number
- Customer account number
- Shipping and billing address
- Purchase order number
- Catalog number and description of product
- Quantity of kits
- Selection of MILLIPLEX® Cytokine Analytes/Serum Matrix Requirements

FAX: (636) 441-8050
Toll-Free US: (800) MILLIPORE
Mail Orders: Millipore Corp.
6 Research Park Drive
St. Charles, Missouri 63304 U.S.A.

For International Customers:

To best serve our international customers in placing an order or obtaining additional information about MILLIPLEX® MAP products, please contact your multiplex specialist or sales representative or email our European Customer Service at customerserviceEU@Millipore.com.

Conditions of Sale

All products are for research use only. They are not intended for use in clinical diagnosis or for administration to humans or animals. All products are intended for *in vitro* use only.

Material Safety Data Sheets (MSDS)

Material Safety Data Sheets for Millipore products may be ordered by fax or phone or through our website at www.millipore.com/techlibrary/index.do

WELL MAP

	1	2	3	4	5	6	7	8	9	10	11	12
A	0 pg/mL Standard (Background)	16 pg/mL Standard	QC-1 Control	Etc.								
B	0 pg/mL Standard (Background)	16 pg/mL Standard	QC-1 Control									
C	0.13 pg/mL Standard	80 pg/mL Standard	QC-2 Control									
D	0.13 pg/mL Standard	80 pg/mL Standard	QC-2 Control									
E	0.64 pg/mL Standard	400 pg/mL Standard	Sample 1									
F	0.64 pg/mL Standard	400 pg/mL Standard	Sample 1									
G	3.2 pg/mL Standard	2000 pg/mL Standard	Sample 2									
H	3.2 pg/mL Standard	2000 pg/mL Standard	Sample 2									

Blood Systems Research Institute
Core Immunology Laboratory 270 Masonic Avenue, SF, CA. 94118
(415) 749-6609 / FAX (415 775-3859

Title: Milliplex Human Cytokine / Chemokine Kit			Page 1 of 4		
Doc#	Imm001	**Revision:**		**Effective Date:**	4/22/10

1	Purpose		To provide instruction on performing the Milliplex Cytokine Kit assay
2	Scope	2.1	To measure cytokines in plasma or serum.
3	Responsibilities	3.1	The Antibody-Immobilized Beads are light sensitive and must be protected from light.
		3.2	Cover the assay plate containing beads with opaque plate lid or aluminum foil duringall incubation steps.
		3.3	Reagents must be at room temperature before use.
		3.4	The bottom of the Microtiter Filter Plate must not be in direct contact with any surface during assay setup or incubation times. Use the plate stand at all times.
		3.5	Blot the bottom of the Microtiter Filter Plate after each wash with a paper towel.
		3.6	Keep vacuum settings at lowest possible level.
		3.7	After hydration, all Standards and Controls must be transferred to polypropylene tubes.
		3.8	Standards prepared by serial dilution must be used within one hour.
		3.9	Plate shaker should be set between 500 to 800 RPM.
		3.10	Frozen plasma or serum must be completely thawed, vortexed and centrifuged before addition to plate.
		3.11	Vortex all reagents well before adding to plate.
4	Materials Required	4.1	LabScan Luminex 100 IS Reader
		4.2	Computer
		4.3	Bio-Plex Manager Software
		4.4	Plate Shaker
		4.5	4° C Refrigerator
		4.6	Pipetman P1000 and P200 with tips
		4.7	Rainin Multichannel Pipette 20 ul – 200 ul
		4.8	Polypropylene Microfuge Tubes

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		4.9	Laboratory Vortex Mixer
		4.10	Vacuum Filtration Unit
		4.11	Vacuum Pump
		4.12	Reagent Reservoirs
		4.13	Plate Stand
		4.14	Plate Map Form
5	Procedure	5.1	Preparation of Reagents
		5.1.1	Preparation of Antibody-Immobilized Beads
		5.1.2	If premixed beads are used, sonicate bottle for 30 seconds then vortex for 1 minute before use.
		5.1.3	For individual beads, sonicate each vial for 30 seconds then vortex for 1 minute.
		5.1.4	Add 60 µl from each bead vial into the mixing bottle and then bring the final volume up to 3.0 mL with Bead Diluent. Vortex the mixed beads well.
		5.1.5	Quality Controls
		5.1.6	Reconstitute QC 1and QC 2 vials with 250 µL deionized water.
		5.1.7	Invert vial several times to mix and vortex.
		5.1.8	Let stand 5 – 10 minutes and transfer to polypropylene tubes.
		5.1.9	Wash buffer
		5.1.10	Dilute 30 mL of 10X Wash Buffer with 270 mL deionized water.
		5.1.11	Store at 2-8°C for up to one month.
		5.2	Preparation of Serum Matrix
		5.2.1	Add 1.0 mL deionized water to the lyophilized Serum Matrix. Mix well.
		5.2.2	Allow at least 10 minutes for complete reconstitution.
		5.3	Preparation of Human Cytokine Standards
		5.3.1	Reconstitute Human Cytokine Standard with 250 µL deionized water.
		5.3.2	Invert vial several times to mix and vortex
		5.3.3	Let stand 5 – 10 minutes and transfer to polypropylene tubes.
		5.3.4	This will be used as the 10,000 pg/mL standard.
		5.3.5	Label five polypropylene microfuge tubes 2000, 400, 80, 16, and 3.2.

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		5.3.6	Add 200 µL Assay Buffer to each tube.

Standard Concentration	Volume of Assay Buffer	Volume of Standard to add
2000	200 µL	50 µL of 10,000 pg/mL
400	200 µL	50 µL of 2000 pg/mL
80	200 µL	50 µL of 400 pg/mL
16	200 µL	50 µL of 80 pg/mL
3.2	200 µL	50 µL of 16 pg/mL

		5.4	Immunoassay Procedure
		5.4.1	Fill in the Milliplex Human Cytokine/Chemokine Plate Map with the tech ID, lot number, date performed, and expiration date of the kit.
		5.4.2	Indicate the location of all the samples to be run on the Plate Map.
		5.4.3	Prewet the plate with 200 µL of Assay Buffer into each well.
		5.4.4	Seal and mix on the plate shaker for 10 minutes.
		5.4.5	Remove Assay Buffer by vacuum. Blot bottom of plate with a paper towel.
		5.4.6	Add 25 µL of each Standard and Control into the appropriate wells.
		5.4.7	Add 25 µL of Assay Buffer to the sample wells.
		5.4.8	Add 25 µL of serum matrix to the background, standards, and control wells.
		5.4.9	Add 25 µL of sample into the appropriate wells.
		5.4.10	Vortex the Bead Bottle and add 25 µL to each well.
		5.4.11	Seal the plate with a plate sealer and cover it with a lid or aluminum foil.
		5.4.12	Place on the Shaker at 4° C for 16 to 18 hours.
		5.4.13	Remove fluid by vacuum
		5.4.14	Wash plate 2 times with 200 µL/well Wash Buffer, removing Wash Buffer by vacuum filtration

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			between each wash.
		5.4.15	Blot plate with paper towel.
		5.4.16	Add 25 µL of Detection Antibodies into each well
		5.4.17	Seal, cover plate with lid, and incubate one hour at room temperature on the shaker.
		5.4.18	Do Not Vacuum After Incubation
		5.4.19	Add 25 µL Streptavidin-Phycoerythin to each well.
		5.4.20	Seal, cover plate with lid, and incubate 30 minutes at room temperature on the shaker. Remove fluid by vacuum.
		5.4.21	Wash plate 2 times with 200 µL/well Wash Buffer, removing Wash Buffer by vacuum filtration between each wash.
		5.4.22	Blot plate with paper towel.
		5.4.23	Add 150 µL of Sheath Fluid to all wells.
		5.4.24	Resuspend the beads on the plate shaker for 5 minutes.
		5.4.25	Run plate on the Luminex 100. See settings below for reader setup
		5.4.26	Save file to the G drive after completion

Human Cytokine/Chemokine Magnetic Bead Panel

96 Well Plate Assay

Cat. # HCYTOMAG-60K
HCYTMAG-60K-PX29
HCYTMAG-60K-PX30
HCYTMAG-60K-PX39
HCYTMAG-60K-PX42

MILLIPLEX® MAP

HUMAN CYTOKINE / CHEMOKINE MAGNETIC BEAD PANEL KIT
96 Well Plate Assay

Cat. # HCYTOMAG-60K
HCYTMAG-60K-PX29 (premixed)
HCYTMAG-60K-PX30 (premixed)
HCYTMAG-60K-PX39 (premixed)
HCYTMAG-60K-PX42 (premixed)

TABLE OF CONTENTS **PAGE**

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES

By purchasing this product, which contains fluorescently labeled microsphere beads authorized by Luminex Corporation (“Luminex”), you, the customer, acquire the right under Luminex’s patent rights, if any, to use this product or any portion of this product, including without limitation the microsphere beads contained herein, only with Luminex’s laser based fluorescent analytical test instrumentation marketed under the name of Luminex 100™ IS, 200™, HTS, FLEXMAP 3D™ MAGPIX®.

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INTRODUCTION

"Cytokine" is a general term used for a diverse group of soluble proteins and peptides which act as regulators under both normal and pathological conditions to modulate the functional activities of individual cells and tissues. These proteins also mediate direct interactions between cells and regulate processes taking place in the extracellular environment. Cytokines differ from hormones in that they act on a wider spectrum of target cells. Also, unlike hormones, they are not produced by specialized cells which are organized in specialized glands. The cytokine group of proteins includes lymphokines, interferons, colony stimulating factors and chemokines. Cytokine and chemokine research plays a significant role in achieving a deeper understanding of the immune system and its multi-faceted response to most antigens, as well as disease states such as inflammatory disease, allergic reactions, IBD, sepsis, and cancer.

To identify specific cytokines involved in any inflammatory or immune response, it might be necessary to screen panels of cytokines, often requiring some level of automation and/or high throughput. Magnetic Beads can make the process of automation and high throughput screening easier with features such as walk-away washing. Advantages outside automation include:

- More flexible plate and plate washer options
- Improved performance with turbid serum/plasma samples
- Assay results equivalent to non-magnetic beads
- Automated washing eliminates technical obstacles (i.e., clogging of wells that contain viscous samples) which may result during vacuum manifold/manual washing

Therefore, the **MILLIPLEX® *MAP*** Human Cytokine / Chemokine panel enables you to focus on the therapeutic potential of cytokines as well as the modulation of cytokine expression. Coupled with the Luminex xMAP® platform in a **magnetic bead** format, you receive the advantage of ideal speed and sensitivity, allowing quantitative multiplex detection of multiple analytes simultaneously, which can dramatically improve productivity.

Millipore's MILLIPLEX *MAP* Human Cytokine / Chemokine panel is the most versatile system available for cytokine and chemokine research.

- MILLIPLEX *MAP* offers you the ability to:
    - Select a 39-plex (for serum/plasma) or 42-plex (for cell culture) pre-mixed kit or
    - Choose any combination of analytes from our panel of 42 analytes to design a custom kit that better meets your needs (Note: RANTES, PDGF-AA, PDGF-BB can't be combined to all other cytokines when measuring serum/plasma due to different dilution need).
- A convenient "all-in-one" box format gives you the assurance that you will have all the necessary reagents you need to run your assay.

INTRODUCTION (continued)

Millipore's MILLIPLEX *MAP* Human Cytokine / Chemokine kit is to be used for the simultaneous quantification of the following 42 human cytokines and chemokines: EGF, Eotaxin, G-CSF, GM-CSF, IFNα2, IFNγ, IL-10, IL-12P40, IL-12P70, IL-13, IL-15, IL-17, IL-1RA, IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IP-10, MCP-1, MIP-1α, MIP-1β, RANTES, TNFα, TNFβ, VEGF,FGF-2, TGF-α, FIT-3L, Fractalkine, GRO, MCP-3, MDC, PDGF-AA, PDGF-BB, sCD40L, sIL-2Ra, and IL-9.

This kit is for research purposes only.

Please read entire protocol before use.

It is important to use same assay incubation conditions throughout your study.

PRINCIPLE

MILLIPLEX MAP is based on the Luminex® xMAP® technology — one of the fastest growing and most respected multiplex technologies offering applications throughout the life-sciences, and is capable of performing a variety of bioassays including immunoassays on the surface of fluorescent-coded magnetic beads known as MagPlex ™-C microspheres.

- Luminex uses proprietary techniques to internally color-code microspheres with two fluorescent dyes. Through precise concentrations of two dyes, 100 distinctly colored bead sets can be created, each of which is coated with a specific capture antibody.
- After an analyte from a test sample is captured by the bead, a biotinylated detection antibody is introduced.
- The reaction mixture is then incubated with Streptavidin PE conjugate, the reporter molecule, to complete the reaction on the surface of each microsphere.
- The microspheres are allowed to pass rapidly through a laser, which excites the internal dyes marking the microsphere set. A second laser excites PE, the fluorescent dye on the reporter molecule.
- Finally, high-speed digital-signal processors identify each individual microsphere and quantify the result of its bioassay, based on fluorescent reporter signals.

The capability of adding multiple conjugated beads to each sample results in the ability to obtain multiple results from each sample. Open-architecture xMAP technology enables multiplexing of many types of bioassays reducing time, labor and costs over traditional methods.

STORAGE CONDITIONS UPON RECEIPT

- Recommended storage for kit components is 2 - 8°C.
- Once the standards and controls have been reconstituted, immediately transfer contents into polypropylene vials. **DO NOT STORE RECONSITUTED STANDARDS OR CONTROLS IN GLASS VIALS**. For long-term storage, freeze reconstituted standards and controls at ≤ -20°C. Avoid multiple (>2) freeze thaw cycles.
- **DO NOT FREEZE Antibody-Immobilized Beads, Detection Antibody, and Streptavidin-Phycoerythrin**.

REAGENTS SUPPLIED

Note: Store all reagents at 2 – 8 ℃

REAGENTS SUPPLIED	CATALOG NUMBER	VOLUME	QUANTITY
Human Cytokine / Chemokine Standard	MXH8060-2 (for 29, 30plex) or MXH8060 (for 39, 42plex)	lyophilized	1 vial
Human Cytokine Quality Controls 1 and 2	MXH6060-2 (for 29, 30plex) or MXH6060 (for 39, 42plex)	lyophilized	2 vials
Serum Matrix Note: Contains 0.08% Sodium Azide	MXHSM	lyophilized	1 vial (required for serum and plasma samples only)
Set of one 96-Well Plates with 2 Sealers	----------	----------	1 plates 2 sealers
Assay Buffer	L-AB	30 mL	1 bottle
10X Wash Buffer Note: Contains 0.05% Proclin	L-WB	30 mL	2 bottles
Human Cytokine Detection Antibodies	MXH1060-1 or MXH1060-2 or MXH1060-3 or MXH1060-4	3.2 mL	1 bottle
Streptavidin-Phycoerythrin	L-SAPE9 *(Use with Cat. # MXH1060-1)* or L-SAPE3 *(Use with Cat. # MXH1060-2)* or L-SAPE10 *(Use with Cat. # MXH1060-3)* or L-SAPE11 *(Use with Cat. # MXH1060-4)*	3.2 mL	1 bottle
Bead Diluent (not provided with premixed panel)	LBD	3.5 mL	1 bottle
Mixing Bottle (not provided with premixed panel)	----------	----------	1 bottle

REAGENTS SUPPLIED (continued)

Human Cytokine / Chemokine Antibody-Immobilized Premixed Magnetic Beads:

Premixed 29-plex Beads	HCYPMX29-MAG	3.5 mL	1 bottle

Premixed 30-plex Beads (Premixed 29plex + RANTES)	HCYPMX29-MAG+HCYRNTS-MAG	3.5 mL	1 bottle + 1 vial

Premixed 39-plex Beads	HCYPMX39-MAG	3.5 mL	1 bottle

Premixed 42-plex Beads (premixed 39-plex + RANTES, PDGF-AA, PDGF-BB)	HCYPMX39-MAG + HCYRNTS-MAG, HPDGFAA-MAG, HPDGFBB-MAG	3.5 mL	1 bottle + 3 vials

Human Cytokine / Chemokine Antibody-Immobilized Magnetic Beads:

Bead/Analyte Name	Luminex Magnetic Bead Region	Customizable 42 Analytes (50X concentration, 90µL) Available	Cat. #	29-Plex Premixed Beads	39-Plex Premixed Beads	42-Plex Premixed Beads
Anti-Human EGF Bead	12	✓	HEGF-MAG	✓	✓	✓
Anti-Human FGF-2 Bead	13	✓	HCYFGF2-MAG		✓	✓
Anti-Human Eotaxin Bead	14	✓	HETXN-MAG	✓	✓	✓
Anti-Human TGF-α Bead	15	✓	HCYTGFA-MAG		✓	✓
Anti-Human G-CSF Bead	18	✓	HGCSF-MAG	✓	✓	✓
Anti-Human Flt-3L Bead	19	✓	HFLT3L-MAG		✓	✓
Anti-Human GM-CSF Bead	20	✓	HGMCSF-MAG	✓	✓	✓
Anti-Human Fractalkine Bead	21	✓	HFKN-MAG		✓	✓
Anti-Human IFNα2 Bead	22	✓	HIFNA2-MAG	✓	✓	✓
Anti-Human IFNγ Bead	25	✓	HCYIFNG-MAG	✓	✓	✓
Anti-Human GRO Bead	26	✓	HGR0-MAG		✓	✓
Anti-Human IL-10 Bead	27	✓	HCYIL10-MAG	✓	✓	✓
Anti-Human MCP-3 Bead	28	✓	HMCP3-MAG		✓	✓
Anti-Human IL-12p40 Bead	29	✓	HIL12P40-MAG	✓	✓	✓
Anti-Human MDC Bead	30	✓	HMDC-MAG		✓	✓
Anti-Human IL-12P70 Bead	33	✓	HIL12P70-MAG	✓	✓	✓
Anti-Human PDGF-AA Bead	34	✓	HPDGFAA-MAG			✓
Anti-Human IL-13 Bead	35	✓	HIL13-MAG	✓	✓	✓
Anti-Human PDGF-BB Bead	36	✓	HPDGFBB-MAG			✓
Anti-Human IL-15 Bead	37	✓	HIL15-MAG	✓	✓	✓
Anti-Human sCD40L Bead	38	✓	HCD40L-MAG		✓	✓
Anti-Human IL-17 Bead	39	✓	HIL17-MAG	✓	✓	✓
Anti-Human IL-1RA Bead	42	✓	HIL1RA-MAG	✓	✓	✓
Anti-Human sIL-2Ra Bead	43	✓	HIL2RA-MAG		✓	✓
Anti-Human IL-1α Bead	44	✓	HIL1A-MAG	✓	✓	✓
Anti Human IL-9 Bead	45	✓	HIL9-MAG		✓	✓
Anti-Human IL-1β Bead	46	✓	HCYIL1B-MAG	✓	✓	✓
Anti-Human IL-2 Bead	48	✓	HIL2-MAG	✓	✓	✓
Anti-Human IL-3 Bead	51	✓	HIL3-MAG	✓	✓	✓
Anti-Human IL-4Bead	53	✓	HIL4-MAG	✓	✓	✓
Anti-Human IL-5 Bead	55	✓	HIL5-MAG	✓	✓	✓
Anti-Human IL-6 Bead	57	✓	HCYIL6-MAG	✓	✓	✓
Anti-Human IL-7 Bead	61	✓	HIL7-MAG	✓	✓	✓

Anti-Human IL-8 Bead	63	✓	HCYIL8-MAG	✓	✓	✓
Anti-Human IP-10 Bead	65	✓	HIP10-MAG	✓	✓	✓
Anti-Human MCP-1 Bead	67	✓	HCYMCP1-MAG	✓	✓	✓
Anti-Human MIP-1αBead	72	✓	HMIP1A-MAG	✓	✓	✓
Anti-Human MIP-1β Bead	73	✓	HMIP1B-MAG	✓	✓	✓
Anti-Human RANTES Bead	74	✓	HCYRNTS-MAG			✓
Anti-Human TNFα Bead	75	✓	HCYTNFA-MAG	✓	✓	✓
Anti-Human TNFβ Bead	76	✓	HTNFB-MAG	✓	✓	✓
Anti-Human VEGF Bead	78	✓	HCYVEGF-MAG	✓	✓	✓

MATERIALS REQUIRED BUT NOT PROVIDED

Reagents

1. Luminex Sheath Fluid (Luminex Catalogue #40-50000) or Luminex Drive Fluid (Luminex Catlogue # MPXDF-4PK)

Instrumentation / Materials

1. Adjustable Pipettes with Tips capable of delivering 25 µL to 1000 µl
2. Multichannel Pipettes capable of delivering 5 µl to 50 µl or 25 µl to 200 µl
3. Reagent Reservoirs
4. Polypropylene Microfuge Tubes
5. Rubber Bands
6. Aluminum Foil
7. Absorbent Pads
8. Laboratory Vortex Mixer
9. Sonicator (Branson Ultrasonic Cleaner Model # B200 or equivalent)
10. Titer Plate Shaker (Lab-Line Instruments, Model #4625, or equivalent)
11. Luminex ^{200,} HTS, FLEXMAP 3D™ or MAGPIX® with xPONENT software by Luminex Corporation
12. Automatic Plate washer for magnetic beads (Bio-Tek ELx405, Millipore catalog #40-015 or equivalent) or Hand held Magnetic Separation Block (Millipore catalog # 40-285 or equivalent)

Note: If a plate washer or hand held magnetic separation block for magnetic beads is not available, one can use Microtiter filter plate (MX-PLATE) to run the assay with the use of Vacuum Filtration Unit (Millipore Vacuum Manifold Catalog #MSVMHTS00, or equivalent. Millipore Vacuum Pump Catalog #WP6111560 or equivalent.)

SAFETY PRECAUTIONS

- All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions as established by the Centers for Disease Control and Prevention and by the Occupational Safety and Health Administration when handling and disposing of infectious agents.
- Sodium Azide or Proclin has been added to some reagents as a preservative. Although the concentrations are low, Sodium Azide and Proclin may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build up.

TECHNICAL GUIDELINES

To obtain reliable and reproducible results, the operator should carefully read this entire manual and fully understand all aspects of each assay step before running the assay. The following notes should be reviewed and understood before the assay is set-up.

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- Do not use beyond the expiration date on the label.
- Do not mix or substitute reagents with those from other lots or sources.
- The Antibody-Immobilized Beads are light sensitive and must be protected from light at all times. Cover the assay plate containing beads with opaque plate lid or aluminum foil during all incubation steps.
- It is important to allow all reagents to warm to room temperature (20-25°C) before use in the assay.
- Incomplete washing can adversely affect the assay outcome. All washing must be performed with the Wash Buffer provided.
- After hydration, all standards and controls must be transferred to polypropylene tubes.
- The standards prepared by serial dilution must be used within 1 hour of preparation. Discard any unused standards except the standard stock which may be stored at ≤ -20°C for 1 month and at ≤ -80°C for greater than one month.
- If samples fall outside the dynamic range of the assay, further dilute the samples with the appropriate diluent and repeat the assay.
- Any unused mixed Antibody-Immobilized Beads may be stored in the Bead Mix bottle at 2-8°C for up to one month.
- During the preparation of the standard curve, make certain to mix the higher concentration well before making the next dilution. Use a new tip with each dilution.
- The plate should be read immediately after the assay is finished. If, however, the plate cannot be read immediately, seal the plate, cover with aluminum foil or an opaque lid, and store the plate at 2-8°C for up to 24 hours. Prior to reading, agitate the plate on the plate shaker at room temperature for 10 minutes. Delay in reading a plate may result in decreased sensitivity for some analytes.
- The titer plate shaker should be set at a speed to provide maximum orbital mixing without splashing of liquid outside the wells. For the recommended plate shaker, this would be a setting of 5-7, which is approximately 500-800 rpm.
- Ensure that the needle probe is clean. This may be achieved by sonication and/or alcohol flushes.
- When reading the assay on Luminex 200™, adjust probe height according to the protocols recommended by Luminex to the kit solid plate using 4 alignment discs. When reading the assay on FLEXMAP 3D™, adjust probe height according to the protocols recommended by Luminex to the kit solid plate using 1 alignment disc. When reading the assay on MAGPIX, adjust probe height according to the protocols recommended by Luminex to the kit solid plate using 2 alignment disc.

- For cell culture supernatants or tissue extraction, use the culture or extraction medium as the matrix solution in blank, standard curve and controls. If samples are diluted in assay buffer, use the assay buffer as matrix.
- For serum/plasma sample that require a dilution instead of “Neat”, use the Serum Matrix provided in the kit as the diluent.
- For cell/tissue homogenate, the final cell or tissue homogenate should be prepared in a buffer that has a neutral pH, contains minimal detergents or strong denaturing detergents, and has an ionic strength close to physiological concentration. Avoid debris, lipids, and cell/tissue chunks. Centrifuge samples before use.
- Vortex all reagents well before adding to plate.

SAMPLE COLLECTION AND STORAGE

A. Preparation of Serum Samples:

- Allow the blood to clot for at least 30 minutes before centrifugation for 10 minutes at 1000xg. Remove serum and assay immediately or aliquot and store samples at ≤ -20°C.
- Avoid multiple (>2) freeze/thaw cycles.
- When using frozen samples, it is recommended to thaw the samples completely, mix well by vortexing and centrifuge prior to use in the assay to remove particulates.
- Neat Serum samples (for measuring 39 cytokines, not including RANTES, PDGF-AA, PDGF-BB) are used. When further dilution is required, use Serum Matrix as the diluent.
- When measuring RANTES, PDGF-AA, PDGF-BB in serum, sample should be diluted 1:100 in the assay buffer and **a standard curve with assay buffer matrix should be used accordingly**. When further dilution beyond 1:100 is required, use Serum Matrix as the diluent.

B. Preparation of Plasma Samples:

- Plasma collection using EDTA as an anti-coagulant is recommended. Centrifuge for 10 minutes at 1000xg within 30 minutes of blood collection. Remove plasma and assay immediately or aliquot and store samples at ≤ -20°C.
- Avoid multiple (>2) freeze/thaw cycles.
- When using frozen samples, it is recommended to thaw the samples completely, mix well by vortexing and centrifuge prior to use in the assay to remove particulates.
- Neat Plasma samples (for measuring 39 cytokines, not including RANTES, PDGF-AA, PDGF-BB) are used. When further dilution is required, use Serum Matrix as the diluent.
- When measuring RANTES, PDGF-AA, PDGF-BB in plasma, sample should be diluted 1:100 in the assay buffer and **a standard curve with assay buffer matrix should be used accordingly**. When further dilution beyond 1:100 is required, use Serum Matrix as the diluent.

C. Preparation of Tissue Culture Supernatant:

- Centrifuge the sample to remove debris and assay immediately or aliquot and store samples at ≤ -20°C.
- Avoid multiple (>2) freeze/thaw cycles.
- Tissue culture supernatant may require a dilution with an appropriate control medium prior to assay.

NOTE:

- A maximum of 25 µL per well of neat or diluted serum or plasma can be used. Tissue culture or other media may also be used.
- All samples must be stored in polypropylene tubes. **DO NOT STORE SAMPLES IN GLASS.**
- Avoid debris, lipids and cells when using samples with gross hemolysis or lipemia.
- Care must be taken when using heparin as an anticoagulant since an excess of heparin will provide falsely high values. Use no more than 10 IU heparin per mL of blood collected.

PREPARATION OF REAGENTS FOR IMMUNOASSAY

A. Preparation of Antibody-Immobilized Beads

If premixed beads are used, sonicate the premixed bead bottle 30 seconds and then vortex for 1 minute before use.
To prepare 42 plex premixed beads, add 70 µL of RANTES, PDGF-AA and PDGF-BB beads to the 39-plex premixed bead bottle. Mix well before use.
(**Note**: Due to high concentration of RANTES, PDGF-AA, PDGF-BB in serum/plasma, they have to be measured separately with **1:100** diluted serum/plasma. 39plex premixed beads are used for measuring all other 39 cytokines in serum/plasma with **Neat** serum/plasma)

For individual vials of beads, sonicate each antibody-bead vial for 30 seconds; vortex for 1 minute. Add 60 µL from each antibody bead vial to the Mixing Bottle and bring final volume to 3.0 mL with Bead Diluent. Vortex the mixed beads well. Unused portion may be stored at 2-8°C for up to one month. (Note: Due to the composition of magnetic beads, you may notice a slight color in the bead solution. This does not affect the performance of the beads or the kit.)

Example 1: When using 20 cytokine antibody-immobilized beads, add 60 µL from each of the 20 bead sets to the Mixing Bottle. Then add 1.8 mL Bead Diluent.

Example 2: When using 9 cytokine antibody-immobilized beads, add 60 µL from each of the 9 bead sets to the Mixing Bottle. Then add 2.46 mL Bead Diluent.

B. Preparation of Quality Controls

Before use, reconstitute Quality Control 1 and Quality Control 2 with 250 µL deionized water. Invert the vial several times to mix and vortex. Allow the vial to sit for 5-10 minutes and then transfer the controls to appropriately labeled polypropylene microfuge tubes. Unused portion may be stored at ≤ -20°C for up to one month.

C. Preparation of Wash Buffer

Bring the 10X Wash Buffer to room temperature and mix to bring all salts into solution. Dilute 30 mL of 10X Wash Buffer with 270 mL deionized water. Store unused portion at 2-8°C for up to one month.

D. Preparation of Serum Matrix

This step is required for serum or plasma samples only.

Add 1.0 mL deionized water to the bottle containing lyophilized Serum Matrix. Mix well. Allow at least 10 minutes for complete reconstitution. Leftover reconstituted Serum Matrix should be stored at ≤ -20°C for up to one month.

E. Preparation of Human Cytokine Standard

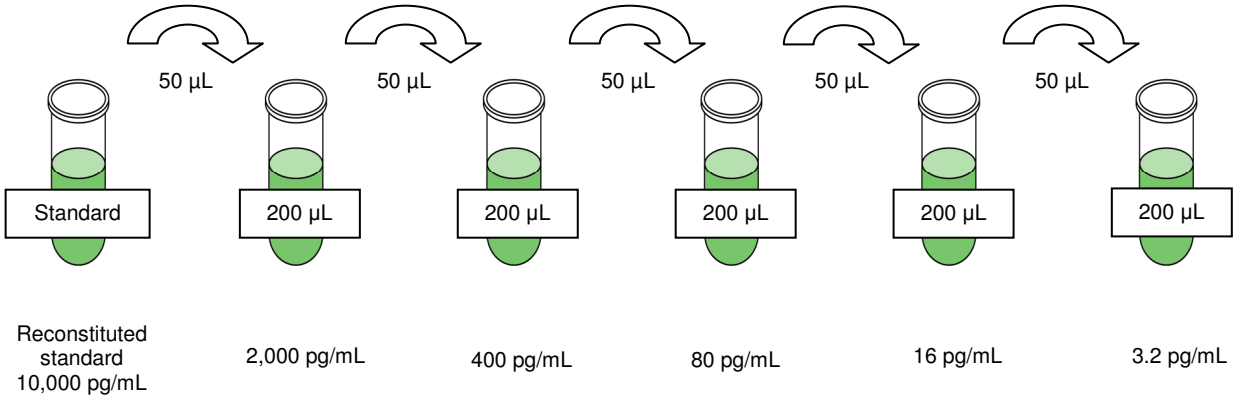
1.) Prior to use, reconstitute the Human Cytokine Standard with 250 µL deionized water to give a 10,000 pg/mL concentration of standard for all analytes. Invert the vial several times to mix. Vortex the vial for 10 seconds. Allow the vial to sit for 5-10 minutes and then transfer the standard to an appropriately labeled polypropylene microfuge tube. This will be used as the 10,000 pg/mL standard; the unused portion may be stored at ≤ -20°C for up to one month.

2). Preparation of Working Standards

Label five polypropylene microfuge tubes 2,000, 400, 80, 16, and 3.2 pg/mL. Add 200 µL of Assay Buffer to each of the five tubes. Prepare serial dilutions by adding 50 µL of the 10,000 pg/mL reconstituted standard to the 2,000 pg/mL tube, mix well and transfer 50 µL of the 2,000 pg/mL standard to the 400 pg/mL tube, mix well and transfer 50 µL of the 400 pg/mL standard to the 80 pg/mL tube, mix well and transfer 50 µL of the 80 pg/mL standard to 16 pg/mL tube, mix well and transfer 50 µL of the 16 pg/mL standard to the 3.2 pg/mL tube and mix well. The 0 pg/mL standard (Background) will be Assay Buffer.

Standard Concentration (pg/mL)	Volume of Deionized Water to Add	Volume of Standard to Add
10,000	250 µL	0

Standard Concentration (pg/mL)	Volume of Assay Buffer to Add	Volume of Standard to Add
2,000	200 µL	50 µL of 10,000 pg/mL
400	200 µL	50 µL of 2000 pg/mL
80	200 µL	50 µL of 400 pg/mL
16	200 µL	50 µL of 80 pg/mL
3.2	200 µL	50 µL of 16 pg/mL



IMMUNOASSAY PROCEDURE

- Prior to beginning this assay, it is imperative to read this protocol completely and to thoroughly understand the Technical Guidelines.
- Allow all reagents to warm to room temperature (20-25°C) before use in the assay.
- Diagram the placement of Standards [0 (Background), 3.2, 16, 80, 400, 2,000, and 10,000 pg/mL], Controls 1 and 2, and Samples on Well Map Worksheet in a vertical configuration. (Note: Most instruments will only read the 96-well plate vertically by default.) It is recommended to run the assay in duplicate.
- If using a filter plate, set the filter plate on a plate holder at all times during reagent dispensing and incubation steps so that the bottom of the plate does not touch any surface.

1. Add 200 µL of Wash Buffer into each well of the plate. Seal and mix on a plate shaker for 10 minutes at room temperature (20-25°C).
2. Decant Wash Buffer and remove the residual amount from all wells by inverting the plate and tapping it smartly onto absorbent towels several times.
3. Add 25 µL of each Standard or Control into the appropriate wells. Assay Buffer should be used for 0 pg/mL standard (Background).
4. Add 25 µL of Assay Buffer to the sample wells.
5. Add 25 µL of appropriate matrix solution to the background, standards, and control wells. When assaying serum or plasma, use the Serum Matrix provided in the kit. When assaying tissue culture or other supernatant, use proper control culture medium as the matrix solution.
6. Add 25 µL of serum/plasma Sample (1:100 dilution for RANTES, PDGF-AA, and PDGF-BB, Neat for all other 39 cytokines) or 25 µl cell culture sample into the appropriate wells.
7. Vortex Mixing Bottle and add 25 µL of the Mixed or Premixed Beads to each well. (Note: During addition of Beads, shake bead bottle intermittently to avoid settling.)
8. Seal the plate with a plate sealer. Wrap the plate with foil and incubate with agitation on a plate shaker overnight at 4°C or 2 hours at room temperature (20-25°C). *An overnight incubation (16-18 hr) may improve assay sensitivity for some analytes.*

Add 200 µL Wash Buffer per well

↓ Shake 10 min, RT

↓ Decant

- Add 25 µL Standard or Control to appropriate wells
- Add 25 µL Assay Buffer to background and sample wells
- Add 25 µL appropriate Matrix Solution to background, standards, and control wells
- Add 25 µL Samples to sample wells
- Add 25 µL Beads to each well

↓ Incubate overnight at 4°C or 2 hours at RT with shaking

9. Gently remove well contents and wash plate 2 times following instructions listed in the **PLATE WASHING** section**.**
10. Add 25 µL of Detection Antibodies into each well. (Note: Allow the Detection Antibodies to warm to room temperature prior to addition.)
11. Seal, cover with foil and incubate with agitation on a plate shaker for 1 hour at room temperature (20-25°C). **DO NOT ASPIRATE AFTER INCUBATION.**
12. Add 25 µL Streptavidin-Phycoerythrin to each well containing the 25 µL of Detection Antibodies.
13. Seal, cover with foil and incubate with agitation on a plate shaker for 30 minutes at room temperature (20-25°C).
14. Gently remove well contents and wash plate 2 times following instructions listed in the **PLATE WASHING** section**.**
15. Add 150 µL of Sheath Fluid (or Drive Fluid if using MAGPIX®) to all wells. Resuspend the beads on a plate shaker for 5 minutes.
16. Run plate on Luminex 200™, HTS, FLEXMAP 3D™ or MAGPIX® with xPONENT software.
17. Save and analyze the Median Fluorescent Intensity (MFI) data using a 5-parameter logistic or spline curve-fitting method for calculating cytokine/chemokines concentrations in samples. (Note: For diluted samples, multiply the calculated concentration by the dilution factor.)

Remove well contents and wash 2X with 200 µL Wash Buffer

Add 25 µL Detection Antibodies per well

Incubate 1 hour at RT

Do Not Aspirate

Add 25 µL Streptavidin-Phycoerythrin per well

Incubate for 30 minutes at RT

Remove well contents and wash 2X with 200 µL Wash Buffer

Add 150 µL Sheath Fluid or Drive Fluid per well

Read on Luminex (100 µL, 50 beads per bead set)

PLATE WASHING

1.) Solid Plate
If using a solid plate, use either a hand-held magnet or magnetic plate washer.

A.) For hand-held magnet, rest plate on magnet for 60 seconds to allow complete settling of magnetic beads. Remove well contents by gently decanting the plate in an appropriate waste receptacle and gently tapping on absorbent pads to remove residual liquid. Wash plate with 200 uL of Wash Buffer by removing plate from magnet, adding Wash Buffer, shaking for 30 seconds, reattaching to magnet, letting beads settle for 60 seconds and removing well contents as previously described after each wash. Repeat wash steps as recommended in Assay Procedure.

B.) For magnetic plate washer, let plate "soak" on magnet for 60 seconds to allow complete settling of the magnetic beads. Remove well contents by aspiration. Wash plate with 200 µL/well of Wash Buffer, letting beads "soak" for 60 seconds and removing Wash Buffer by aspiration after each wash. Repeat wash steps as recommended in Assay Procedure. **Note:** If using the recommended plate washer for magnetic beads (Bio-Tek ELx405) follow the appropriate equipment settings outlined in **EQUIPMENT SETTINGS.**

2.) Filter Plate (Millipore Cat# MX-PLATE)
If using a filter plate, use a vacuum filtration manifold to remove well contents. Wash plate 200 µL/well of Wash Buffer, removing Wash Buffer by vacuum filtration after each wash Repeat wash steps as recommended in the Assay Procedure.

EQUIPMENT SETTINGS

Bio-Tek ELx405:

The general recommended wash protocol (Link Protocol) is as follows:

Soak Program: Wash Program:
Soak → Aspirate→Dispense→Soak→Aspirate→Dispense→Soak→Aspirate

1.) Soak program:
1. Soak duration: 60 sec
2. Shake before soak?: NO

2.) Wash program:

Method:
1. Number of cycles: 2
2. soak/shake: YES
3. Soak duration: 60 sec
4. Shake before soak: NO
5. Prime after soak: NO

Dispense:
1. Dispense volume: 200 µL/well
2. Dispense flow rate: 5
3. Dispense height: 130 (16.51 mm)
4. Horizontal disp pos: 00 (0 mm)
5. Bottom Wash first?: NO
6. Prime before start?: NO

Aspiration:

1. Aspirate height: 35 (4.445 mm)
2. Horizontal Asp Pos: 30 (1.372 mm)
3. Aspiration rate: 06 (15.0 mm/sec)
4. Aspiration delay: 0
5. Crosswise Aspir: NO
6. Final Aspir: YES
7. Final Aspir delay: 0 (0 msec)

3.) Link program: (**Note:** this is the program to use during actual plate washing). Link together the Soak and Wash programs outlined above.

Note: After the final aspiration, there will be approximately 25 µl of residual Wash Buffer in each well. This is expected when using the BioTek Plate washer and this volume does not need to be aspirated from the plate.

If using an automatic plate washer other than BioTek ELx405, please refer to the manufacturer's recommendations for programming instructions.

These specifications are for the Luminex 200™ xPONENT™, FlexMAP 3D™, MAGPIX® and Luminex HTS. Luminex instruments with other software (e.g. MasterPlex, StarStation, LiquiChip, Bio-Plex, LABScan100) would need to follow instrument instructions for gate settings and additional specifications from the vendors for reading Luminex Magnetic Beads.

For magnetic bead assays, the Luminex 200™and HTS instruments must be calibrated with the xPonent 3.1 compatible Calibration Kit (Millipore Cat.# 40-275) and performance verified by Verification Kit (Millipore Cat. # 40-276). The Luminex FlexMAP 3D™instrument must be calibrated with the FlexMAP 3D™ Calibration Kit (Millipore cat# 40-028) and performance verified with the FlexMAP 3D™ Performance Verification Kit (Millipore cat# 40-029). The Luminex MAGPIX® instrument must be calibrated with the MAGPIX® Calibration Kit (Millipore cat# 40-049 and performance verified with the MAGPIX® Performance Verification Kit (Millipore cat# 40-050).

NOTE: These assays cannot be run on Luminex 100™ instruments or any instruments using the Luminex IS 2.3 or Luminex 1.7 software.

The Luminex probe height must be adjusted to the plate provided in the kit. Please use Cat.# MAG-PLATE, if additional plates are required for this purpose.

Events:	50, per bead	
Sample	100 µL	
Gate Settings:	8,000 to 15,000	
Reporter	Default (low PMT)	
Time Out:	60 seconds	
Bead Set:	Customizable 42-Plex Beads	
	EGF	12
	FGF-2	13
	Eotaxin	14
	TGF-α	15
	G-CSF	18
	Flt-3L	19
	GM-CSF	20
	Fractalkine	21
	IFNα2	22
	IFNγ	25
	GRO	26
	IL-10	27
	MCP-3	28
	IL-12P40	29
	MDC	30
	IL-12P70	33
	PDGF-AA	34
	IL-13	35
	PDGF-BB	36
	IL-15	37
	sCD40L	38
	IL-17	39
	IL-1RA	42
	sIL-2RA	43
	IL-1α	44
	IL-9	45
	IL-1β	46
	IL-2	48
	IL-3	51
	IL-4	53
	IL-5	55
	IL-6	57
	IL-7	61
	IL-8	63
	IP-10	65
	MCP-1	67
	MIP-1α	72
	MIP-1β	73
	RANTES	74
	TNFα	75
	TNFβ	76
	VEGF	78

QUALITY CONTROLS

The ranges for each analyte in Quality Control 1 and 2 are provided on the card insert or can be located at the Millipore website www.millipore.com/techlibrary/index.do using the catalog number as the keyword.

ASSAY CHARACTERISTICS

Cross-Reactivity

There was no or negligible cross-reactivity between the antibodies and any of the other analytes in this panel.

Assay Sensitivities (minimum detectable concentrations, pg/mL)

Mind: Minimum Detectable Concentration is calculated by the Statelier® Immunoassay Analysis Software from Brendan Technologies. It measures the true limits of detection for an assay by mathematically determining what the empirical MinDC would be if an infinite number of standard concentrations were run for the assay under the same conditions.

Cytokine	MinDC (pg/ml)	MinDC+2SD (pg/ml)
EGF	2.8	4.6
FGF-2	7.6	11.8
Eotaxin	4.0	6.8
TGFα	0.8	1.2
G-CSF	1.8	3.3
Flt-3L	5.4	7.0
GM-CSF	7.5	15.0
Fractalkine	22.7	37.7
IFNα2	2.9	4.8
IFNγ	0.8	1.1
GRO	9.9	14.1
IL-10	1.1	1.6
MCP-3	3.8	6.4
IL-12P40	7.4	12.7
MDC	3.6	7.1
IL-12P70	0.6	1.0
IL-13	1.3	1.9
IL-15	1.2	1.7
sCD40L	5.1	9.9
IL-17	0.7	1.2
IL-1RA	8.3	17.1
sIL-2Rα	11.2	20.5
IL-1α	9.4	12.6
IL-9	1.2	2.0
IL-1β	0.8	1.0
IL-2	1.0	1.6
IL-3	0.7	1.0
IL-4	4.5	7.1
IL-5	0.5	0.7
IL-6	0.9	1.3
IL-7	1.4	2.4
IL-8	0.4	0.7
IP-10	8.6	14.0
MCP-1	1.9	3.4
MIP-1α	2.9	6.2
MIP-1β	3.0	4.8
TNFα	0.7	1.1
TNFβ	1.5	1.9
VEGF	26.3	47.9
PDGF-AA	0.4	0.7
PDGFAB-BB	2.2	2.7
RANTES	1.2	1.9

Precision

Intra-assay precision is generated from the mean of the % CV's from sixteen reportable results across two different concentration of cytokines in a single assay. Inter-assay precision is generated from the mean of the % CV's from four reportable results across two different concentrations of cytokines across six different experiments.

Cytokine	Intra-assay %CV	Inter-assay %CV (N=6 assays)
EGF	2.3	5.8
FGF-2	2.3	4.8
Eotaxin	7.2	10.8
TGFα	4.1	9.5
G-CSF	1.8	15.5
Flt-3L	2.4	6.6
GM-CSF	3.1	10.1
Fractalkine	4.5	9.4
IFNα2	2.4	13.3
IFNγ	1.6	12.0
GRO	2.1	9.2
IL-10	1.6	16.8
MCP-3	1.6	6.4
IL-12P40	2.8	12.4
MDC	1.6	7.2
IL-12P70	2.2	16.7
IL-13	2.2	9.2
IL-15	2.7	8.1
sCD40L	3.7	18.9
IL-17	2.2	7.9
IL-1RA	2.1	10.7
sIL-2Rα	2.4	8.0
IL-1α	3.3	12.8
IL-9	2.4	8.4
IL-1β	2.3	6.7
IL-2	2.1	6.3
IL-3	3.4	6.1
IL-4	2.9	14.2
IL-5	2.6	10.8
IL-6	2.0	18.3
IL-7	1.7	16.1
IL-8	1.9	3.5
IP-10	2.6	15.3
MCP-1	1.5	7.9
MIP-1α	1.9	14.5
MIP-1β	2.4	8.8
TNFα	2.6	13.0
TNFβ	1.6	11.4
VEGF	3.7	10.4
PDGF-AA	4.3	16.7
PDGFAB-BB	2.1	12.3
RANTES	1.9	5.0

Accuracy

Spike Recovery: The data represents mean recovery of three concentration levels (low, medium and high) of spiked standards ranging from 3-10,000pg/mL in serum matrix.

Cytokine	% Recovery in matrix
EGF	97.5
FGF-2	99.0
Eotaxin	100.5
TGFα	91.7
G-CSF	100.3
Flt-3L	98.2
GM-CSF	100.7
Fractalkine	87.2
IFNα2	93.9
IFNγ	98.1
GRO	97.5
IL-10	97.7
MCP-3	97.0
IL-12P40	93.3
MDC	102.3
IL-12P70	104.0
IL-13	95.0
IL-15	95.3
sCD40L	95.2
IL-17	103.8
IL-1RA	93.5
sIL-2Rα	95.2
IL-1α	92.9
IL-9	99.4
IL-1β	94.9
IL-2	95.4
IL-3	101.0
IL-4	94.5
IL-5	99.9
IL-6	96.1
IL-7	93.0
IL-8	98.3
IP-10	93.8
MCP-1	98.3
MIP-1α	105.0
MIP-1β	92.4
TNFα	97.8
TNFβ	97.5
VEGF	91.8
PDGF-AA	97.9
PDGFAB-BB	102.0
RANTES	93.8

TROUBLESHOOTING GUIDE

Problem	Probable Cause	Solution
Insufficient Bead Count	Plate Washer aspirate height set too low	Adjust aspiration height according to manufacturers instructions.
	Bead mix prepared inappropriately	Sonicate bead vials and vortex just prior to adding to bead mix bottle according to protocol. Agitate bead mix intermittently in reservoir while pipetting this into the plate.
	Samples cause interference due to particulate matter or viscosity	See above. Also sample probe may need to be cleaned with Alcohol flush, Back flush and washes; or if needed probe should be removed and sonicated.
	Probe height not adjusted correctly	When reading the assay on Luminex 200™, adjust probe height according to the protocols recommended by Luminex to the kit solid plate using 4 alignment discs. When reading the assay on FLEXMAP 3D™, adjust probe height according to the protocols recommended by Luminex to the kit solid plate using 1 alignment disc. When reading the assay on MAGPIX, adjust probe height according to the protocols recommended by Luminex to the kit solid plate using 2 alignment disc.
Background is too high	Background wells were contaminated	Avoid cross-well contamination by using sealer appropriately, and pipeting with Multichannel pipets without touching reagent in plate.
	Matrix used has endogenous analyte or interference	Check matrix ingredients for cross reacting components (e.g. interleukin modified tissue culture medium).
	Insufficient washes	Increase number of washes.
Beads not in region or gate	Luminex not calibrated correctly or recently	Calibrate Luminex based on Instrument Manufacturer's instructions, at least once a week or if temperature has changed by >3^{o}C.
	Gate Settings not adjusted correctly	Some Luminex instruments (e.g. Bioplex) require different gate settings than those described in the Kit protocol. Use Instrument default settings.
	Wrong bead regions in protocol template	Check kit protocol for correct bead regions or analyte selection.
	Incorrect sample type used	Samples containing organic solvents or if highly viscous should be diluted or dialyzed as required.
	Instrument not washed or primed	Prime the Luminex 4 times to rid of air bubbles, wash 4 times with sheath fluid or water if there is any remnant alcohol or sanitizing liquid.

	Beads were exposed to light	Keep plate and bead mix covered with dark lid or aluminum foil during all incubation steps.
Signal for whole plate is same as background	Incorrect or no Detection Antibody was added	Add appropriate Detection Antibody and continue.
	Streptavidin-Phycoerythrin was not added	Add Streptavidin-Phycoerythrin according to protocol. If Detection Antibody has already been removed, sensitivity may be low.
Low signal for standard curve	Detection Antibody may have been removed prior to adding Streptavidin Phycoerythrin	May need to repeat assay if desired sensitivity not achieved.
	Incubations done at inappropriate temperatures, timings or agitation	Assay conditions need to be checked.
Signals too high, standard curves are saturated	Calibration target value set too high	With some Luminex Instrument (e.g. Bio-plex) Default target setting for RP1 calibrator is set at High PMT. Use low target value for calibration and reanalyze plate.
	Plate incubation was too long with standard curve and samples	Use shorter incubation time.
Sample readings are out of range	Samples contain no or below detectable levels of analyte	If below detectable levels, it may be possible to use higher sample volume. Check with tech support for appropriate protocol modifications.
	Samples contain analyte concentrations higher than highest standard point.	Samples may require dilution and reanalysis for just that particular analyte.
	Standard curve was saturated at higher end of curve.	See above.
High Variation in samples and/or standards	Multichannel pipet may not be calibrated	Calibrate pipets.
	Plate washing was not uniform	Confirm all reagents are removed completely in all wash steps.
	Samples may have high particulate matter or other interfering substances	See above.
	Plate agitation was insufficient	Plate should be agitated during all incubation steps using a vertical plate shaker at a speed where beads are in constant motion without causing splashing.
	Cross well contamination	Check when reusing plate sealer that no reagent has touched sealer. Care should be taken when using same pipet tips that are used for reagent additions and that pipet tip does not touch reagent in plate.
FOR FILTER PLATES ONLY		
Filter plate will not vacuum	Vacuum pressure is insufficient	Increase vacuum pressure such that 0.2mL buffer can be suctioned in 3-5 seconds.
	Samples have insoluble particles	Centrifuge samples just prior to assay setup and use supernatant.

	High lipid concentration	After centrifugation, remove lipid layer and use supernatant.
Plate leaked	Vacuum Pressure too high	Adjust vacuum pressure such that 0.2mL buffer can be suctioned in 3-5 seconds. May need to transfer contents to a new (blocked) plate and continue.
	Plate set directly on table or absorbent towels during incubations or reagent additions	Set plate on plate holder or raised edge so bottom of filter is not touching any surface.
	Insufficient blotting of filter plate bottom causing wicking	Blot the bottom of the filter plate well with absorbent towels after each wash step.
	Pipette touching plate filter during additions	Pipette to the side of plate.
	Probe height not adjusted correctly	Adjust probe to 3 alignment discs in well H6.
	Sample too viscous	May need to dilute sample.

REPLACEMENT REAGENTS	**Cat #**
Human Cytokine / Chemokine Standard	MXH8060
Human Cytokine / Chemokine Standard	MXH8060-2
Human Cytokine Quality Controls 1 and 2	MXH6060
Human Cytokine Quality Controls 1 and 2	MXH6060-2
Human Cytokine Detection Antibodies	MXH1060-1
Human Cytokine Detection Antibodies	MXH1060-2
Human Cytokine Detection Antibodies	MXH1060-3
Human Cytokine Detection Antibodies	MXH1060-4
Serum Matrix	MXHSM
Bead Diluent	LBD
Assay Buffer	L-AB
Streptavidin-Phycoerythrin	L-SAPE9
Streptavidin-Phycoerythrin	L-SAPE3
Streptavidin-Phycoerythrin	L-SAPE10
Streptavidin-Phycoerythrin	L-SAPE11
Set of two 96-Well Black plates with sealers	MAG-PLATE
10X Wash Buffer	L-WB

Antibody-Immobilized Magnetic Beads

Cytokine	Bead #	Cat. #
EGF	12	HEGF-MAG
FGF-2	13	HCYFGF2-MAG
Eotaxin	14	HETXN-MAG
TGF-α	15	HCYTGFA-MAG
G-CSF	18	HGCSF-MAG
Flt-3L	19	HFLT3L-MAG
GM-CSF	20	HGMCSF-MAG
Fractalkine	21	HFKN-MAG
IFNα2	22	HIFNA2-MAG
IFNγ	25	HCYIFNG-MAG
GRO	26	HGR0-MAG
IL-10	27	HCYIL10-MAG
MCP-3	28	HMCP3-MAG
IL-12P40	29	HIL12P40-MAG
MDC	30	HMDC-MAG
IL-12P70	33	HIL12P70-MAG
PDGF-AA	34	HPDGFAA-MAG
IL-13	35	HIL13-MAG
PDGF-BB	36	HPDGFBB-MAG
IL-15	37	HIL15-MAG
sCD40L	38	HCD40L-MAG
IL-17	39	HIL17-MAG
IL-1RA	42	HIL1RA-MAG
sIL-2RA	43	HIL2RA-MAG
IL-1α	44	HIL1A-MAG
IL-9	45	HIL9-MAG
IL-1β	46	HCYIL1B-MAG
IL-2	48	HIL2-MAG
IL-3	51	HIL3-MAG
IL-4	53	HIL4-MAG
IL-5	55	HIL5-MAG
IL-6	57	HCYIL6-MAG
IL-7	61	HIL7-MAG
IL-8	63	HCYIL8-MAG
IP-10	65	HIP10-MAG
MCP-1	67	HCYMCP1-MAG
MIP-1α	72	HMIP1A-MAG
MIP-1β	73	HMIP1B-MAG
RANTES	74	HCYRNTS-MAG
TNFα	75	HCYTNFA-MAG
TNFβ	76	HTNFB-MAG
VEGF	78	HCYVEGF-MAG
Premixed 29 Plex Beads HCYPMX29-MAG		Premixed 29 Plex Beads
Premixed 39 Plex Beads HCYPMX39-MAG		Premixed 39 Plex Beads

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- Quantity of kits
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WELL MAP

	1	2	3	4	5	6	7	8	9	10	11	12
A	0 pg/mL Standard (Background)	400 pg/mL Standard	QC-2 Control									
B	0 pg/mL Standard (Background)	400 pg/mL Standard	QC-2 Control									
C	3.2 pg/mL Standard	2,000 pg/mL Standard	Sample 1									
D	3.2 pg/mL Standard	2,000 pg/mL Standard	Sample 1									
E	16 pg/mL Standard	10,000 pg/mL Standard	Sample 2									
F	16 pg/mL Standard	10,000 pg/mL Standard	Sample 2									
G	80 pg/mL Standard	QC-1 Control	Etc.									
H	80 pg/mL Standard	QC-1 Control										

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Title: Pre-Amplification Protocol			Page 1 of 3		
Doc#	MTC-0001	**Revision:**		**Effective Date:**	08/31/1995

1	Purpose	1.1	To provide guidelines and procedures to be followed in pre-amplification work areas. This protocol is designed to prevent contamination of equipment, supplies, reagents and samples.
2	Scope	2.1	This protocol applies to all Molecular Transfusion Core personnel.
		2.2	This protocol applies to pre-amplification work areas, which includes the reagent preparation laboratory and the sample preparation laboratory.
3	Responsibilities	3.1	It is the responsibility of all Molecular Transfusion Core personnel to adhere to the universal precautions.
		3.2	It is the responsibility of each Molecular Transfusion Core personnel working in the specified areas to adhere to these guidelines and procedures.
4	Materials and Equipment	4.1	Disposable gloves
		4.2	Disposable lab coats
		4.3	Bleach, 10%
		4.4	Alcohol, 70%
		4.5	Plastic wash bottles
		4.6	Gauze sponges, 4 x 4 inches
		4.7	Soak containers
5	Basic Principles	5.1	Physical separation of reagent, sample and amplification laboratories prevents contamination of “clean” areas from “hot” areas.
		5.2	Supplies, instruments and personal protective equipments are not interchangeable between laboratories.
		5.2	Use of dedicated equipment assigned to each workstation allows containment of possible contamination.
		5.3	Unidirectional flow of worksheets, from “clean” to “hot” areas, prevents contamination of “clean” areas.
		5.4	Unidirectional flow of racks, from “clean” to “hot” areas, prevents contamination of “clean” areas, until decontamination is performed.
		5.5	Decontamination procedures during and at the end of the day provides prevents spread and accumulation of

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			contaminating substances.
6	Supplies and Reagents	6.1	Supplies will originate from a “clean” area.
		6.2	Instruments, calculators, pens, pipettes will not be shared between laboratories.
		6.3	Instruments, calculators, pens, pipettes are dedicated for specific tasks.
		6.4	Each workstation is designated by color according to the level of use.
7	Gloves and Lab coats	7.1	Gloves will be worn before touching any item in the pre-amplification areas.
		7.2	Lab coats will be worn whenever working in the pre-amplification areas
		7.3	Gloves and lab coats will not be worn from one laboratory to another.
		7.4	Gloves and lab coats will be discarded when soiled.
8	Housekeeping, Clean-up, Decontamination	8.1	The workstation garbage bins will be lined by a Ziploc bag before use.
		8.2	Discard the used Ziploc bag daily.
		8.2	When soiled and after use, the workstation, centrifuges, pipettes, pens will be decontaminated with 10% bleach and 70% alcohol.
		8.3	Racks will be soaked in 10% bleach and rinsed immediately after use.
9	Unidirectional Workflow of Paperwork	9.1	Paperwork, like experimental designs and worksheets, must follow a one way-flow, from the Reagent Prep Lab to Sample Prep Lab to PCR Lab.
		9.2	All paperwork entering the Reagent Prep Lab must be faxed. Paperwork faxed to the Reagent Prep Lab may be moved to the Sample Prep Lab but once in the Sample Prep Lab, may not go back to the Reagent Prep Lab.
		9.3	All paperwork entering the Sample Prep Lab must be faxed, unless the paper originated from the Reagent Prep Lab. Paperwork faxed to the Sample Prep Lab may not be moved to the Reagent Prep Lab.
		9.4	All paperwork which entered the PCR Lab may not be moved back to either Reagent or Sample Prep Labs.
		9.5	Speed dials to both fax numbers are set-up in the basement fax machine. a. Reagent Prep Lab Fax: 749-6689 b. Sample Prep Lab Fax: 749-6666

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10	Racks Reverse Flow- Bleach Twice	10.1	Racks that were moved to the PCR lab must be immersed in 10% bleach, for 5 minutes, and rinsed with water before they can be moved to the Sample Prep Lab. Once in the Sample Prep Lab, the racks should be immediately immersed in 10% bleach, for 5 minutes, and rinsed again before use.
		10.2	Racks that were moved to the Sample Prep Lab must be immersed in 10% bleach, for 5 minutes, and rinsed with water before they can be moved to the Reagent Prep Lab. Once in the Reagent Prep Lab, the racks should be immediately immersed in 10% bleach, for 5 minutes, and rinsed again before use.

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Title: Quality Control Procedures for the Quantitative Reverse Transcription Real-Time PCR Assays Using SyBr Green			Page 1 of 2		
Doc#	MTC-0002	**Revision:**		**Effective Date:**	04/01/10

1	Purpose	1.1	To provide the reverse transcription assays a method for evaluating the efficiency of the RNA extraction, reverse transcription and PCR amplification.
2	Scope	2.1	This protocol applies to all Molecular Transfusion Core personnel.
		2.2	This protocol serves as a QC procedure for the quantitative reverse transcription real-time PCR assays using SyBr Green.
		2.3	This protocol applies to assays using Qiagen columns as RNA extraction procedure.
		2.4	This protocol applies to assays which quantifies RNA viruses.
3	Responsibilities	3.1	It is the responsibility of the supervisor to ensure that the technical staff performing the assay is trained to include and analyze the QC samples in every run.
		3.2	It is the responsibility of the supervisor to ensure that variances or deviance are documented and addressed.
		3.3	It is the responsibility of the staff performing the assay to include the QC samples in each run.
		3.4	It is the responsibility of the staff performing the assay to document deviance to the protocol.
		3.5	It is the responsibility of all Molecular Transfusion Core personnel to adhere to the universal precautions and MTC-0001.
4	Materials and Equipment	4.1	Quantitative Positive QC Plasma Standards: Plasma spiked with RNA virus, (1000 copies/100µL, 100 copies/100µL, 10 copies/100µL, 1 copy/100µL)
		4.2	Negative QC Plasma samples: Unspiked plasma negative for either Dengue Virus or West Nile Virus
		4.3	Negative QC sample: No template control (Solution A and B)
		4.4	Real-time Thermal Cycler
5	RNA Extraction	5.1	One each of positive QC plasma standard will be added during RNA extraction of experimental samples.
		5.2	One negative QC plasma samples unspiked with virus will

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			be added during RNA extraction of experimental samples.
6	Reverse Transcription	6.1	The positive standards and the negative control will be processed along with the experimental samples.
7	PCR Amplification	7.1	PCR amplification will include two wells containing Solution A and B, a no template control reagent.
		7.2	The positive standards and the negative control will be processed along with the experimental samples.
8	Evaluation of RNA Extraction and Reverse Transcription Efficiency	8.1	The quantitative standards will be evaluated for linearity and efficiency.
		8.2	The standards will be compared to values in the control chart. The control chart will have values of at least 20 standards ran before the assay is used.
		8.3	Experimental unknowns will be quantified by interpolation using the quantitative standards.
		8.4	Negative Control Plasma sample will be used to evaluate non-specific amplification.
		8.5	No template controls will be used to evaluate generation of primer dimers.
9	Evaluation of PCR Specificity	9.1	The melting temperatures of the experimental unknowns will be compared to melting temperatures of the positive controls. Experimental unknowns with the same melting temperature will be counted as positive.
		9.2	The melting temperatures of the experimental unknowns will be compared to melting temperatures of the negative control. Experimental unknowns with the same melting temperature will be counted as negative.
		9.3	The melting temperatures of the experimental unknowns will be compared to melting temperatures of the no template control. Experimental unknowns with the same melting temperature will be counted as negative.
10	Deviance	10.1	A run where the quantitative standards are outside the acceptable values of the control chart will be invalidated.
		10.2	A run where the melting temperatures of the quantitative standards are inconsistent with expected melting temperatures will be invalidated.
		10.3	A run where the linearity of standards is below the acceptable value will be invalidated.

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Title: Preventative Maintenance, Calibration and Validation of Equipment Performed By External Service Contractors			Page 1 of 2		
Doc#	MTC-0003	**Revision:**		**Effective Date:**	04/29/10

1	Purpose	1.1	To provide information on the schedule of preventative maintenance, calibration and validation of equipments. To provide instructions on how to handle the documentations provided by external service contractors.
2	Scope	2.1	This protocol applies to equipments used by the Molecular Transfusion Core laboratory.
		2.2	This protocol applies to equipments which are serviced by companies certified to perform validation and calibration. Specifically, the equipments pertain to: Applied BioSystems 7500, Roche LightCycler 480, centrifuges, michrocentrifuges and pipettes.
3	Responsibilities	3.1	It is the responsibility of the supervisor to ensure that the procedure is performed on a regular schedule.
		3.2	It is the responsibility of the supervisor to ensure that documentations are filed in the designated binder or folder.
4	Materials and Equipment	4.1	Service Reports or Calibration Certificates
		4.2	Equipment specific folders
5	Thermal Cyclers: ABI 7500 and Roche LC 480	5.1	The ABI 7500 and Roche LC 480's are scheduled for annual maintenance by a service engineer from Applied BioSystems, Inc. and Roche Diagnostics Corp, respectively.
		5.2	The service engineer will perform the preventative maintenance per instrument requirement.
		5.2	A service report will be provided by the service engineer to indicate that the instrument passed all required parameters.
		5.3	The service report will be filed in their respective folders.
6	Centrifuges and microcentrifuges	6.1	Centrifuges and microcentrifuges will be maintained annually by a qualified laboratory service repair company.
		6.2	Service stickers are placed on the centrifuges to indicate that the instrument passed the maintenance quality controls.
		6.3	A service report will be provided by the service engineer to indicate that the instrument passed all required

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			parameters.
		6.4	The service report will be filed in its respective folders.
7	Pipettes	7.1	Pipettes are scheduled for service annually.
		7.2	Pipettes are sent out to qualified pipette service laboratories.
		7.3	Each pipette will be provided a sticker on the pipette indicating that the pipette had been validated. Each pipette will also be provided a calibration certificate.
		7.4	The calibration certificate will be filed in its respective folder.

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Title: Creation of a Control Chart			Page 1 of 2		
Doc#	MTC-0004	**Revision:**		**Effective Date:**	04/01/10

1	Purpose	1.1	To create a control chart to be used to generate an acceptable range and standard deviations for positive controls.
2	Scope	2.1	This protocol applies to all Molecular Transfusion Core personnel.
		2.2	This protocol applies to control charts to be used for evaluating acceptability of standard curves and qualitative positive controls.
3	Responsibilities	3.1	It is the responsibility of the supervisor to ensure that the technical staff performing the assay is trained to perform the assay.
		3.2	It is the responsibility of the supervisor to ensure that variances or deviance are documented and addressed.
		3.3	It is the responsibility of the staff performing the assay to document deviance to the protocol.
		3.4	It is the responsibility of the technical staff performing the assays to abide by the universal precaution and Protocol number MTC-0001.
4	Materials and Equipment	4.1	20 Positive control samples
		4.2	Negative sample controls
		4.3	No template controls (Solution A and B)
		4.4	Real-time Thermal Cycler
		4.5	All materials and equipment to run the assay. See specific assay procedure.
5	Running the assay	5.1	The 20 positive controls should be placed on 5 different runs, four controls per run.
		5.2	Negative sample controls should be included in each run.
		5.3	No template controls should be included in each run.
6	Evaluation of PCR Specificity	6.1	The melting temperatures of the amplicons of the positive controls will be compared to each other for homogeneity.
		6.2	The melting temperatures of the positive controls will be compared to melting temperatures of the negative control. Positive controls with the same melting temperature as the negative control will be invalidated.
		6.3	The melting temperatures of the positive controls will be

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			compared to melting temperatures of the no template controls. Positive controls with the same melting temperature as the no template controls will be invalidated.
7	Control Chart	7.1	At least twenty data points will be collected before generating the control chart.
		7.1	The cycle threshold for each data will be charted.
		7.2	Acceptability will be set at 2x standard deviation.

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Title: West Nile Virus RT-PCR Assay			Page 1 of 4		
Doc#	MTC-0006	**Revision:**		**Effective Date:**	04/01/10

1	Purpose	1.1	To provide instructions on how to perform the West Nile Virus RT-PCR assay.
2	Scope	2.1	This protocol applies to testing samples to be interrogated for the presence of West Nile Virus.
3	Responsibilities	3.1	This procedure is to be performed by personnel from the Molecular Transfusion Core.
		3.2	It is the responsibility of the Molecular Transfusion Core supervisor to ensure that the laboratory personnel have been trained in properly handling human specimens and wearing PPE.
		3.3	It is the responsibility of all Molecular Transfusion Core personnel to adhere to the universal precautions and MTC-0001.
4	Materials and Equipment	4.1	Roche 480
		4.2	PCR workstation
		4.3	Pipettes
		4.4	Heat block
		4.5	Centrifuge
		4.6	Microcentrifuge
		4.7	QIAamp Viral RNA Mini Kit
		4.8	Pipette tips
		4.9	Ethanol (200 proof)
		4.10	RNase-free 1.5 mL tubes
		4.11	96 well PCR plate
		4.12	Buffer
		4.13	dNTPs
		4.14	Primers
		4.15	Probe
		4.15	Probe
		4.16	FastStart Taq
		4.17	10x Solution A+B
		4.18	RNase inhibitor
		4.19	MuLV reverse transcriptase
		4.20	Lab coat
		4.21	Gloves

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5	RNA Extraction via QIAamp Viral RNA Spin Protocol	5.1	Pipet 800 uL of prepared Buffer AVL containing Carrier RNA into a 1.5 mL microcentrifuge tube.
		5.2	Add 200 uL of plasma to the Buffer AVL/Carrier RNA in the microcentrifuge tube. Mix by pulse-vortexing for 15 sec.
		5.3	Incubate at room temperature (15-25^{o}C) for 10 min.
		5.4	Briefly centrifuge the 1.5 mL microcentrifuge tube to remove drops from the inside of the lid.
		5.5	Add 800 uL of ethanol (96-100%) to the sample, and mix by pulse-vortexing for 15 sec. After mixing, briefly centrifuge the 1.5 mL microcentrifuge tube to remove drops from inside the lid.
		5.6	Carefully apply 600 uL of the solution from step 5 to the QIAamp spin column without wetting the rim. Close the cap, and centrifuge at 8,000 rpm for 1 min. Place the QIAamp spin column into a clean 2 mL collection tube, and discard the tube containing the filtrate.
		5.7	Carefully open the QIAamp spin column, and repeat step 6.
		5.8	Carefully open the QIAamp spin column and add 500 uL of Buffer AW1. Close the cap and centrifuge at 8,000 rpm for 1 min. Place the QIAamp spin column in a clean 2 mL collection tube and discard the tube containing the filtrate.
		5.9	Carefully open the QIAamp spin column and add 500 uL of Buffer AW2. Close the cap and centrifuge at full speed (14,000 rpm) for 3 min.
		5.10	Place the QIAamp spin column in a new 2 mL collection tube and discard the old collection tube with the filtrate. Centrifuge at full speed (14,000 rpm) for 1 min.
		5.11	Place the QIAamp spin column in a clean 1.5 mL microcentrifuge tube. Discard the old collection tube containing the filtrate.
		5.12	Add 120 uL of H_2O equilibrated to room temperature. Close the cap, and incubate at room temperature for 1 min. Centrifuge at 10,000 rpm for 2 min.
6	Reverse Transcription	6.1	Thaw dGTP, dATP, dTTP, dCTP, and downstream primer to room temperature and vortex.
		6.2	Add the following to a labeled 1.5 mL screwcap tube: 12.0 uL 10X Solution A+B 1.2 uL total 100 mM dNTPs (without dUTP)

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			3.0 uL RNase inhibitor (40U/uL) 1.5 uL MuLV reverse transcriptase (100 U/uL) 0.45 uL of downstream primer
		6.3	Add sample to bring final volume up to 120 uL. (To dilute the 10X Sol A+B to 1X Sol A+B)
		6.4	Vortex mixture and centrifuge.
		6.5	Reverse transcribe at 42^{o}C for 40 min in heat block.
		6.6	Vortex mixture and centrifuge.
		6.7	Incubate at 100^{o}C for 10 min.
		6.8	Vortex and centrifuge.
7	PCR Reaction Mix Preparation	7.1	Add 30 uL of mineral oil to each well of a 96-well amplification plate.
		7.2	Thaw primers and probe to room temperature and vortex.
		7.3	Add the following to a labeled 1.5 mL screwcap tube: 50 uL/rxn Buffer 52 0.5 uL/rxn Forward primer (VWNVA1) 0.5 uL/rxn Reverse primer (VWNVA2) 1.0 uL/rxn Probe (WNV PROBE) 1.0 uL/rxn FastStart Taq
		7.4	Add 50 uL of reaction mix to each well of amplification plate.
		7.5	Add 25 uL of sample to each well.
		7.6	Centrifuge plate at 1600 rpm for 1 min
8	Set up of Roche 480	8.1	Push button on instrument to open plate holder drawer. Both lights have to be steady green. Place plate in the instrument and close the plate holder drawer.
		8.2	Go to Overview screen. Click on “New Experiment” button.
		8.3	From drop-down menu choose “Dual Color Hydrolysis Probe.”
		8.4	Choose “Apply Template” then select template “PCR with Probes” in the Run Template folder. Cycle conditions are: 1 cycle of 95^{o}C for 1 min followed by 45 cycles of 95^{o}C for 30 sec and 56^{o}C for 1 min.
		8.5	Save in WNV folder and hit “Start Run” button.
9	Analysis on Roche 480	9.1	Click on “Analyze” button.

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		9.2	Select “Absolute Quant/Fit Point.”
		9.3	Adjust the threshold bar, moving it above the background signal.
		9.4	Choose dye for signal such as “FAM” for WNV probe.
		9.5	Click on “Calculate.”
		9.6	Click on “Save” (floppy disk icon on right side).
		9.7	Click on “Report” and choose parameters to be reported.
		9.8	Click on “Generate.”
		9.9	Print report by clicking printer icon on top left of the report generated.

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Title: Quality Control Procedures for the Quantitative Reverse Transcription Real-Time PCR Assays Using a Fluorescent Probes			Page 1 of 2		
Doc#	MTC-0007	**Revision:**		**Effective Date:**	04/01/10

1	Purpose	1.1	To provide the reverse transcription assays a method for evaluating the efficiency of the RNA extraction, reverse transcription and PCR amplification.
2	Scope	2.1	This protocol applies to all Molecular Transfusion Core personnel.
		2.2	This protocol serves as a QC procedure for the quantitative reverse transcription real-time PCR assays using fluorescent probes.
		2.3	This protocol applies to assays using Qiagen columns as RNA extraction procedure.
		2.4	This protocol applies to assays which quantifies RNA viruses.
3	Responsibilities	3.1	It is the responsibility of the supervisor to ensure that the technical staff performing the assay is trained to include and analyze the QC samples in every run.
		3.2	It is the responsibility of the supervisor to ensure that variances or deviance are documented and addressed.
		3.3	It is the responsibility of the staff performing the assay to include the QC samples in each run.
		3.4	It is the responsibility of the staff performing the assay to document deviance to the protocol.
		3.5	It is the responsibility of all Molecular Transfusion Core personnel to adhere to the universal precautions and MTC-0001.
4	Materials and Equipment	4.1	Quantitative Positive QC Plasma Standards: Plasma spiked with RNA virus, (1000 copies/100µL, 100 copies/100µL, 10 copies/100µL, 1 copy/100µL)
		4.2	Negative QC Plasma samples: Unspiked plasma negative for either Dengue Virus or West Nile Virus
		4.3	Negative QC sample: No template control (Solution A and B)
		4.4	Real-time Thermal Cycler
5	RNA Extraction	5.1	One each of positive QC plasma standard will be added during RNA extraction of experimental samples.

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		5.2	One negative QC plasma samples unspiked with virus will be added during RNA extraction of experimental samples.
6	Reverse Transcription	6.1	The positive standards and the negative control will be processed along with the experimental samples.
7	PCR Amplification	7.1	PCR amplification will include two wells containing Solution A and B, a no template control reagent.
		7.2	The positive standards and the negative control will be processed along with the experimental samples.
8	Evaluation of RNA Extraction and Reverse Transcription Efficiency	8.1	The quantitative standards will be evaluated for linearity and efficiency.
		8.2	The standards will be compared to values in the control chart. The control chart will have values of at least 20 standards ran before the assay is used.
		8.3	Experimental unknowns will be quantified by interpolation using the quantitative standards.
		8.4	Negative Control Plasma sample will be used to evaluate non-specific amplification.
		8.5	No template controls will be used to evaluate generation of primer dimers.
9	Deviance	9.1	A run where the quantitative standards are outside the acceptable values of the control chart will be invalidated.
		9.2	A run where the linearity of standards is below the acceptable value will be invalidated.

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Title: West Nile Virus RT-PCR Assay for Whole Blood Samples			Page 1 of 4		
Doc#	MTC-0008	**Revision:**		**Effective Date:**	04/01/10

1	Purpose	1.1	To provide instructions on how to perform the West Nile Virus RT-PCR assay on whole blood samples.
2	Scope	2.1	This protocol applies to testing samples to be interrogated for the presence of West Nile Virus.
3	Responsibilities	3.1	This procedure is to be performed by personnel from the Molecular Transfusion Core.
		3.2	It is the responsibility of the Molecular Transfusion Core supervisor to ensure that the laboratory personnel have been trained in properly handling human specimens and wearing PPE.
		3.3	It is the responsibility of all Molecular Transfusion Core personnel to adhere to the universal precautions and MTC-0001.
4	Materials and Equipment	4.1	Roche 480
		4.2	PCR workstation
		4.3	Pipettes
		4.4	Heat block
		4.5	Centrifuge
		4.6	Microcentrifuge
		4.7	QIAamp Viral RNA Mini Kit
		4.8	Pipette tips
		4.9	Ethanol (200 proof)
		4.10	RNase-free 1.5 mL tubes
		4.11	96 well PCR plate
		4.12	Buffer
		4.13	dNTPs
		4.14	Primers
		4.15	Probe
		4.15	Probe
		4.16	FastStart Taq
		4.17	10x Solution A+B
		4.18	RNase inhibitor
		4.19	MuLV reverse transcriptase
		4.20	Lab coat

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		4.21	Gloves
5	RNA Extraction via QIAamp Viral RNA Spin Protocol	5.1	Pipet 400 uL of prepared Buffer AVL containing Carrier RNA into a 1.5 mL microcentrifuge tube.
		5.2	Add 100 uL of plasma to the Buffer AVL/Carrier RNA in the microcentrifuge tube. Mix by pulse-vortexing for 15 sec.
		5.3	Incubate at room temperature (15-25^{o}C) for 10 min.
		5.4	Briefly centrifuge the 1.5 mL microcentrifuge tube to remove drops from the inside of the lid.
		5.5	Add 400 uL of ethanol (96-100%) to the sample, and mix by pulse-vortexing for 15 sec. After mixing, briefly centrifuge the 1.5 mL microcentrifuge tube to remove drops from inside the lid.
		5.6	Carefully apply 450 uL of the solution from step 5 to the QIAamp spin column without wetting the rim. Close the cap, and centrifuge at 8,000 rpm for 1 min. Place the QIAamp spin column into a clean 2 mL collection tube, and discard the tube containing the filtrate.
		5.7	Carefully open the QIAamp spin column, and repeat step 6.
		5.8	Carefully open the QIAamp spin column and add 500 uL of Buffer AW1. Close the cap and centrifuge at 8,000 rpm for 1 min. Place the QIAamp spin column in a clean 2 mL collection tube and discard the tube containing the filtrate.
		5.9	Repeat step 8.
		5.10	Carefully open the QIAamp spin column and add 500 uL of Buffer AW2. Close the cap and centrifuge at full speed (14,000 rpm) for 3 min.
		5.11	Repeat step 10.
		5.12	Place the QIAamp spin column in a new 2 mL collection tube and discard the old collection tube with the filtrate. Centrifuge at full speed (14,000 rpm) for 1 min.
		5.13	Place the QIAamp spin column in a clean 1.5 mL microcentrifuge tube. Discard the old collection tube containing the filtrate.

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		5.14	Add 60 uL of H_2O equilibrated to room temperature. Close the cap, and incubate at room temperature for 1 min. Centrifuge at 8,000 rpm for 1 min.
6	Reverse Transcription	6.1	Thaw dGTP, dATP, dTTP, dCTP, and downstream primer to room temperature and vortex.
		6.2	Add the following to a labeled 1.5 mL screwcap tube: 12.0 uL 10X Solution A+B 1.2 uL total 100 mM dNTPs (without dUTP) 3.0 uL RNase inhibitor (40U/uL) 1.5 uL MuLV reverse transcriptase (100 U/uL) 0.45 uL of downstream primer
		6.3	Add sample to bring final volume up to 120 uL. (To dilute the 10X Sol A+B to 1X Sol A+B)
		6.4	Vortex mixture and centrifuge.
		6.5	Reverse transcribe at 42^{o}C for 40 min in heat block.
		6.6	Vortex mixture and centrifuge.
		6.7	Incubate at 100^{o}C for 10 min.
		6.8	Vortex and centrifuge.
7	PCR Reaction Mix Preparation	7.1	Add 30 uL of mineral oil to each well of a 96-well amplification plate.
		7.2	Thaw primers and probe to room temperature and vortex.
		7.3	Add the following to a labeled 1.5 mL screwcap tube: 50 uL/rxn Buffer 52 0.5 uL/rxn Forward primer (VWNVA1) 0.5 uL/rxn Reverse primer (VWNVA2) 1.0 uL/rxn Probe (WNV PROBE) 1.0 uL/rxn FastStart Taq
		7.4	Add 50 uL of reaction mix to each well of amplification plate.
		7.5	Add 25 uL of sample to each well.
		7.6	Centrifuge plate at 1600 rpm for 1 min
8	Set up of Roche 480	8.1	Push button on instrument to open plate holder drawer. Both lights have to be steady green. Place plate in the instrument and close the plate holder drawer.
		8.2	Go to Overview screen. Click on “New Experiment” button.

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		8.3	From drop-down menu choose “Dual Color Hydrolysis Probe.”
		8.4	Choose “Apply Template” then select template “PCR with Probes” in the Run Template folder. Cycle conditions are: 1 cycle of 95°C for 1 min followed by 45 cycles of 95°C for 30 sec and 56°C for 1 min.
		8.5	Save in WNV folder and hit “Start Run” button.
9	Analysis on Roche 480	9.1	Click on “Analyze” button.
		9.2	Select “Absolute Quant/Fit Point.”
		9.3	Adjust the threshold bar, moving it above the background signal.
		9.4	Choose dye for signal such as “FAM” for WNV probe.
		9.5	Click on “Calculate.”
		9.6	Click on “Save” (floppy disk icon on right side).
		9.7	Click on “Report” and choose parameters to be reported.
		9.8	Click on “Generate.”
		9.9	Print report by clicking printer icon on top left of the report generated.

KINETIC PCR AMPLIFICATION LAYOUT AND CONDITIONS

Project Name_____ Specificity_____

Experiment Title_____ Date of Amplification_____ By_____

Notes/Comments:

A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12
B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12
C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12
D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12
F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12
H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12

Kinetic Software

File Saved As_____

Temp	Time
95 C	10 min
95 C	30 sec
___C	30 sec
72 C	45 sec

Threshold value:_____

Normalization Range:_____ to _____

Reaction Mix

Buffer Number_____ Date/Initial of Buffer Prep_____

# of Samples_____

Total Buffer Volume Req._____

	Conc.		Lot#	Vol.
dNTPs	50 uL/mL		_____	_____
Primer A___	_____	_____	_____	_____
Primer B____	_____	_____	_____	_____
Syber Green	0.15uL/rxn		_____	_____
Dilution 1:400 FastStart	0.14uL/rxn		_____	_____

Relative distribution of West Nile virus RNA in blood compartments: implications for blood donor nucleic acid amplification technology screening

Lori Lai, Tzong-Hae Lee, Leslie Tobler, Li Wen, Ping Shi, Jeff Alexander, Helen Ewing, and Michael Busch

BACKGROUND: Despite implementation of targeted individual-donor nucleic acid test (NAT) screening of blood donors for West Nile virus (WNV), three "breakthrough" WNV transfusion transmission cases were reported (2004-2008), suggesting that current plasma-based assays are unable to detect all WNV-infectious donations. A 2007 report found that 19 of 20 red blood cell components from WNV-infected donors contained 1 log higher viral load than plasma components. This study's aim was to further establish the value of screening whole blood relative to plasma for WNV RNA by generating differential viral loads on paired samples derived from blood screening tubes.

STUDY DESIGN AND METHODS: WNV RNA–positive donors identified by routine NAT screening were enrolled and quantitative viral data were generated using cross-sectional (index-donation) and longitudinal (follow-up) specimens. A real-time reverse transcription–polymerase chain reaction viral load assay was used on both study sample sets and replicate qualitative NAT screening assays were also used on the longitudinal study samples.

RESULTS: For the cross-sectional study, seronegative index donations (n = 29) had WNV RNA concentrations fourfold higher in plasma than in whole blood, whereas for seropositive donations (n = 13), the WNV RNA concentrations were 10-fold higher in whole blood than in plasma. All 10 longitudinal study participants were seropositive throughout the follow-up study; whole blood viral load was consistently greater than plasma viral load (mean difference, 343 copies; $p < 0.001$) up to 200 days after index.

CONCLUSION: The improved sensitivity of WNV NAT using whole blood instead of plasma was confirmed, but appears to be limited to better detection in seropositive stages. However, the implication of these findings for blood screening requires further study to establish the infectivity of persistent whole blood viremia.

In 1999, West Nile virus (WNV), a mosquito-borne flavivirus, was reported as the causative agent linked to a cluster of viral encephalitis cases in Queens, New York.[1] The first WNV transfusion-transmitted infection was observed in 2002.[2] Later that year, 22 additional transfusion-transmitted infections were confirmed.[3]

After these transfusion transmission reports, the Food and Drug Administration (FDA) asked blood screening test manufacturers to develop WNV nucleic acid amplification technology (NAT) assays to detect infected donors at the earliest seronegative stages.[4] By July 2003, two investigational qualitative WNV NAT assays were released, and by July 2005, more than 1000 viremic blood donors detected by these assays were reported.[3]

WNV NAT screening was initially implemented in minipool (MP) test formats employed for NAT screening for other viruses.[5] Cost-effective and logistically advantageous, MP-NAT screening involved pooling specimens from 6 to 24 donors and screening the combined specimen pool for viral RNA.[6] After six transfusion transmission cases linked to MP-NAT screened donors

ABBREVIATIONS: BSRI = Blood Systems Research Institute; Ct = cycle threshold; ID = individual donation; MP = minipool; S/CO = signal to cutoff; TMA = transcription-mediated amplification; WNV = West Nile virus.

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were reported in 2003, "triggering" strategies were developed and implemented that involved switching from MP-NAT to more sensitive individual-donation (ID) NAT when criteria indicating a higher risk of WNV infection in donors were met.[7] In addition, systems were developed to enable regional communication of WNV yield data among donor centers to support rapid triggering of ID-NAT when adjacent regions experience epidemic activity. Despite implementation of targeted ID-NAT, three more WNV transfusion-transmitted infections were reported between 2004 and 2008; all were linked to MP-NAT screened donations that were missed because ID-NAT triggering criteria were not reached at the screening sites.[7,8]

One approach to reducing WNV transfusion transmission risk could be to develop an alternative specimen preparation protocol that would increase the clinical sensitivity of current WNV NAT screening assays. In 2007, FDA investigators found an order of magnitude greater viral loads in the red blood cell (RBC) components collected from WNV-infected blood donors compared to that in the donors' corresponding plasma components.[9] This FDA study also reported that RBC-associated WNV was infectious in Vero cell cultures and another report from this group indicated that WNV antibody–positive specimens could also infect cells in vitro.[10]

Typically intended for transfusion, blood components are generally not initially screened for pathogens. Instead, plasma samples from vacutainer blood collection tubes collected at the time of donation are routinely screened. The 2007 FDA report suggested that further research exploring the use of whole blood as a screening sample is needed, potentially enhancing the extraction of cell-associated viral nucleic acids.[9] To this study group's knowledge, no research has been conducted to develop a blood donor screening method for WNV RNA in whole blood samples derived from blood collection tube specimens.

This study's objective was to further establish the sensitivity of screening whole blood relative to plasma for WNV RNA by generating differential viral loads using paired whole blood and plasma samples prepared from blood collection tubes instead of blood components. Our hypothesis was that, consistent with the 2007 FDA report, we would find 1 log higher viral loads in whole blood compared with plasma.[9] We also expected to see variations in the whole blood and plasma viral loads according to the stage of infection as defined by serology status, similar to our recent findings of compartmentalization in parvovirus B19 in infected blood donors.[11]

MATERIALS AND METHODS

Blood donor populations

This retrospective analysis was designed to gather quantitative viral data on cross-sectional and longitudinal specimens from WNV-infected donors that had been processed and stored in a repository at Blood Systems Research Institute (BSRI). The cross-sectional portion of the study involved using a previously described real-time reverse transcription–polymerase chain reaction (RT-PCR) assay to quantify viral loads in plasma and whole blood samples derived from specimens collected from donors on the index donation or index date.[12] The cross-sectional study samples were from donations that had been confirmed positive for WNV after reactive WNV RNA screening using a transcription-mediated amplification (TMA) NAT assay.[13] This TMA assay was used for blood screening at Creative Testing Solutions (Tempe, AZ). Viral loads were also determined in longitudinal follow-up whole blood and plasma specimens from enrolled participants whose WNV infections were confirmed by TMA reactivity at index and later seroconversion.

All samples were tested "blind" with no donor identification or outcome information and no data on bleed sequence or predicate WNV results provided during the testing process. Longitudinal study participants signed an informed consent form approved by the University of California at San Francisco Committee on Human Research to allow blood specimens to be collected during specific time intervals after their index date. This study's research protocol is compliant with the Declaration of Helsinki principles and was also approved by the A.T. Still University Mesa Institutional Review Board.

Serologic assay

Stage of infection for both the cross-sectional study participants and the longitudinal study participants was established at each specimen collection date using anti-WNV immunoglobulin (Ig)M and IgG immunoassays (Focus Diagnostics, Cypress, CA). The specimens were divided into seronegative (IgM negative and IgG negative) and seropositive (IgM positive and/or IgG positive) groups.

Sample preparation

Whole blood and plasma samples derived from specimens collected from both study groups were prepared by BSRI previous to this investigation. Anticoagulated blood specimens in collection tubes containing ethylenediaminetetraacetate (EDTA) were centrifuged at $872 \times g$ for 10 minutes. Plasma was removed and divided into aliquots for storage in a biospecimen bank at –70°C. The buffy coat was resuspended with the remaining RBCs and plasma, and this volume, now called the whole blood sample, was also divided into aliquots and stored.

Sample aliquots from the longitudinal study group were further processed for replicate qualitative NAT testing using the TMA assay. These follow-up sample aliquots were thawed and diluted 1:5 by adding four parts

of phosphate-buffered saline (PBS) to one part of whole blood or plasma. The samples were diluted to prevent the TMA inhibition that would likely occur if undiluted whole blood was tested.[14] To directly compare the proportion of replicate TMA assays that were positive using 1:5 diluted whole blood with corresponding plasma aliquots, the plasma was also diluted 1:5 in PBS before replicate TMA testing in parallel with diluted whole blood.

Real-time RT-PCR assay

The WNV RNA real-time PCR assay used in this study was based on methods reported in detail in a US patent.[12] A real-time RT-PCR assay using primer pairs described in the patent was previously validated by testing its ability to detect both US and Ugandan WNV strains.[12]

In this study, the assay protocol involved RNA extraction step using RNA kits (QIAamp, Qiagen, Valencia, CA) using procedures slightly modified from the package insert. (Of note, use of this RNA extraction method enabled testing of whole blood and plasma-derived RNA without predilution of samples before extraction, as was necessary before the target capture step TMA assay.) Real-time RT-PCR used primers and probes that targeted highly conserved sequences within the capsid region or the NS1/NS2 region of the WNV genome. After amplification, the mean cycle threshold (C_t) values from two replicate tests were determined for whole blood and plasma-derived samples processed in parallel.

WNV RNA–positive plasma with a known concentration, originally sourced from an FDA stock of WNV isolate (NY99) culture supernatant spiked into plasma, was obtained from CBER/FDA and used as the standard for viral load testing.[15] This standard was serially diluted into negative plasma or whole blood and replicate tests were performed on the serial dilutions to determine the limit of detection of the real-time RT-PCR assay on both sample types using Probit analysis.

TMA assay

A WNV assay (Procleix, NVD, Emeryville, CA) used in this study is FDA-licensed for donor screening.[13] The assay consists of three major steps including sample preparation with magnetic particle–based target capture, viral RNA target amplification based on TMA technology, and amplification product detection with chemiluminescent probes using a hybridization assay.[13]

To determine the specificity of the TMA assay on PBS-diluted specimens, 15 paired whole blood and plasma specimens were collected from donors who were not infected with WNV. These donors were anti-WNV IgM negative and shown to have no detectable WNV RNA in their plasma and whole blood using the real-time RT-PCR assay. Their specimens were diluted with PBS using the above protocol and tested in replicates along with the diluted follow-up specimens with the TMA assay using the automated NAT instrument platform (Procleix TIGRIS, NVD).

Statistical analysis

For the cross-sectional study specimens, the difference in C_t values (ΔC_t) between whole blood and plasma was calculated and used to estimate the differential viral load in the sample types. A ΔC_t of 3.3 is equivalent to a 10-fold viral load difference. The Mann-Whitney rank sum test (JMP, Version 7, SAS Institute, Inc., Cary, NY) was used to assess the difference in ΔC_t between the seronegative and seropositive groups—all tests were two-tailed ($t = 0.05$). In addition to calculating the proportion of replicate TMA-reactive results on the diluted whole blood and plasma samples at serial time periods after the index donation date, the data analysis for the longitudinal cohort also included plotting viral load data relative to time postindex donation date and serology results.

RESULTS

Probit analysis established that the 50 and 95% detection limits for the WNV RNA real-time RT-PCR assay were 9.5 and 73.7 copies/mL, respectively (Table 1). The detection limits for whole blood were 15.5 copies/mL (50%) and 89.0 copies/mL (95%). Since there were only one or two viral load levels that were within the range of 0% to 100% positive rate, our replicate dilution data did not contain

TABLE 1. WNV detection limits determined by Probit analysis using a CBER/FDA WNV standard dilution panel with a real-time RT-PCR assay

Dilution matrix	Copies/mL	Number reactive/ number tested	% Reactive	50% detection limit in copies/mL*	95% detection limit in copies/mL*
Plasma	10^5-10^2	16/16	100	9.5	73.7
	10^1	7/16	44		
	10^0	1/18	6		
Whole blood	10^5-10^2	16/16	100	15.5	89.0
	10^1	5/16	31		
	10^0	0/18	0		

* Determined by probit analysis.

TABLE 2. C_t values of paired whole blood (WB) and plasma (PL) specimens in the WNV cross-sectional study obtained using a real-time RT-PCR assay*

Antibody stage	IgM–/IgG– (n = 29)	IgM+/IgG– (n = 3)	IgM+/IgG+ (n = 9)	IgM+/IgG+ (n = 1)
WB WNV RNA (% detectable)	83	100	89	100
PL WNV RNA (% detectable)	86	0	11	0
WB C_t (mean)	37.2	36.3	36.9	34.9
PL C_t (mean)	35.2	40.0	39.9	40.0
ΔC_t = plasma C_t – whole blood C_t	–2.0	3.7	3.0	5.1
p value	0.0002	0.06	0.002	NA†
WB WNV fold difference vs. PL	–4	+12	+8	+34

* All specimens collected on the index date. All C_t values 40 or greater are indicated as 40 (undetectable).
† Not applicable; unable to calculate p value due to data from only one participant.

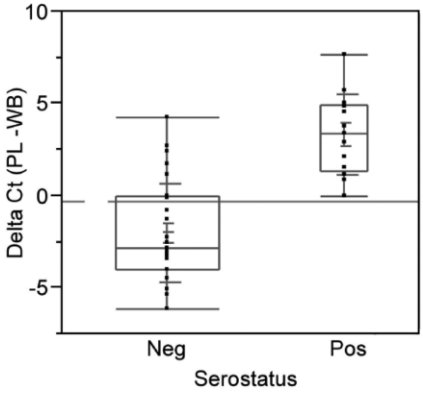

Fig. 1. Box-and-whisker plot of the ΔC_t values (plasma C_t – whole blood C_t [PL – WB]) based on serology status, negative (IgM– and IgG–, n = 29) and positive (IgM+ and/or IgG+, n = 13) for the cross-sectional study. The ΔC_t values are plotted on the y-axis and the serologic status (Neg and Pos) is plotted on the x-axis.

enough information to estimate confidence intervals (CIs) for these limit of detection values. All fivefold diluted whole blood samples from 15 non–WNV-infected donors tested nonreactive by replicate TMA assays, indicating that the TMA assay demonstrated 100% specificity when used with this sample set.

The cross-sectional study results revealed that the viral load was fourfold lower in whole blood compared to plasma in seronegative donor specimens (29/42), indicating that the virus was mainly suspended in the plasma and not cell associated during the viremic seroconversion stage of infection (Table 2). In contrast, we observed an approximately 10-fold higher viral load in whole blood versus plasma in seropositive index donation specimens (13/42). As Fig. 1 indicates, several seronegative specimens (3/29) showed slightly higher viral loads in whole blood compared to plasma, although this differential viral load distribution was less than that seen in whole blood relative to plasma samples from WNV-seropositive specimens.

All 10 longitudinal study participants were seropositive throughout follow-up, and their viral load results showed that more WNV RNA was typically detected in whole blood (mean difference, 343 copies; $p < 0.001$) than in plasma up to 200 days after index (Fig. 2). Plasma viral load levels were detectable only within 15 days after index for five of the participants, while the other five participants had no plasma viremia detectable by the real-time RT-PCR assay during their entire follow-up period. For six participants with follow-up specimens collected at least 90 days after index, five had detectable WNV RNA in whole blood to 3 months after index donation. All five study participants for whom sixth month specimens were available tested negative for WNV RNA in both the whole blood and the plasma compartments; by this point their IgG levels had reached a plateau while their IgM levels were decreasing or under the cutoff value of 1. As the IgM signal-to-cutoff (S/CO) values decreased, the whole blood viral load generally decreased (Fig. 2). Figure 3A presents a summary of the more sensitive qualitative RNA screening test results based on an mean percentage of reactive TMA tests out of five replicates performed on the diluted whole blood and plasma samples derived from specimens collected during six follow-up date ranges. These data confirm that there was a higher probability of detecting WNV in whole blood compared to plasma up to 3 months after index. The viral load mean for the same six follow-up date ranges show a pattern similar to that of the replicate TMA results (Fig. 3B).

DISCUSSION

Our retrospective study, using tube-derived specimens, revealed significantly higher viral loads in plasma than whole blood during the seronegative stage, in contrast to an earlier study that demonstrated higher levels of WNV RNA in RBC components compared to plasma irrespective of donation viral load or serologic status.[9] Although these different results may be due to a number of varying factors, the persistent whole blood viremia detected in this study may also present an opportunity for further studies exploring the diagnostic implications of this finding.

In the earlier study, confirmed WNV RNA–reactive samples from leukoreduced RBC units and their corresponding components were used—the RBC samples

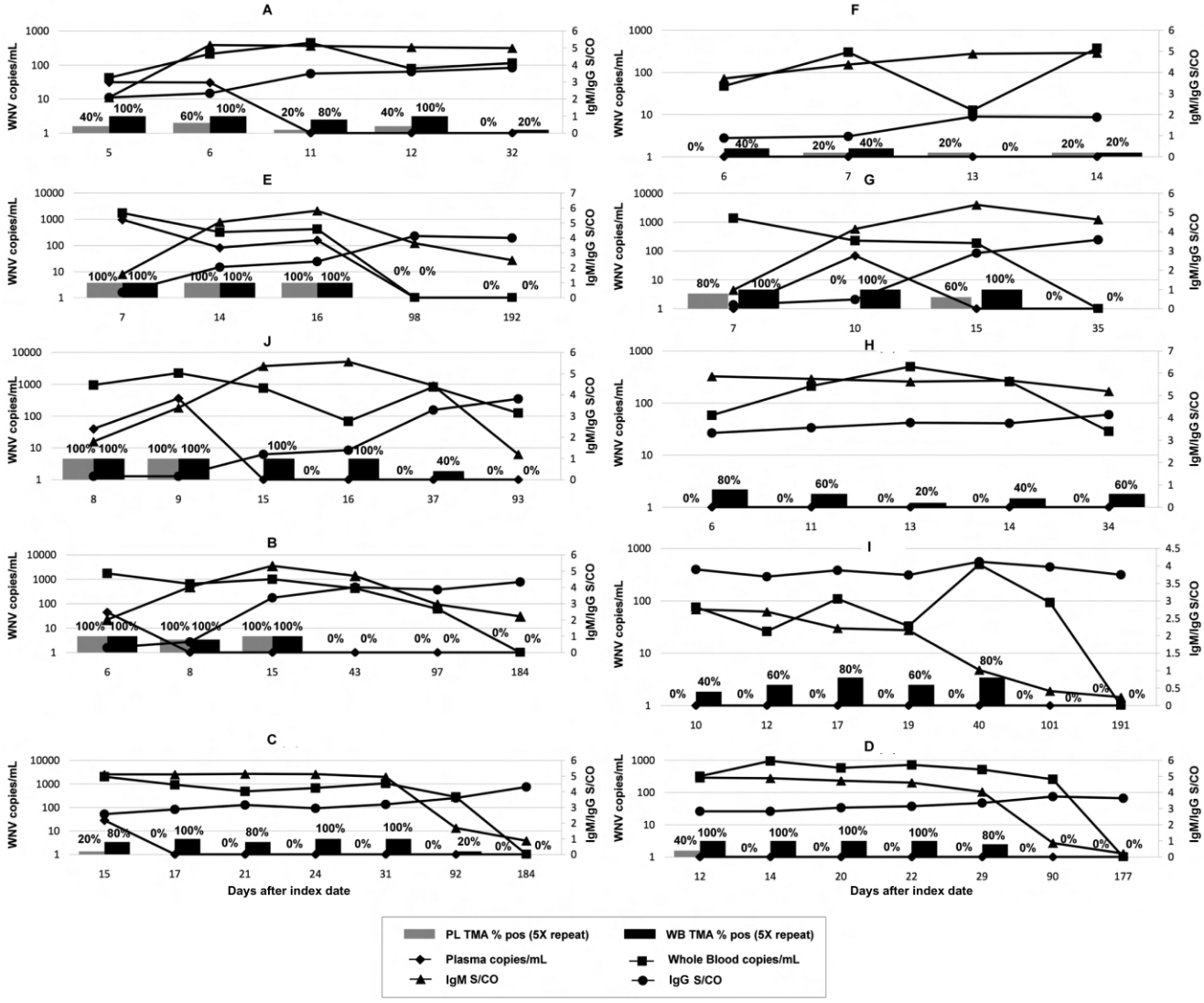

Fig. 2. WNV RNA concentration and serologic status over the follow-up period after index donation date for each of the 10 WNV-infected blood donors who participated in the longitudinal study. The corresponding plasma and whole blood WNV RNA concentrations in copies/mL is plotted on the left y-axis. IgM and IgG S/CO ratio is plotted on the right y-axis. The days after index donation date is plotted on the x-axis. The percentage of diluted plasma and whole blood samples that were TMA positive out of five replicate tests is indicated above the gray (plasma) and black (whole blood) bars above each follow-up collection day. The 10 participants are assigned a letter from A to J.

derived from fresh blood components were washed twice before extraction with Trizol (Life Technologies Corporation, Carlsbad, CA), whereas plasma was extracted with QiaAmp. In our study, the whole blood and plasma samples were prepared from blood donor specimen tubes routinely used for screening and then frozen and thawed before viral load quantification. The thawed whole blood in our study contained lysed RBCs, white blood cells (WBCs), and platelets (PLTs) while the leukoreduced RBC units most likely contained a greater amount of intact RBCs along with a minimal amount of WBCs and PLTs before sample processing for RT-PCR. The impact of starting with thawed, lysed RBCs, in addition to more WBCs and PLTs in our unwashed whole blood samples on real-time RT-PCR results relative to the RBC samples used in the earlier study, was not clearly understood. No specific stability data regarding WNV RNA in frozen whole blood samples could be found after a literature search; however, an earlier human immunodeficiency virus Type 1 RNA stability study found no significant change in mean viral loads in EDTA-anticoagulated plasma samples for at least 6 months when processed promptly and stored at –70°C.[16] The longitudinal sample pairs were thawed and tested at least 1 year after they were prepared and frozen so it may

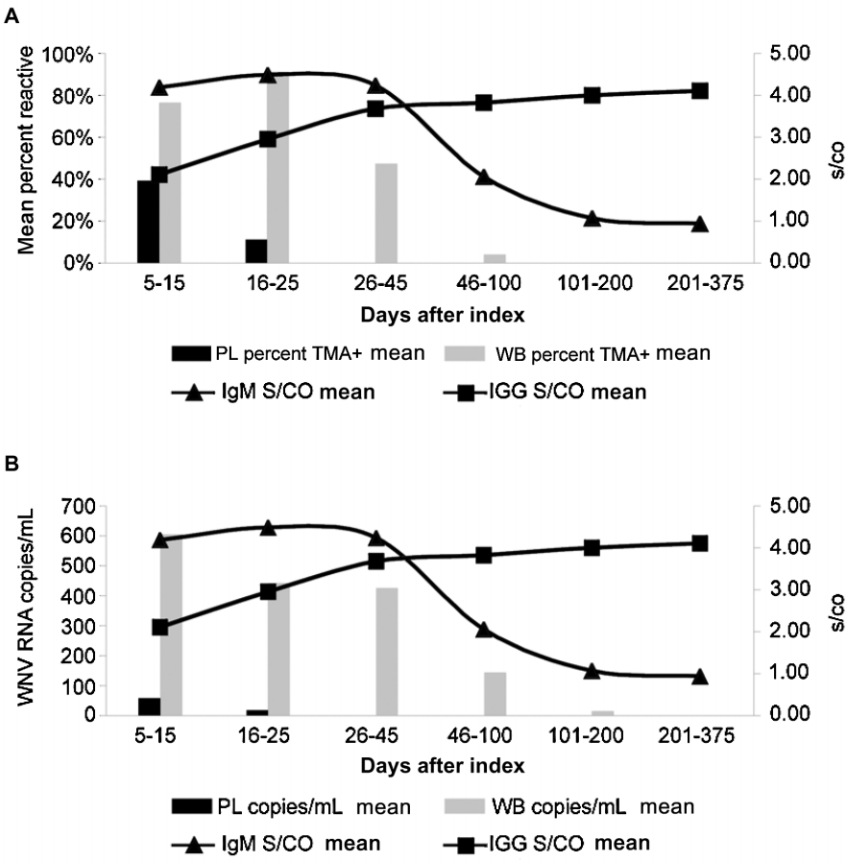

Fig. 3. (A) Mean percentage of TMA-reactive (Procleix WNV Assay, Novartis Diagnostics) for all particpants' corresponding plasma (PL) and whole blood (WB) samples collected at designated time periods after index and the mean S/CO value for IgM and IgG for those samples over the same time periods. The mean percentages of TMA reactive (+) plasma and whole blood 1:5 diluted samples are plotted on the left y-axis as bar graphs and the mean S/CO values for IgM and IgG are plotted on the right y-axis. The time periods (5-15, 16-25, 26-45, 46-100, 101-200, and 201-375 days) of the follow-up collection days after index donation are plotted on the *x*-axis. (B) WNV RNA concentration (real-time RT-PCR assay used at BSRI) for all participants' corresponding plasma and whole blood samples collected at designated time periods after index and the mean S/CO value for IgM and IgG for those samples over the same time periods. The mean copies/mL for neat whole blood and neat plasma are plotted on the left y-axis as bar graphs and the mean S/CO values for IgM and IgG are plotted on the right y-axis. The time periods (5-15, 16-25, 26-45, 46-100, 101-200, and 201-375 days) of the follow-up collection days after index donation are plotted on the x-axis.

have been possible that some RNA degradation occurred in the both the plasma and the whole blood samples during storage; however, we could not demonstrate this because the whole blood and plasma samples were not tested for viral loads before being frozen. Per our protocol, our objective was to test samples without introducing further preservatives that could impact the findings of the study.

In our study, RNA extraction was done with RNA kits (QIAamp, Qiagen). We speculated that the different extraction methods might have resulted in different RNA extraction efficiencies, which may at least partially explain the different findings between the two studies. One study reported with foot-and-mouth disease virus spiked plasma samples, the Trizol extraction procedure detected 1 log less viral RNA 10^{-8} dilution compared with 10^{-7} dilution) than other extraction methods, including QIAamp RNA kit, using one RT-PCR assay. With another assay, the QIAamp RNA kit was approximately 2 log more sensitive than the Trizol method.[17]

Another possibility for the contrasting findings may be the different primers and probes used in each study. It is possible that primers and probes used in the earlier study could have been more efficient in detecting WNV RNA in RBC samples than those used in this study. If this could be demonstrated in a future study, the consistently higher RBC viral loads found in the earlier study, regardless of serostatus, could be explained.

Although there are no data to indicate that the lower percentage (83%) of seronegative samples with detectable whole blood viremia compared to that of plasma viremia (86%) was due to false-negative results, one of our current study's limitations was that there was no internal control in our real-time RT-PCR assay (the WNV TMA assay used in our study did include an internal control that had to be reactive for the results to be considered valid and included in our analysis; no such invalid results were observed with the 1:5 diluted whole blood and plasma samples tested from WNV-positive or control donor specimens). For real-time RT-PCR assays without a negative control, detecting false-negative results may be less likely compared to assays with an internal control. No internal control was used in the real-time RT-PCR assay in this study due to technical challenges associated with validating and optimizing the internal control input value. Future research using our real-time RT-PCR assay may include optimizing this assay to incorporate an internal control.

Our real-time RT-PCR assay's 95% limit of detection, established by probit analysis, was higher for whole blood (89.0 copies/mL) compared to plasma (73.7 copies/mL)

but it is not clear if this difference was significant since CIs could not be estimated. Future studies may must include more replicate testing of viral load levels between 10^2 and 10^0 copies/mL to obtain better limit of detection estimates and associated CIs for our real-time RT-PCR assay. Another published real-time RT-PCR assay was shown to reliably detect WNV RNA at a concentration of 10 to 30 copies/mL[18] so there may be opportunities to further improve the analytical sensitivity of our assay in future studies.

In our study, the viral loads in whole blood were approximately 1 log higher compared to plasma after seroconversion. Similar to the findings in our recent study of parvovirus B19 viremia in donors, WNV RNA levels varied with infection stage.[11] A potential explanation for the detection of higher viral loads in the plasma samples relative to the whole blood samples in seronegative donations is that without induced IgM specific for WNV, the dissemination of virus into the plasma compartment cannot be significantly slowed.[19] Again, similar to our recent study of Parvovirus B19,[11] whole blood WNV levels decreased considerably as soon as IgM became undetectable by the sixth month postindex follow-up collection date. Potential explanations of these findings could include WNV being preferentially bound to RBCs when the virus is present in IgM immune complexes, more WNV tending to bind at higher plasma concentration due to steric effects on receptor-mediated binding or WNV being present in high levels within a subset of peripheral blood RBCs in earlier compared to later stages of WNV infection. The last hypothesis is interesting because no sixth month follow-up whole blood sample was WNV RNA reactive and all available third month samples had detectable WNV RNA, which would be consistent with the 120-day survival period for RBCs.

From our study, we surmise that the clinical value of screening whole blood instead of plasma for WNV RNA may be limited to better detection during the seropositive stages that have shown little to no transfusion transmission risk to date. Only one seropositive (IgM-positive/IgG-negative) donation with a low plasma RNA load has been implicated in the 32 WNV transfusion transmission cases documented by the CDC.[10] Our group reported that in 2007, 9 of 34 ID-NAT only yield cases were IgM-positive/IgG-negative donations.[7] Another group had reported that in 2003 to 2004, a median viral load of 100 copies/mL was detected in 143 anti-WNV IgM-positive donations (including both donations without and with WNV-specific IgG).[6] Two inconclusive investigations of patients identified as having possible WNV transfusion-transmitted infection involved donors who were IgM positive but had plasma found WNV RNA negative by PCR, findings that are consistent with our observations in this study.[3] Based on the findings of this study, it may be possible to speculate that had the whole blood compartment of these two donors been tested, there is a greater likelihood that WNV RNA would have been detected in this compartment. Although not typically observed to date in vivo, another previous study showed that over 50% (15/28) of WNV antibody–positive samples, including some with high viral loads (8/15) and some with low viral loads (7/15), demonstrated infectivity for Vero cell culture and/or human monocyte-derived macrophage culture.[10]

Persistent whole blood viremia observed in some participants up to 90 days after index, combined with earlier findings of in vitro infectivity of RBC-associated WNV, suggest the need for future studies to evaluate related blood safety implications. Since plasma viremia can only be detected a few days after infection, diagnosis of WNV infection in humans is typically based on serologic test results.[20] Positive serologic results need to be confirmed by viral neutralization studies to rule out the presence of cross-reactive antigens such as Japanese encephalitis complex in the clinical sample; these neutralization studies require a Biosafety Level 3 facility.[20] If a whole blood NAT assay could be developed for diagnostic use, this could complement the currently used diagnostic tools and provide an opportunity for WNV RNA detection for several months instead of several days after infection.

More research is also needed to explore if whole blood screening could enhance the yield of MP-NAT during the early postseroconversion stages, extending the detection window of an active WNV infection for up to 90 days after infection as opposed to the current window period of approximately 6 to 7 days using current testing methods. Further longitudinal studies using a larger number of WNV-infected participants are ongoing to further explore these open research questions.

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CONFLICT OF INTEREST

LL and PS are NVD employees; MB is a Gen-Probe, Inc., and NVD grant recipient; LT, JA, HE, LW, and THL have nothing to disclose.

REFERENCES

1. Lanciotti RS, Roehrig JT, Deubel V, Smith J, Parker M, Steele K, Crise B, Volpe KE, Crabtree MB, Scherret JH, Hall RA, MacKenzie JS, Cropp CB, Panigrahy B, Ostlund E, Schmitt B, Malkinson M, Banet C, Weissman J, Komar N, Savage HM, Stone W, McNamara T, Gubler DJ. Origin of the West Nile virus responsible for an outbreak of

encephalitis in the northeastern United States. Science 1999;286:2333-7.
2. Harrington T, Kuehnert MJ, Kamel H, Lanciotti RS, Hand S, Currier M, Chamberland ME, Petersen LR, Marfin AA. West Nile virus infection transmitted by blood transfusion. Transfusion 2003;43:1018-22.
3. Pealer LN, Marfin AA, Petersen LR, Lanciotti RS, Page PL, Stramer SL, Stobierski MG, Signs K, Newman B, Kapoor H, Goodman JL, Chamberland ME. Transmission of West Nile virus through blood transfusion in the United States in 2002. N Engl J Med 2003;349:1236-45.
4. Montgomery SP, Brown JA, Kuehnert M, Smith TL, Crall N, Lanciotti RS, Macedo de Oliveira A, Boo T, Marfin AA. Transfusion-associated transmission of West Nile virus, United States 2003 through 2005. Transfusion 2006;46: 2038-46.
5. Busch MP, Caglioti S, Robertson EF, McAuley JD, Tobler LH, Kamel H, Linnen JM, Shyamala V, Tomasulo P, Kleinman SH. Screening the blood supply for west nile virus RNA by nucleic acid amplification testing. N Engl J Med 2005;353:460-7.
6. Stramer SL, Fang CT, Foster GA, Wagner AG, Brodsky JP, Dodd RY. West Nile virus among blood donors in the United States, 2003 and 2004. N Engl J Med 2005;353:451-9.
7. Kleinman SH, Williams JD, Robertson G, Caglioti S, Williams RC, Spizman R, Morgan L, Tomasulo P, Busch MP. West Nile virus testing experience in 2007: evaluation of different criteria for triggering individual-donation nucleic acid testing. Transfusion 2009;49:1160-70.
8. Centers for Disease Control and Prevention (CDC). West Nile virus transmission via organ transplantation and blood transfusion—Louisiana, 2008. MMWR Morb Mortal Wkly Rep 2009;58:1263-7.
9. Rios M, Daniel S, Chancey C, Hewlett IK, Stramer SL. West Nile virus adheres to human red blood cells in whole blood. Clin Infect Dis 2007;45:181-6.
10. Rios M, Daniel S, Dayton AI, Wood O, Hewlett IK, Epstein JS, Caglioti S, Stramer SL. In vitro evaluation of the protective role of human antibodies to West Nile virus (WNV) produced during natural WNV infection. J Infect Dis 2008; 198:1300-8.
11. Lee TH, Kleinman SH, Wen L, Montalvo L, Todd DS, Wright DJ, Tobler LH, Busch MP. Distribution of parvovirus B19 DNA in blood compartments and persistence of virus in blood donors. Transfusion 2011 (in press).
12. Shyamala V. Identification of Oligonucleotides for the Capture, Detection, and Quantitation of West Nile Virus. U.S.P. Office. United States of America, Novartis Vaccines and Diagnostics. 2006; US 7,132,233 B2: 28.
13. Linnen JM, Deras ML, Cline J, Wu W, Broulik AS, Cory RE, Knight JL, Cass MM, Collins CS, Giachetti C. Performance evaluation of the PROCLEIX West Nile virus assay on semi-automated and automated systems. J Med Virol 2007;79: 1422-30.
14. Tobler LH, Dockter J, Stramer SL, Kleinman SH, Todd D, Giachetti C, Busch MP. Use of the Procleix HIV-1 and HCV discriminatory assays to detect HIV and HCV RNA in whole blood. Transfusion 2002;42:1525-7.
15. Rios M, Srinivasan K, Wood O, Daniel S, Tomioka K, Williams A, Hewlett IK. West Nile virus: correlation between infectivity and RNA copy number. Transfusion 2003;43:3A.
16. Ginocchio CC, Wang XP, Kaplan MH, Mulligan G, Witt D, Romano JW, Cronin M, Carroll R. Effects of specimen collection, processing, and storage conditions on stability of human immunodeficiency virus type 1 RNA levels in plasma. J Clin Microbiol 1997;35:2886-93.
17. Goris N, Vandenbussche F, Herr C, Villers J, Van der Stede Y, De Clercq K. Validation of two real-time RT-PCR methods for foot-and-mouth disease diagnosis: RNA-extraction, matrix effect, uncertainty of measurement and precision. J Virol Methods 2009;160:157-62.
18. Tang Y, Anne Hapip C, Liu B, Fang CT. Highly sensitive TaqMan RT-PCR assay for detection and quantification of both lineages of West Nile virus RNA. J Clin Virol 2006;36: 177-82.
19. Diamond MS, Sitati EM, Friend LD, Higgs S, Shrestha B, Engle M. A critical role for induced IgM in the protection against West Nile virus infection. J Exp Med 2003;198:1853-62.
20. Gea-Banacloche J, Johnson RT, Bagic A, Butman JA, Murray PR, Agrawal AG. West Nile virus: pathogenesis and therapeutic options. Ann Intern Med 2004;545-53.

Protocol for infection of Vero cells with frozen samples from BSRI

Samples:
For V1 passage:
Refer to sample list sent by BSRI.
Negative control (media)
Positive control (plasma from sample NY03-11)

For M1V1 passage:
Day 7 supernatants from macrophage passage 1 from all samples, including positive and negative controls
Negative control (media)

Media for WNV infection:
MEM, with Pen-Strep and L-glutamine 1:100 (cMEM)

Other materials:
FBS
dPBS w/o Ca++/Mg++
5 ml serological pipets
P1000 and tips
Qiagen buffer AVL with carrier RNA (from kit 52926)
Labeled 15 ml centrifuge tubes

Prepare T-25 flasks to be 70% confluent at the time of infection.
Thaw plasma and whole blood samples to be used for culture at 37º. Warm PBS and culture media to 37º.
Remove culture media and wash once with 3 ml sterile prewarmed PBS. Remove PBS immediately prior to adding CCB or plasma.
For each flask, add 250 ul of thawed CCB or plasma. Rock flask to ensure that cell surface is fully covered by the 250 ul.
Incubate cells for 1 hour at 37º, 5% CO_2, rocking every 15 minutes.
After 1 hour, add 5 ml of prewarmed cMEM + 2% FBS to each flask without removing the inoculum. Return cells to 37º, 5% CO_2.
Remaining plasma or CCB (if any) will be stored at -70 in BSL3.

For infection of Veros with macrophage passage 1 supernatants, the above protocol will be followed, except that a sample volume of 500 ul of supernatant will be used.

On day 7, CPE will be assessed and supernatants will be harvested.
2 x 140 ul aliquots of each supernatant will be mixed with 560 ul Qiagen buffer AVL and stored at -20º until ready to extract. The remainder of each supernatant will be stored at -70º in BSL3. Supernatant samples will be extracted using the Qiagen ViralAmp RNA mini kit (52926) in a QiaCube instrument and eluted in a volume of 50 ul.

Viral loads will be quantified by qRT-PCR using the Applied Biosystems One-Step RNA to Ct kit (cat # 4392938) in an AB Taqman 7300 instrument. The qRT-PCR assays will use primers and probes specific for the WNV 3' noncoding region: WN3ncF, 5′-CAGCCACGCTACGGCG-3′; WN3ncR, 5′-CAGTCCTCCTGGGGCACTA-3′; and WN3ncP, 5′-TCTGCGGAGAGTGCAGTCTGCGAT-3′ and amplification conditions were previously described in (Rios M, et al *Transfusion 2006;46:659-67)*. Samples will be run in a volume of 10 ul, in duplicate in two independent assays for a total of four replicates for each sample. Data will be analyzed using SDS software v. 1.4 (Applied Biosystems).

Protocol for infection of monocyte-derived macrophages (MDM) with frozen samples from BSRI

Samples:
Refer to sample list sent by BSRI.
Negative control (media)
Positive control (plasma from sample NY03-11)

Media for WNV infection:
MEM, with Pen-Strep and L-glutamine 1:100 (cMEM)

Other materials for culture:
FBS
M-CSF (Sigma M6518), 10 ug/500 ml media
dPBS w/o Ca++/Mg++
5 ml serological pipets
P1000 and tips
Qiagen buffer AVL with carrier RNA (from kit 52926)
Labeled 15 ml centrifuge tubes

Plate elutriated monocytes from NIH Division of Transfusion Medicine at 1x10^6 cells/flask in 5 ml cMEM +10% FBS + M-CSF. Cells will be used for infection on days 7 and 8.
Thaw plasma and whole blood samples to be used for culture at 37º. Warm PBS and culture media to 37.
Remove culture media and wash once with 3 ml sterile prewarmed PBS. Remove PBS immediately prior to adding CCB or plasma.
For each flask, add 250 ul of thawed CCB or plasma. Rock flask to ensure that cell surface is fully covered by the 250 ul.
Incubate cells for 1 hour at 37º, 5% CO_2, rocking every 15 minutes.
After 1 hour, add 5 ml of prewarmed cMEM + 5% FBS to each flask without removing the inoculum. Return cells to 37º, 5% CO_2. Most supernatants will gel due to combining plasma/CCB with complete media.
Remaining plasma or CCB (if any) will be stored at -70 in BSL3.

On day 7, cultures will be observed on a phase contrast microscope, supernatants will be harvested (tapping corner of flask gently to remove any supernatants that are still gelled), and cultures fed with 5 ml fresh prewarmed cMEM + 5% FBS. On day 13, cultures will be observed on a phase contrast microscope, and supernatants will be harvested.
2 x 140 ul aliquots of each supernatant will be mixed with 560 ul Qiagen buffer AVL and stored at -20º until ready to extract. The remainder of each supernatant will be stored at -70º in BSL3. Supernatant samples will be extracted using the Qiagen ViralAmp RNA mini kit (52926) in a QiaCube instrument and eluted in a volume of 50 ul.

Viral loads will be quantified by qRT-PCR using the Applied Biosystems One-Step RNA to Ct kit (cat # 4392938) in an AB Taqman 7300 instrument. The qRT-PCR assays will use primers and probes specific for the WNV 3' noncoding region: WN3ncF, 5′-CAGCCACGCTACGGCG-3′; WN3ncR, 5′-CAGTCCTCCTGGGGCACTA-3′; and WN3ncP, 5′-TCTGCGGAGAGTGCAGTCTGCGAT-3′ and amplification conditions were previously described in (Rios M, et al *Transfusion 2006;46:659-67)*. Samples will be run in a volume of 10 ul, in duplicate in two independent assays for a total of four replicates for each sample. Data will be analyzed using SDS software v. 1.4 (Applied Biosystems).

10. WNV DATABASE MANAGEMENT SYSTEM

The WNV database management system will be handled through Freezerworks for the Specimen Tracking system and through MYSQL for the database related to the specimen characterization.

10.1 Tracking system - Freezerworks :

Freezerworks is a relational database designed for the tracking of repository aliquots. All created aliquots in the WNV arm of this project will be tracked using Freezerworks.

10.2 Database system – *MYSQL database system*:

10.2.1 System Overview

This database management system will be established to handle all data related to the characterization of the samples. This will include data from symptom questionnaires, viral (viral loads, TMA reactivity, genotyping, infectivity studies), and immunologic parameters (cytokines and chemokines). At the end of the project, the system will be able to accept all data exported from Freezerworks if necessary. It provides a convenient and powerful platform for our researchers to integrate data, assays, and various data analyses and approaches on immunology, epidemiology, and virology (Figure 1).

The system has two functions: data storage and data analysis. It consists of two major components: (1) **MYSQL database system** for data storage with various features such as data entry, edit, modification, data import, data export, etc. The Freezerworks tracking system can be integrated into the MYSQL database. (2) **Web interface** for data analysis, such as data processing, data mining, and various statistical analyses. It includes different types of computing tools such as scatter plot, regression, anova, and other analyzing and visualizing applications. A pilot system has been developed for the database management.

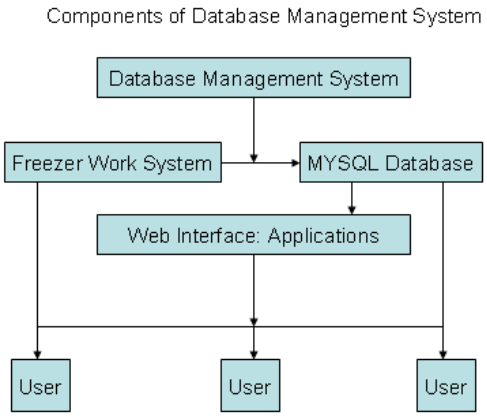


Figure 10A. Relations between components. Users access database (including Freezer work tracking system) by web interfaces or Linux system.

10.2.2 MYSQL Database

MYSQL is used for the database system as an extended database protocol, containing almost all features of SQL and Access that can be easily linked to web interfaces, web-based applications, R/BioConductor and other computational software. Besides, it is free of charge for license. The database interfaces may look like the following with options in interface for parameters with various purposes (Figure 2).

Cytokine Database and Analysis

Database Disease Cytokine Data Log Yes Imputing No Submit

No	ID	Disease	CD4	Race	Age	HCV	IL-1beta	IL-2	IL-4	IL-5	IL-6	IL-7	IL-8	IL-10	IL-12(p70)	IL-13	IFN-gamma	GM
1	10100242	HIVNeg	1307	AAm	57	HCV.Pos	0.86	9.75	0.07	0.31	3.6	3.36	57.01	7.5	0.07	2.15	1.64	2.64
2	10100521	HIVNeg	705	AAm	37	HCV.Neg	0.14	3.91	0.07	0.11	2.81	7.12	24.84	9.57	1.66	1.86	2.4	1.7
3	10100622	HIVNeg	1054	AAm	41	HCV.Pos	1.21	21.77	355.11	1.35	40.08	7.41	105.26	12.85	6.5	151.48	22.39	5.59
4	10205484	HAART	601	AAm	42	HCV.Pos	0.56	0.07	0.07	0.07	8.22	5.45	203.29	1.31	0.07	0.07	0.07	1.65
5	10310259	HAART	785	AAm	34	HCV.Pos	0.07	0.4	8.47	0.57	5.15	6.11	25.34	12.54	0.26	8.22	0.51	0.29
6	10310817	HIVNeg	688	NonAAm	53	HCV.Neg	0.39	1.03	170.44	0.84	10.8	7.95	636.31	7.17	0.18	50.68	1.64	5.97
7	12121449	HAART	788	AAm	48	HCV.Pos	0.07	0.51	0.07	0.16	1.8	4.79	15.05	27.23	0.9	0.07	0.72	0.17
8	12121637	NC	825	AAm	33	HCV.Neg	0.07	0.07	0.07	0.15	3.13	4.86	22.17	26.6	0.07	0.07	0.07	0.16
9	12330237	HIVNeg	703	AAm	40	HCV.Neg	0.1	0.07	0.07	0.07	2.6	12.9	8.64	5.28	0.22	1.87	0.07	0.26
10	20100244	HAART	1130	AAm	35	HCV.Neg	0.07	0.07	0.07	0.25	1.03	5.95	6.41	3.34	0.07	0.07	0.07	0.14
11	20100915	NC	569	AAm	34	HCV.Neg	0.58	3.47	10.42	0.6	5.32	7.54	6.81	10.1	0.18	9.18	17.24	1.5
12	20102527	HAART	553	NonAAm	28	HCV.Neg	6.09	84.83	0.32	0.07	1.39	20.77	4.04	5.65	0.5	0.45	49.34	18.6

Figure 10B. Database management interface

Freezer work tracking system This system is an important part of the database management system. It contains the records of all samples. The records include locations, dates, sample types, lab information, enrollment status, visit status, active visits, and so on. The information can be

imported into the MYSQL database system through web interfaces and then can be traced by users through MYSQL databases and the web interfaces.

The database can be accessed in three ways. (1) One is the standard Unix/Linux-based MYSQL command system. Queries can be made through general MYSQL query functions or programming. (2) The second one is PhpMyAdmin, which is web-based software for creating and maintaining MYSQL databases. People need certain training for using these two methods to access the database system. (3) The third way is our web interface methods specially designed for non-professional users who have no or very little training on database applications, data entry, edit, import/export data with other databases.

10.2.3 Data security

A formal database policy system will be set up for the database management. Data in the database system are password protected and can only be accessed by registered users with user names and passwords. General database security policy will be applied in the database management. Non-users can not access the database system. Regular users can only read the data. All data can only be edited and changed by designed database managers. Data are owned by their owners, who can control whether the data can be shared, read or modified by other users.

Computers linked to the database system will be password protected with automatic timeout mechanism. User system will be setup for the database management. All users are organized into different levels (each of which may have different levels to access the data) and different groups based on their professional backgrounds and research interests. Research group or news groups can be setup by users for research purpose.

10.2.4 Data exchange

Data exchange is one of the major features of our database (Figure 3). It is time-consuming for people to import or export data from or to other databases. Applications are designed to deal with the issue. Text or Excel files will mainly be used for data exchanges.

At the end of the project MYSQL will be able to accept all data from the Freezerworks tracking system regarding the location of the biospecimen (recording locations, dates, sample types, lab information, etc.). The information can be imported into the MYSQL database system through web interfaces and then can be traced by users through the web. Reports on Freezerworks can be exported from MYSQL database system via the web interfaces.

All data from MYSQL can also be exported to any other database management system.

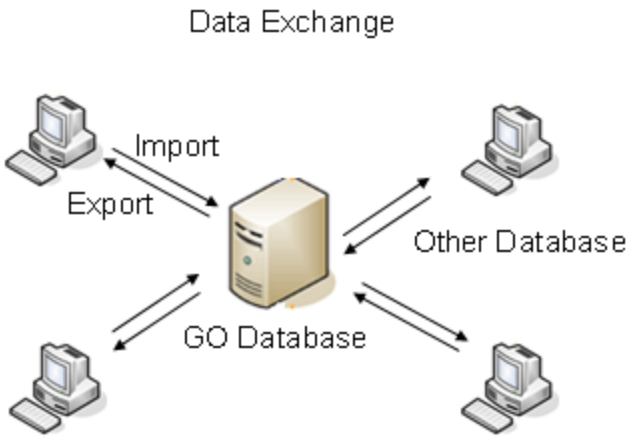


Figure 10C. Data exchange between GO database and other external databases

10.2.5 Data import

An application in Perl, php, and R is developed for data import purpose. Text or Excel files for data sets are put into a folder that is specially setup for data exchange. The program first checks for accuracy and errors for QA/QC issues. Next, it setup the databases or tables for the data files. Then it imports the data from the text files into the databases or tables. Finally, the text files are removed from the folder after data import is finished. Database dictionary or change Log (or history) will be updated so that the changes can be traced in the future. Notices will automatically be sent to users to announce the availability of new data.

10.2.6 Data export

Another similar program is designed for data exports. It provides options for users to export data from databases or tables. The data are saved into text files in a folder setup for data export. Accuracy and errors are also checked and text files will also be removed after this step is finished.

10.2.7 Data storage

All data will be stored in the database system under different databases and tables based on data categories. Database sizes or table sizes depend on practical applications. Although tables may contain a large number of records (MANY rows and columns), we will still organize tables in reasonable sizes for efficient data accessing, data processing, and data analysis. The database management will use not only primary keys, but also other reasonable keys for efficient data accessing purpose. The data will be organized as a data warehouse system.

The data will be backup periodically (e.g. daily or weekly) on the server. Un-used data will be cleaned up periodically.

10.2.8 Data usage

All data are available to registered users with password protection. However, some of the data may become public after certain limit time, e.g. six months, one year, five years, or after the research is published.

Data will be available for inspection by authorized personnel.

Data summary will be reported periodically on data usage and other status on databases and tables.

10.2.9 Reports

Reports on freezer work and samples can be export from MYSQL database system via the web interfaces. Users have various options to manage reports. Report management contains the following main parts: (1) Users access the MYSQL database system, including freezer work tracking system. This can be done by standard MYSQL query functions using Linux operating system or our web interfaces; (2) Users run our report generating applications on the databases; (3) primary reports are generated with information on enrollment, visits, and other data; (4) A validation system then checks for possible errors on the reports. If all information is correct and accurate, the final reports are printed.

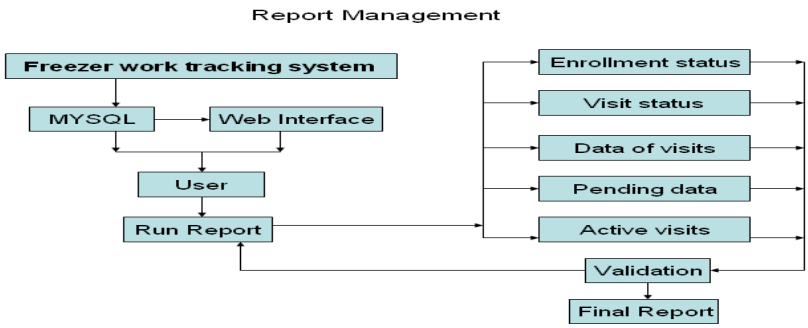


Figure 10D. Report management process

10.3 Web interface

Web interfaces will be an important part in the system and developed in up-to-dated programming languages, such as php, html, javascript, CGI, CSS, XML, etc. Computational applications will be implemented in R, perl, C/C++, and so on for statistical analyses. Essential

web-based applications include data Log chart, bar chart, pie chart, box plot, scatter plot, heatmap, regression, anova, and etc. Other new analysis tools will be periodically designed and available for the community if requested by researchers.

Examples of interfaces from our pilot system with their features are listed in the following. These applications aim to meet our essential needs and provide powerful tools for data analysis in practical applications.

10.3.1 Data Log chart

Data history is displayed across data points for single or multiple variables and subjects.

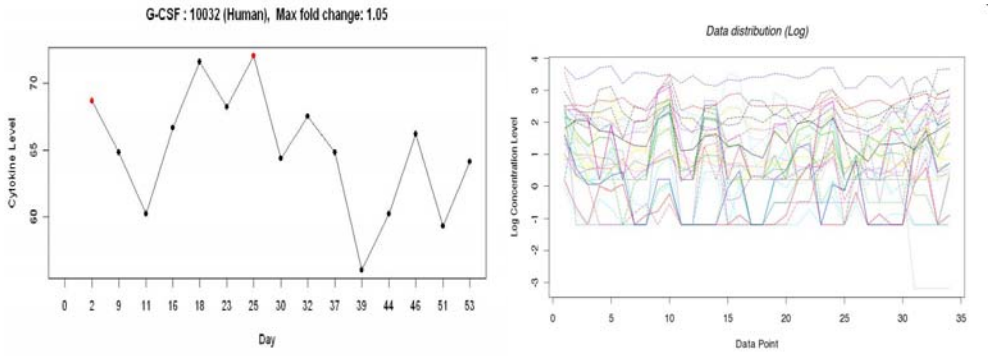


Figure 10E. Concentration levels for one single cytokine (left) or multiple cytokines (right).

10.3.2 Heatmap

Clusters can be defined through heatmaps on cytokines and subjects/patients. Data errors and outliers can be identified through the clusters, which can also be used as a tool for QA/QC issue.

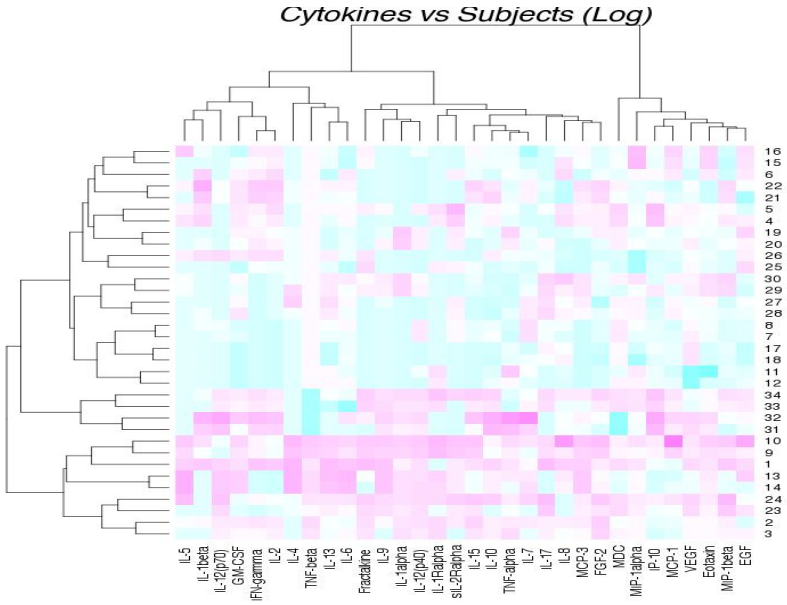


Figure 10F. Heatmap on cytokines and subjects for clusters

10.3.3 Boxplot

Box plot can be generated by our web applications to compare differences between various groups of subjects. It shows significances from Anova for data analysis on groups.

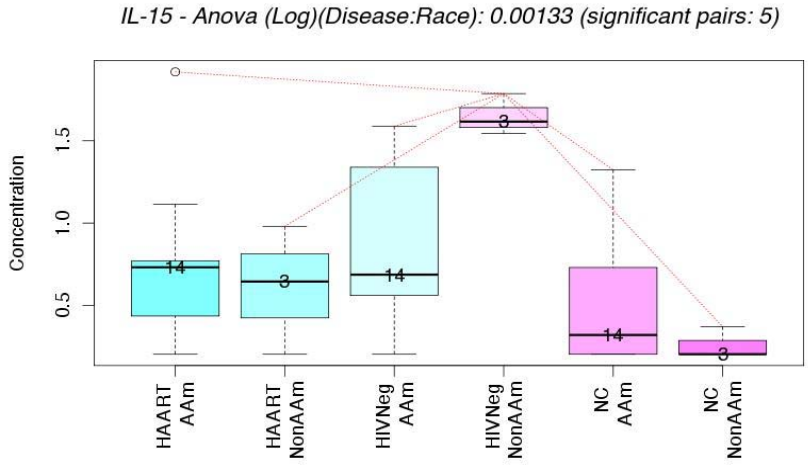
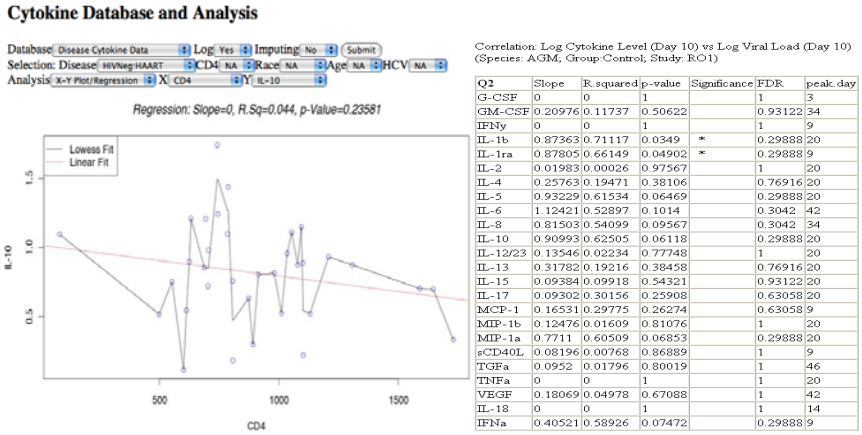


Figure 10G. Boxplot for cytokine groups with links for significant differences

10.3.4 Regression

Our web interface applications provide regression tools to find significant correlations.

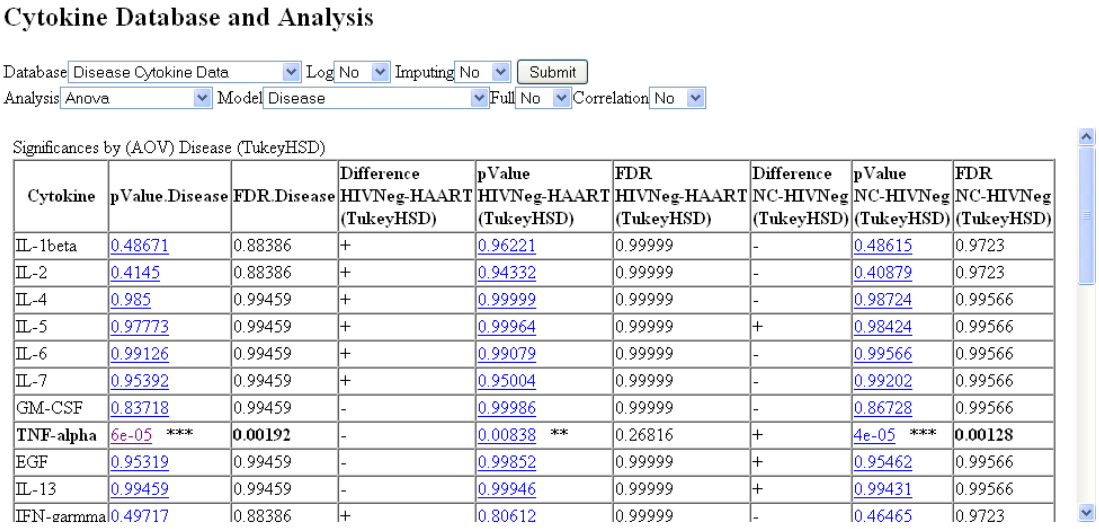


Q2	Slope	R.squared	p-value	Significance	FDR	peak.day
G-CSF	0	0	1		1	3
GM-CSF	0.20976	0.11737	0.50622		0.93122	34
IFNy	0	0	1		1	9
IL-1b	0.87363	0.71117	0.0349	*	0.29888	20
IL-1ra	0.87805	0.66149	0.04902	*	0.29888	9
IL-2	0.01983	0.00026	0.97567		1	20
IL-4	0.25763	0.19471	0.38106		0.76916	20
IL-5	0.93229	0.61534	0.06469		0.29888	20
IL-6	1.12421	0.52897	0.1014		0.3042	42
IL-8	0.81503	0.54099	0.09567		0.3042	34
IL-10	0.90993	0.62505	0.06118		0.29888	20
IL-12/23	0.13546	0.02234	0.77748		1	20
IL-13	0.31782	0.19216	0.38458		0.76916	20
IL-15	0.09384	0.09918	0.54321		0.93122	20
IL-17	0.09302	0.30156	0.25908		0.63058	20
MCP-1	0.16531	0.29775	0.26274		0.63058	9
MIP-1b	0.12476	0.01609	0.81076		1	20
MIP-1a	0.7711	0.60509	0.06853		0.29888	20
sCD40L	0.08196	0.00768	0.86889		1	9
TGFa	0.0952	0.01796	0.80019		1	46
TNFa	0	0	1		1	20
VEGF	0.18069	0.04978	0.67088		1	42
IL-18	0	0	1		1	14
IFNa	0.40521	0.58926	0.07472		0.29888	9

Figure 10H. Regression to identify significant correlations

10.3.5 Anova

Our web interface application also provides convenient anova tools to find significant differences or correlations through various models. Anova is usually followed by Tukey HSD tests to identify significant differences by pairwise comparisons. Detailed tables are generated for information on significance as follows.



Cytokine	pValue.Disease	FDR.Disease	Difference HIVNeg-HAART (TukeyHSD)	pValue HIVNeg-HAART (TukeyHSD)	FDR HIVNeg-HAART (TukeyHSD)	Difference NC-HIVNeg (TukeyHSD)	pValue NC-HIVNeg (TukeyHSD)	FDR NC-HIVNeg (TukeyHSD)
IL-1beta	0.48671	0.88386	+	0.96221	0.99999	-	0.48615	0.9723
IL-2	0.4145	0.88386	+	0.94332	0.99999	-	0.40879	0.9723
IL-4	0.985	0.99459	+	0.99999	0.99999	-	0.98724	0.99566
IL-5	0.97773	0.99459	+	0.99964	0.99999	+	0.98424	0.99566
IL-6	0.99126	0.99459	+	0.99079	0.99999	-	0.99566	0.99566
IL-7	0.95392	0.99459	+	0.95004	0.99999	-	0.99202	0.99566
GM-CSF	0.83718	0.99459	-	0.99986	0.99999	-	0.86728	0.99566
TNF-alpha	6e-05 ***	**0.00192**	-	0.00838 **	0.26816	+	4e-05 ***	**0.00128**
EGF	0.95319	0.99459	-	0.99852	0.99999	+	0.95462	0.99566
IL-13	0.99459	0.99459	-	0.99946	0.99999	+	0.99431	0.99566
IFN-gamma	0.49717	0.88386	+	0.80612	0.99999	-	0.46465	0.9723

Figure 10I. Anova

12. QUALITY ASSURANCE/QUALITY CONTROL AT BSRI

12.1 Overview

As it is critical for repositories to carefully track each of the specimens that is received, processed and disseminated from our facility. Accuracy and timeliness are critical to ensure their effective future use. Systems have been established to verify that all specimens and linked data are handled appropriately.

These systems involve the accurate descriptions of tasks performed documented in Research Operating Procedures (**ROP**s) that have been reviewed. Regular checking of records will be required to ensure that appropriate steps are being followed.

12.2 Quality Assurance / Quality Control programs - Definition:

Quality Assurance (QA) is an integrated system of management activities involving planning, implementation, documentation, assessment, and improvement to ensure that a process, or biospecimen, is of the type and quality expected for the repository.

Quality Control (QC) is the system of technical activities that measures the attributes and performance of a process, or biospecimen, against defined standards, to verify that the stated requirements are fully met.

Requirements: Each repository needs a Quality Assurance Program/Quality Management System (QA/QMS) or adheres to the QA program of the organization with which the repository is associated. The program describes the repository's commitment to its QA and QC programs, and describes approaches for ensuring that the requirements of the QA and QC programs are met. Should it not be possible to have a formal Quality Assurance Program with dedicated staff, a program should be in place to review procedures and records to assess the efficacy and quality of repository operations. This review will be conducted on an annual basis.

12.3 Quality management system

12.3.1 Standard Operating Procedures Manual

Our repository has developed procedures in a standardized written format that have been incorporated into a Standard Operating Procedures (ROP) manual. The

ROPs contain therein define and describe in detail, all procedures. **These ROPs should be utilized to ensure that all samples are appropriately collected and stored so that they are effectively disseminated for subsequent uses.** Research investigators will be able to access and take advantage of the specimen collected as part of this project.

ROPs serve as the description of how tasks pertaining to repository operations should be handled by staff assigned to those specific responsibilities. ROPs will allow for uniformity and reproducibility in specimen handling. ROPs have been written and reviewed before they were finalized. On these ROPs you will find:

- Title – Each ROP has been given a unique name which captures the essence of the practice described.
- Number – Each ROP has been given a unique number that will be used for easy reference. The numbering system should include the revision number for the practice so that the most recent version can be easily identified.
- Date – The date the procedure was first introduced as well as the date of the most recent version. The date format is based on the dd/mm/yyyy system where d represents day, m represents month and y represents year.
- Department/Division/Staff Covered – The individuals to whom the ROP applies.
- Protective Wear – Protective equipment that should be worn by staff when performing the procedure described.
- Equipment – A list and description of the equipment needed to perform the procedure (name, model, serial number, inventory tracking number, and manufacturer).
- Supplies – All materials and supplies should be recorded. The ROP may ask for a record of the lots and expiration dates for the materials and supplies utilized.
- Step-by-Step Guidance – The procedure has been written in specific detail to ensure that the procedure can be repeated in a reproducible fashion to include the order of steps that should be followed, the times allowed for each step (as needed) and the temperatures at which the steps are performed.

12.3.2 Critical Topics covered by the Standard Operating Procedures Manual

You will find in the Research Operating Procedure (ROP) manual the following:

- Specimen handling
- Laboratory procedures for tests performed in-house and any specimen aliquoting or other specimen processing privacy and confidentiality protections, and other legal, ethical and cultural issues.
- Access and sharing of specimens and associated data.
- Shipping and receiving of specimens.
- Records management practices.
- Quality assurance (QA) and quality control (QC) for instruments, reagents, labels, and processes employed in sample collection, processing and retrieval.
- Equipment qualification, maintenance, repair and calibration.
- Safety programs including reporting of near miss incidents, injuries and exposure to potential bloodborne pathogens.
- Investigation, documentation and reporting of incidents, or errors
- Disposal of medical and other hazardous waste.
- Training programs.

12.3.3. Implementation and modifications

Once reviewed and approved, all ROPs and associated procedures should be followed as written starting on the dates of implementation. All procedures have document control policies in place that govern retention and modifications or revisions to ROPs. For each modification, the head of department will have to make sure that only the most current versions of documents are available for use and that previous revisions have been removed when new revisions are issued. ROPs will be reviewed regularly to be sure that the current method for performing the procedure is described.

12.3.4 Staff Access and Review

Current copies of the ROP manual will be stored in designated locations in the lab and available to the staff at all times. New and revised policies and procedures will be reviewed by the staff prior to implementation. Training associated with ROPs will be maintained in the training record

12.4 List of ROPs and procedures

Table 13.1 Checklist of ROPs, procedures, and forms for each department

#	Type	Date implemented	Version control	Topic	Department
VRLRC0001	ROP	11/02/09	Yes	**Preparation of WNV "Ready-to-go-shippers" for the Natural history and pathogenesis of WNV in viremic donor study.**	VRLRC
VRLRC0022	ROP	05/12/10	Yes	**VRLRC Laminar Flow Biological Safety Cabinet maintenance**	VRLRC
VRLRC0002	ROP	11/09/09	Yes	**Sample Collection for the Natural history and pathogenesis of WNV in viremic donors study**	VRLRC
VRLRC0014	ROP	3/29/10	Yes	**Procedure for logging the Receipt and Shipping of specimens**	VRLRC
VRLRC0003	ROP	04/26/10	Yes	**VRLRC procedure for receiving specimens**	VRLRC
VRLRC0004	ROP	10/26/09	Yes	**Separation and Preservation of Plasma**	VRLRC
VRLRC0005	ROP	11/02/09	Yes	**Separation and Preservation of Whole Blood Aliquots**	VRLRC
VRLRC0006	ROP	4/27/10	Yes	**Separation and Preservation of Serum**	VRLRC
VRLRC0007	ROP	10/05/09	Yes	**Weekly Reagent Control Record Procedure**	VRLRC
VRLRC0008	ROP	10/19/09		**EDTA or ACD PBMC Cell Separation Overlay Procedure**	VRLRC

VRLRC0013	ROP	03/29/10	Yes	**Using Leucosep® tubes for the isolation of PBMCs**	VRLRC
VRLRC0015	ROP	03/29/10	Yes	**Usage of Specimen Processing and Storage Forms**	VRLRC
VRLRC0016	ROP	04/26/10	Yes	**Using the Coulter Counter Z1 Procedure**	VRLRC
VRLRC0010	ROP	10/19/09	Yes	**CoolCell PBMC Freezing Process**	VRLRC
VRLRC0012	ROP	03/29/10	Yes	**Using Tempus Blood RNA Tubes**	VRLRC
VRLRC0018	ROP	04/26/10	Yes	**Procedure for the requisition of study specific specimens stored at -40C and -80C**	VRLRC
VRLRC0019	ROP	5/17/10	Yes	**Procedure for the Requisition of Study Specific Specimens Stored in Liquid Nitrogen**	VRLRC
VRLRC0020	ROP	04/26/10	Yes	**Daily Monitoring of Mechanical and Liquid Nitrogen Freezers as well as Teledyne Oxygen Monitors Procedure**	VRLRC
VRLRC0021	ROP	5/12/10	Yes	**Oxygen Monitor Documentation and Alarm Response Procedure**	VRLRC
VRLRC0011	ROP	04/27/10	Yes	**VRLRC Shipping of Frozen Infectious Samples**	VRLRC
VRLRC0023	ROP	5/17/10	Yes	**Procedure for Freezerworks Unlimited Database Sample Check-out Process**	VRLRC
VRLRC0024	ROP	5/17/10	Yes	**GO grant WNV Sample ID assignment**	VRLRC

VRLRC0025	ROP	5/26/10	Yes	**Freezerwork's Data Entry for the GO Grant**	VRLRC
MTC-0001	ROP and Protocol	08/31/1995	Yes	**Pre-Amplification Protocol**	MTC
MTC-0002	ROP and Protocol	04/01/10	Yes	**Quality Control Procedures for the Quantitative Reverse Transcription Real-Time PCR Assays Using SyBr Green**	MTC
MTC-0003	ROP and Protocol	04/29/10	Yes	**Preventative Maintenance, Calibration and Validation of Equipment Performed By External Service Contractors**	MTC
MTC-0004	ROP and Protocol	04/01/10	Yes	**Creation of a Control Chart**	MTC
MTC-0005	ROP and Protocol	04-01-10	Yes	**Dengue Fever Virus RT-PCR Assay**	MTC
MTC-0006	ROP and Protocol	04-01-10	Yes	**West Nile Virus RT-PCR Assay**	MTC
MTC-0002	ROP and Protocol	04/01/10	Yes	**Quality Control Procedures for the Quantitative Reverse Transcription Real-Time PCR Assays Using Fluorescent Probes**	MTC
MTCFrm-0001	Form	04-10-10	No	Kinetic PCR Worksheet	MTC

Imm004	ROP	12/11/09	Yes	Luminex Calibration	Immunology
Imm002	ROP	4/22/10	Yes	Milliplex High Sensitivity Human Cytokine / Chemokine Kit	Immunology
Imm001	ROP	4/22/10	Yes	Milliplex Human Standard Sensitivity Cytokine / Chemokine Kit	Immunology
Imm003	ROP	12/11/09	Yes	LabScan Luminex Reader Maintenance	Immunology
	Form	12/11/09	No	Luminex Data Recording Sheet	Immunology

12.5 Records management

Records maintained will include training documents, protocols, standard operating procedures (ROPs), informed consent documentation, procurement documentation, processing records, testing, equipment maintenance, storage location information, sample distribution, and quality control activities.

Security systems will ensure the confidentiality and security of all stored records. Computers operated by repository staff are all password protected and use automatic timeout mechanisms that lock the computer.

Permission levels have been created for staff at different operational levels as well as for users who are not repository staff, where this access is allowed. Access to records will be on a “need to know” basis.

If it is necessary to either destroy or remove specimens at the request of study participants, records will be appropriately amended to indicate that the specimen is no longer part of the collection and the information management system will be adequately updated to reflect this event.

Paper files containing confidential subject information will be stored in locked, fire and water proof enclosures with controlled access.

12.6 Information Security

Pursuant to the Federal Information Security Management Act of 2002, BSI has developed, documented and implemented an information security program, across all divisions, to safeguard information and information systems that support operations. BSI enforces a documented high standard of network security and privacy policies throughout our enterprise. This program includes standardized workstations, network restrictions, secure virtual private network (vpn), and security enabled logons for each level and degree of access.

Our IT permissions are requested by the department heads recognizing the requirements based on the needs of the individual job functions. They are evaluated and scrutinized by the BSI Security Officer. Upon authorization, the employee permissions are enabled and documented by the BSI / BSRI IT Dept. Secure remote access to the BSRI network is provided by a Juniper 2700 VPN Concentrator on the BSI network. Remote vpn connections through this unit are encrypted for secure data access.

Our hardware environment includes servers with specific and shared roles including domain controller, email server, print server, file server, and applications server. Incremental backups are run every night and full backups are completed each weekend on all network servers using the CommVault Media Agent Backup system. The initial backup goes to an external SAN drive to be kept for several weeks. This speeds the backup process and eases immediate restore requests. The older data is backed up to SDLT tape for archival purposes. For hardware redundancy, all server hard drives are installed in a RAID 5 configuration.

BSRI computers are protected using Enterprise Symantec Endpoint Protection suite. Real Time protection keeps the computer secure as you use your computer. Individual computer scans are set to run daily by the network administrator. Virus file updates occur automatically from the Symantec LiveUpdate site. Incoming email is scanned as it arrives on the BSRI email server. Any virus files detected are quarantined and a message is sent to the original recipient notifying them of the detected virus. Viruses are cleaned or averted before reaching the end user’s computer.

BSRI Internet for web browsing and research uses a dedicated full T1 connection that utilizes access-lists (filters) to reject unsolicited incoming traffic. The Internet connection is further protected by secure routers and a Websense firewall that verifies users and packets to ensure that only authorized traffic to and from the network is

allowed. If necessary, BSRI Internet traffic can be re-routed through the Blood Systems (BSI) Internet connection in Scottsdale, Arizona. This full T1 connection has the same highly secure configuration.

Our donor database is run separately on a highly secured Unix based Oracle Database system with its own level of network and multi-tiered access security.

All components of hardware, data, and points of access have secure routers and switches utilizing Cisco, Lucent, Nortel, Juniper, and other known industry leaders to give a diverse and complex matrix of protection of our research data, donor information, and our enterprise integrity. All of these are password specific and bound in checking of permissions granted based on an employee identification code with an industry-approved secured password requirement.

If awarded a contract, BSRI assures that, commensurate with their responsibilities for performing work under the terms and conditions of their contractual agreements, each contractor and subcontractor employee will complete the NIH Computer Security Awareness Training prior to performing any contract work. In addition, each employee, as noted, shall complete the NIH-specified fiscal year refresher course during the period of performance of the contract. BSRI shall maintain a list of all individuals who complete the training and submit it to the Contacting Officer's Technical Representative. Any additional training requirements as defined by NIST Special Publication 800-16 will be met. Written policies are available.

<u>The BSI Security Officer is:</u>
Dale Shakatko
Blood Systems, Inc.
dshakatko@bloodsystems.org

<u>The BSRI information security liaison is:</u>
David Meronuck
Blood Systems Research Institute
dmeronuck@bloodsystems.org

List of departments:	# of pages	from page X to Y of binder
BSI	57 pages	from page 1 to 57
CTS	57 pages	from page 58 to 62
Medical Affairs	6 pages	from page 63 to 67
BSRI Viral Reference Laboratory and Repository Core	78 pages	from page 69 to 146
BSRI Core Immunology laboratory	15 pages	from page 147 to 185
BSRI Molecular Transfusion Core Laboratory	20 pages	from page 162 to 181
Maria Rios Laboratory for infectivity studies	4 pages	from page 182 to 185

Blood Systems, Inc.

DOCUMENT	REV.	SPONSOR	
SM0038	4	RM	Page 1 of 3

Bloodborne Pathogens Information and Training

Purpose

All employees who have the potential for exposure to bloodborne pathogens shall receive information and training to eliminate or minimize risk.

Related Documents

Responsibility Levels for the Exposure Control Plan, SM0031

Materials

- Independent Outside Contractors Safety Checklist, BSI 152
- Independent Contractors Informational Safety Guide, BS 153

Training Provided

All Category I employees who have occupational exposure to bloodborne pathogens (including Category I volunteers and Temporary employees) will receive training:

- At the time of initial assignment to tasks where occupational exposure may take place
  - Refer to Responsibility Levels for the Exposure Control Plan, SM0031.
- At least annually thereafter
- Free of cost and during working hours

Each facility/center within Blood Systems, Inc. shall provide additional training when changes such as modification of tasks or procedures or institution of new tasks or procedures affect employee occupational exposure.

Contractors with a reasonable anticipation of exposure to blood or other potentially infectious material must provide documentation of compliance with OSHA training requirements (e.g., Bloodborne pathogens, Hazard Communication, etc.) prior to the start of work.

NOTE: Refer to Independent Outside Contractors Safety Checklist, BSI 152 and Independent Contractors Informational Safety Guide, BS 153.

The BSI employee who administers the contract shall be responsible for the completion of the Independent Outside Contractors Safety Checklist, BSI 152 and the Independent Contractors Informational Safety Guide, BS 153

Blood Systems, Inc.

DOCUMENT	REV.	SPONSOR	
SM0038	4	RM	Page 2 of 3

Training Content

The training program shall be conducted by a person who is knowledgeable in the subject matter and shall include, but not be limited to the following elements:

- The OSHA Bloodborne Pathogens Standard (29 CFR 1910.1030).
- An explanation of the epidemiology and symptoms of bloodborne diseases.
- An explanation of the modes of transmission of bloodborne pathogens.
- An introduction to Blood Systems, Inc. Exposure Control Plan.
- An explanation of the appropriate methods for recognizing tasks and other activities that may involve exposure to blood and other potentially infectious materials.
- An explanation of the use and limitations of methods that will prevent or reduce exposure including appropriate engineering controls, work practices, and personal protective equipment.
- Information on the types, proper use, location, removal, handling, decontamination and disposal of personal protective equipment.
- An explanation of the basis for selection of personal protective equipment.
- Information on the hepatitis B vaccine, including information on its efficacy, safety, method of administration, benefits of vaccination, and that the vaccine and vaccination will be offered free of charge to Category I employees.
- Actions to take and persons to contact in an emergency (e.g., Center Medical Director or Licensed Medical Professional) involving blood or other potentially infectious pathogens.
- Information on procedures to follow if an exposure occurs and regarding post-exposure evaluation and follow-up.
- An explanation of visual warning of biohazardous materials within the facility, including labels, signs and color-coded containers.
- An opportunity for interactive questions and answers with the trainer, who shall be knowledgeable in the subject matter.

NOTE: Category I employees in a blood center must be made aware of risk factors associated with the transmission of HBV, other hepatitis viruses, HIV, and appropriate precautions.

Training Records

Each facility/center within Blood Systems, Inc. shall maintain records of employee training as required.

Monitoring

Each facility/center's Safety Officer shall monitor the effectiveness of work practices, engineering controls and personal protective equipment used. The monitoring program includes, but is not limited to:

- § Investigating reported occupational exposures to establish the conditions surrounding the exposure and to improve training, work practices, or personal protective equipment to prevent future occurrences.
- § Surveying the workplace to ensure that required work practices are observed and that personal protective equipment are provided and properly used.

Revision History

The following table represents the revision history of this document.

REVISION	ISSUED	IMPLEMENTED	REASON
4	ACR-09-017	07/06/09	§ Updated Training Content block. § Updated Training Provided block. § Changed title of SM0031.
3	COD-010-07-RM	04/02/07	§ Added Related Documents block. § Added Materials block. § Specified emergency contacts. § Modified Training Provided block. § Removed requirement for semi-annual audits.
2	COD-215-05-RM	1/6/06	§ Changed title of BSI 195.
1	COD-150-05-RM	8/15/05	§ Initial release under Document Control.

Blood Systems, Inc.

DOCUMENT	REV.	SPONSOR	
TE0104	1	T&E	Page 1 of 2

Bloodborne Pathogens

Overview

The "Bloodborne Pathogens" training module is designed to provide a basic understanding of bloodborne pathogens, common modes of their transmission, and methods of prevention for the manufacturing employees of Blood Systems.

Objectives

Upon completion of this program the participant will be able to:
- § Discuss OSHA's bloodborne pathogen standard
- § Define bloodborne pathogen and give an example
- § Explain Blood Systems Hepatitis Vaccination Program
- § Understand Blood Systems exposure control plan
- § Explain what is meant by the term "Universal Precautions"
- § Differentiate between an "engineering control" and a "work practice control"
- § List what to do if an exposure occurs

Audience

All Category I manufacturing employees, as defined by Exposure Determination, SM0032

Time Frame

Must be completed by all Category I manufacturing new hires prior to starting his/her work duties in a manufacturing area and annually thereafter, based on 12 months since the date of prior training.

Documentation

Training Event Attendance Record – TED 100
- § LMS Title: Bloodborne Pathogens Annual Training
- § LMS ID: SAFEBBPT

Implementation Notes

All incumbent staff were required to complete this training by June 30, 2009.

Blood Systems, Inc.

DOCUMENT	REV.	SPONSOR	
TE0104	1	T&E	**Page 2 of 2**

Revision History

The following table represents the revision history of this document.

REVISION	ISSUED	IMPLEMENTED	REASON
1	ACR-09-025	08/03/09	§ Initial release.

Blood Systems, Inc.

DOCUMENT	REV.	SPONSOR	
SM0005	5	RM	Page 1 of 7

Workers' Compensation Reporting Procedures

Overview

These are the guidelines for reporting work-related injuries.

Purpose

The purpose of the program is to ensure Workers' Compensation claims are reported and managed in an accurate and timely manner.

Related Documents

- Accident/Incident Investigation Program, SM0008
- Bloodborne Pathogens Program, SM0039
- Management of Blood/Body Fluid Exposure Incidents, SM0040
- Return to Work Program (RTW), SM0012
- Nurse Triage Program, SM0096

Materials

- Medical Incident or Report of Occupational Exposure Form, BSI 149
- Incident Report Form, BS 521
- Nurse Triage Contact Report, BS 543
- Restrictions Form, BS 533
- BSI's Workers' Compensation Insurance Card

Definitions

- **Licensed Medical Professional**: For the purposes of the SOP, the term "Licensed Medical Professional" is defined as a Registered Nurse (RN) or a Medical Doctor (MD).
- **OSHA Definition of First Aid**: Refers to medical attention that is usually administered immediately after the injury occurs and at the location where it occurred. It often consists of a one-time, short-term treatment and requires little technology or training to administer. First aid can include cleaning minor cuts, scrapes, or scratches; treating a minor burn; applying bandages and dressing; the use of non-prescription medicine; draining blisters; removing debris from the eyes; massage; and drinking fluids to relieve heat stress. Source: www.osha.gov.
- **OSHA Definition of Occupational Exposure:** Reasonably anticipated skin, eye, mucous membrane, or parenteral contact with blood or other potentially infections materials that may result from the performance of an employee's duties. Source: www.osha.gov.

Forms

NOTE: If an injured employee wants to seek medical attention and it is an emergency, dial 911.

Incident Report Form, BS 521

§ If an employee suffers a work-related injury but does not need treatment beyond general 1st aid, the event can be noted on the Incident Report Form, BS 521 and does not need to be called in to the insurance carrier (See OSHA's definition of First Aid in the Definitions block).

Nurse Triage Contact Report, BS 543

§ If the injured employee wants to seek medical treatment or is unsure whether or not they want to seek medical treatment, a local RN or MD can be consulted or Nurse Triage can be contacted. Supervisors should utilize this form to notate the recommendations of the licensed medical professional. Reference Nurse Triage Program, SM0096

Medical Incident or Report of Occupational Exposure Form, BSI 149

§ To be completed if the injured employee needs to seek medical attention for their work-related injury.

§ To be completed in the event of an occupational exposure (See OSHA definition of Occupational Exposure in Definitions block).

NOTE: If the employee later decides to seek medical treatment for the same injury, a Medical Incident or Report of Occupational Exposure Form, BSI 149 must be filled out and the insurance carrier notified within 24 hours.

Changing a claim from Incident Only to Medical will not be counted against a center as "LAG" on the Key Indicator (KI) Report.

Claim Filing Procedures

- An employee who sustains an occupational (work-related) injury, or becomes ill due to work-related reasons or sustains a needlestick/ exposure must report the injury/illness or exposure to a supervisor immediately.
  - Refer to SM0039 and SM0040 for needlestick/exposure process
- Safety Officers must be notified within one business day of an employee reporting an accident.
- When necessary, supervisors may contact the Nurse Triage Hotline with the employee to assist in determining if medical treatment is required
  - Reference Nurse Triage Program, SM0096.

Appropriate Response

Use the table below to determine the appropriate response.

If	Then
Employee does <u>not</u> seek medical treatment and Nurse Triage <u>is not</u> contacted.	§ Complete Incident Report Form, BS 521. § <u>Do not</u> call claim into insurance carrier.
Employee does <u>not</u> seek medical treatment and Nurse Triage <u>is</u> contacted.	§ Complete Medical Incident or Report of Occupational Exposure Form, BS 149. § Complete Nurse Triage Contact Report, BS 543. § <u>Do not</u> call claim into insurance carrier. **NOTE**: **Claim will be considered an "incident-only" claim by the insurance carrier.**
Medical treatment <u>is</u> necessary but Nurse Triage <u>is not</u> contacted	§ Complete Medical Incident or Report of Occupational Exposure Form, BS 149. § Call claim into insurance carrier. § Send employee to the designated occupational health clinic with a BSI Workers' Compensation Insurance Card and a Restrictions Form, BS 533 for the treating physician to complete.

Continued on next page

Blood Systems, Inc.

DOCUMENT	REV.	SPONSOR	
SM0005	5	RM	Page 4 of 7

Appropriate Response (continued)

If	Then
Medical treatment <u>is</u> necessary and Nurse Triage <u>is</u> contacted	§ Complete Medical Incident or Report of Occupational Exposure Form, BS 149. § Complete Nurse Triage Contact Report, BS 543. § <u>Do not</u> call claim into insurance carrier. § Send employee to the designated occupational health clinic with a BSI Workers' Compensation Insurance Card and a Restrictions Form, BS 533 for the treating physician to complete. **NOTE**: **If Nurse Triage is contacted, a claim is automatically generated with our insurance carrier (for non-monopolistic states only).**
Life-Threatening Injury	§ Immediately call 911 if the injury is life threatening. § If the injury results in a fatality, permanent disability, loss of a body part, hospitalization of any employee, or injury of more than two employees, Risk Management must be contacted immediately at (888) 874-7017. § Refer to Accident/Incident Investigation Program, SM0008 for major accidents.

NOTE: Monopolistic States (Wyoming, Washington & North Dakota) may choose to utilize the nurse triage program, but all claims must be reported according to individual state requirements.

NOTE: Refer to Return to Work Program (RTW), SM0012 for employees who have sought medical treatment and have been released by their treating physician to temporary modified duty with restrictions.

NOTE: For employees who are unable to complete the BSI 149, Supervisors will complete the injured employee's portion and report the claim to the insurance carrier. When possible, the injured employee will be required to review and sign the form.

NOTE: The statute of limitations on a Workers' Compensation (WC) claim is state regulated. Monopolistic states must refer to Center Specific reporting procedures.

Responsibilities

The responsibility for successfully filing a claim and managing a Workers' Compensation claim is shared by employees, Supervisor, Safety Officer or designee, and the Risk Management Department.

Employee Responsibilities

Employee must:

- Report all (work-related) injuries, illnesses or needlesticks/exposures to a supervisor on duty immediately.
- Complete the appropriate reporting forms with his or her supervisor (See Forms block).
- Notify Supervisor if choosing to seek medical treatment.
  - Must provide Supervisor with updated status reports following each medical visit.
- Must maintain communication with Supervisor regarding medical treatment of work-related injury.

Supervisor Responsibilities

Supervisor must:

- Complete the appropriate reporting forms (See Forms block).
  - Refer to SM0039 and SM0040 for needlestick/exposure process.
- Contact local Safety Officer or designee within one business day of an employee reporting an injury/illness.
- If an employee chooses to seek medical treatment for the injury/illness, the Supervisor must send the employee to the designated occupational clinic or other medical facility (depending on state requirements) with a Restrictions Form, BS 533 and BSI's Workers' Compensation Insurance Card.
- Send a copy of the appropriate completed reporting forms to the Safety Officer or designee.
- Conduct an accident investigation, if applicable
  - Refer to Accident Investigation Program, SM0008 for further information.

Safety Officer or Designee Responsibilities

Safety Officer or designee must:

- Review all BSI 149s, BS 521s, and BS 543s and maintain on file.
- Report medical claims and exposures to the insurance carrier (or State) as needed.
- Assist the Supervisor in conducting an accident investigation, if applicable.
  - Refer to Accident Investigation Program, SM0008 for further information.
- Contact employee if he/she sought medical treatment.
- Review all BSI 149s and BS 521s with the Safety Committee.
- Alert Risk Management whenever a claim reaches lost-time status.
- Provide copies of claim information to Risk Management upon request.
- Maintain a WC file for all Medical Only or Lost Time claims.
  - All correspondence related to the injury must be kept in the file (e.g. BSI WC forms, medical documentation, medical bills, etc.).
  - Post-termination is sent to CO Risk Management.

Risk Management

Risk Management maintains overall responsibility for Workers' Compensation claims and programs and may choose to manage claims on a case-by-case basis as needed.

Blood Systems, Inc.

Revision History

The following table represents the revision history of this document.

REVISION	ISSUED	IMPLEMENTED	REASON
5	ACR-09-026	01/04/10	§ Changed where to store post-termination files. § Added Definitions block. § Added table to determine appropriate response to injury/illness or exposure. § Updated responsibilities.
4	ACR-08-031	12/29/08	§ Revised overview. § Added BSI's Workers' Compensation Insurance Card. § Added Responsibilities blocks. § Revised Medical Treatment block.
3	COD-010-07-RM	04/02/07	§ Changed title. § Added Related Documents block. § Added requirement for Supervisor to contact Insurance Carrier. § Added requirement for completion of BSI 149. § Clarified note in Filing a Claim block.
2	COD-215-05-RM	1/6/06	§ Changed title of BS 521.
1	COD-150-05-RM	8/15/05	§ Initial release under Document Control.

Blood Systems, Inc.

DOCUMENT	REV.	SPONSOR	
SM0030	3	RM	Page 1 of 6

Exposure Control Plan (ECP)

Introduction

The major goal of the Occupational Safety and Health Administration (OSHA) is to regulate facilities where work is carried out and to promote safe work practices in an effort to minimize the incidence of illness and injury experienced by employees. Relative to this goal, OSHA has enacted the Bloodborne Pathogens Standard, code 29 CFR 1910.1030, and amendments to the act on 11/06/2000 known as the Needlestick Safety & Prevention Act. The purpose of the Bloodborne Pathogen Standard is to "reduce occupational exposure to Hepatitis B Virus (HBV), Human Immunodeficiency Virus (HIV) and other bloodborne pathogens" which employees may encounter in their work place.

Overview

The Exposure Control Plan (ECP) provides a work environment in which occupational exposure to blood and other potentially infectious materials is minimized or eliminated. The ECP identifies measures employed for follow-up whenever an employee sustains a potential exposure to blood, body fluids, or other potentially infectious materials (OPIM) during the course of work related activities.

All Blood Systems employees and staff must be aware that there are a number of general principles that should be followed when working with bloodborne pathogens. These include:

- Full appreciation of the risks of bloodborne pathogen exposure.
- Each facility shall institute engineering and work practice controls to eliminate or minimize employee exposure to bloodborne pathogens.
- Each facility shall provide appropriate medical evaluation and follow-up to employees in the event of an exposure, and exposure incidents shall be appropriately investigated and documented.

Purpose

Blood Systems has implemented this ECP to comply with the intent of the OSHA Bloodborne Pathogens Standard and the above principles. The objectives of this plan are:

- To protect employees from the health hazards associated with bloodborne pathogens by requiring the use of appropriate personal protective equipment, appropriate work practices, and recommending vaccinations where indicated.
- To identify job classifications and/or occupational tasks that might expose an employee to bloodborne pathogens.
- To appropriately identify and classify exposure incidents.
- To appropriately investigate exposure incidents including, but not limited to, documentation of required information on the Sharps Injury Log, BS 190 (California only – use BS 190CA instead).
- To provide appropriate treatment and counseling in the event that an employee be exposed to bloodborne pathogens.
- To identify work practices and technological/engineering changes to be implemented which will reduce or eliminate potential exposures to bloodborne pathogens. This includes at least annual review of the frequency of use of the types and brands of sharps involved in the exposure incidents documented on the Sharps Injury Log; refer to Methods of Compliance with the Exposure Control Plan, SM0033.
- To obtain employee feedback in reviewing and updating the ECP with respect to the procedures performed by employees in their respective work areas or departments through the encouragement of SOP suggestions.
- To train employees about bloodborne pathogens prior to offering indicated vaccinations, prior to beginning work duties and within 10 days of hire.
  - To ensure annual bloodborne pathogens training be provided at least annually thereafter.
- To maintain readily available records of employee training and records of exposure incidents including: evaluation of circumstances surrounding an exposure event, medical evaluation, follow-up and treatment, if appropriate or indicated.
- To assign specific responsibilities for implementing this Plan.
- To maintain a schedule and method of implementation for each of the applicable subsections, including, if applicable: Methods of Compliance; HIV, HBV, and HCV Research Laboratories and Production Facilities; Hepatitis B Vaccination and Post-exposure Evaluation and Follow-up; Communication of Hazards to Employees; and Recordkeeping.

Related Documents

Methods of Compliance with the Exposure Control Plan, SM0033

Materials

- § Independent Outside Contractors Safety Checklist, BSI 152
- § Independent Contractors Informational Safety Guide, BSI 153
- § Sharps Injury Log, BS 190
- § California Sharps Injury Form, BS 190CA

Scope of ECP

The ECP for Bloodborne Pathogens (BBP) outlines federal regulations and general policy with respect to bloodborne pathogens for all employees and staff at each center or facility of Blood Systems, Inc., including sub-centers and mobile units.

Contractors with a reasonable anticipation of exposure to blood or other potentially infectious materials must be informed of hazards (e.g., Biological, Chemical, etc.) that may be present in the facility. This information can be provided by the contracting employer and must be indicated in the contract for services.

- § Refer to Independent Outside Contractors Safety Checklist, BSI 152 and Independent Contractors Informational Safety Guide, BSI 153.

Volunteers (interns, externs, students, etc.) and temporary employees that will be involved in Category I (Manufacturing) tasks will also be offered the Hepatitis B vaccine and must go through Manufacturing Safety and BBP training prior to vaccination.

Availability

Blood Systems, Inc. Exposure Control Plan is available to our employees at any time. Employees are advised of this availability during their education/ training/orientation sessions. A copy of the Exposure Control Plan is kept in the Safety Manual as well as online via Blood Systems' Electronic Document Management System (EDMS).

Revision and Updating

Blood Systems, Inc. recognizes that it is important to keep the ECP updated and current. To ensure this, the plan will be reviewed and updated under the following circumstances:

- § Annually
- § Whenever new or modified tasks and procedures are implemented which effect occupational exposure of employees
- § Whenever new functional positions are established within the facility that may involve exposure to bloodborne pathogens

Definitions

Blood - Human blood, blood components and products made from human blood.

Bloodborne Pathogens - Pathogenic microorganisms that are present in human blood and can cause disease in humans. These pathogens include, but are not limited to, hepatitis B virus (HBV), hepatitis C virus (HCV) and human immunodeficiency virus (HIV).

Clinical Laboratory - A workplace where diagnostic or other screening procedures are performed on blood or other potentially infectious materials.

Contaminated - The presence or the reasonably anticipated presence of blood or other potentially infectious materials on an item or surface.

Contaminated Sharps - Any contaminated object that can penetrate the skin, including but not limited to, needles, scalpels, broken glass, broken capillary tubes or blood sampling equipment.

Contaminated Laundry - Any laundry that has been soiled with blood or other potentially infectious materials or may contain sharps.

Decontamination - The use of physical or chemical means to remove, inactivate, or destroy bloodborne pathogens on a surface or item to the point where they are no longer capable of transmitting infectious particles, and the surface or item is rendered safe for handling, use or disposal.

Engineering Controls - Controls that isolate or remove the bloodborne pathogens hazard from the workplace (e.g., sharps disposal containers, needle less systems, self-sheathing needles, and other sharps with engineered sharps injury protection systems).

Continued on next page

Definitions (continued)

Exposure Incident/Occupational Exposure - A specific eye, mouth or other mucous membrane, non-intact skin, or parenteral contact with blood or other potentially infectious materials that may result from the performance of an employee's duties.

Handwashing Facilities - A facility that provides an adequate supply of running, drinkable or clean water, soap and single use towels or hot air drying machines.

Other Potentially Infectious Materials (OPIM) - The following human body fluids: semen, vaginal secretions, cerebrospinal fluid, synovial fluid, pleural fluid, pericardial fluid, peritoneal fluid, amniotic fluids, saliva in dental procedures, any body fluid that is visibly contaminated with blood, and all body fluid in situations where it is difficult or impossible to differentiate between body fluids. Any unfixed tissue or organ (other than intact skin) from a human (living or dead).

Parenteral - Piercing mucous membranes or the skin barrier through such events as needlesticks, human bites, cuts and abrasions.

Percutaneous – Exposure via needlestick, laceration or bite.

Permucosal – Exposure via ocular or mucous membranes (eyes, mouth, etc).

Personal Protective Equipment - Specialized clothing or equipment worn by an employee for protection against hazard. General work clothes (e.g., uniforms, pants, shirts or blouses) not intended to function as protection against a hazard are not considered to be personal protective equipment.

Regulated Waste (Biohazardous Waste or Infectious Waste) - Liquid or semi-liquid blood or other potentially infectious materials; contaminated items that would release blood or other potentially infectious materials in a liquid or semi-liquid state if compressed; items that are caked with dried blood or other potentially infectious materials and are capable of releasing these materials during handling; contaminated sharps; and pathological and microbiological wastes containing blood or other potentially infectious materials.

Source Individual - Any individual living or dead, whose blood or other potentially infectious materials may be a source of occupational exposure to the employee.

Continued on next page

Blood Systems, Inc.

(continued)

Sterilize - The use of a physical or chemical procedure to destroy all microbial life including highly resistant bacterial endospores.

Universal Precautions - An approach to infection control. According to the concept of Universal Precautions, all human blood and certain human body fluids are treated as if known to be infectious for HIV, HBV, HCV and other bloodborne pathogens.

Work Practice Controls - Procedures or policies that reduce the likelihood of exposure by altering the manner in which a task is performed (e.g., prohibiting the recapping of needles by a two-handed technique).

Revision History

The following table represents the revision history of this document.

REVISION	ISSUED	IMPLEMENTED	REASON
3	ACR-09-017	07/06/09	§ Major revision. § Added Overview block. § Added to Purpose, Materials, Scope, Availability, and Definition blocks.
2	COD-010-07-RM	04/02/07	§ Added Materials block. § Removed Biological Safety from Plan name. § Clarified Introduction, Purpose Scope and Availability blocks. § Corrected form number.
1	COD-150-05-RM	8/15/05	§ Initial release under Document Control.

Blood Systems, Inc.

DOCUMENT	REV.	SPONSOR	
SM0034	4	RM	**Page 1 of 7**

Hepatitis B Vaccination Program

Purpose

To implement measures within Blood Systems, Inc. which can reduce or eliminate the risk of acquiring hepatitis B infection through occupational exposure.

Materials

- § Standing Order for the Administration of Hepatitis B Vaccine, BS 572
- § Employee Immunization/Treatment Record, BSI 134
- § Informed Consent for Hepatitis B Vaccination, BSI 124
- § Informed Consent for Terminating BSI Employee to Complete the Hepatitis B Vaccination Series, BSI 124G
- § Special Testing Request Form – Hepatitis Related Testing, BSI 123H
- § Notification Letter for 2x Anti-HBs Non-Responders, BS 197
- § Counseling for HBV vaccine Non-responder, BSI 116
- § Centers for Disease Control and Prevention Hepatitis B Vaccine Information Statement (VIS), VF-053
- § Medical Incident or Report of Occupational Exposure Form, BSI 149

Related Documents

- § Overview of Hepatitis B Virus, Vaccination, and Immune Globulin, SM0070
- § Categorization of Employee Exposure Risk, SM0032
- § Management of Blood/Body Fluid Exposure Incidents, SM0040
- § Hepatitis B Vaccination Administration, SM0035

Policy

Annually, each facility must complete a Standing Order for the Administration of Hepatitis B Vaccine, BS 572, by having the Medical Director of the facility sign and date the form. This form serves as authorization for the administration of the Hepatitis B vaccine.

- § Unless the Medical Director for the main facility is also licensed to practice medicine in the adjacent state(s), then this authorization serves only the state in which the main facility is located. Facilities with sub-centers located in adjacent states must have a BS 572 on file for each adjacent state. Such authorization must be signed by a physician licensed to practice medicine in the adjacent state(s).

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Policy (continued)

- § If the sub-center is unable to obtain authorization from a state-licensed medical practitioner, the Executive Director of the main facility may elect to have sub-center employees receive Hepatitis B vaccine at the main center facility, or from a licensed medical facility located near the sub-center.

- § The original, signed BS 572 should be maintained on file by the individual responsible for the Employee Protection Program or designee.
  - § A photocopy of the BS 572 should be maintained on file by the Safety Officer or designee.

- § Blood Systems, Inc. will provide, at no cost, hepatitis B vaccination to all employees at risk of occupational exposure during the course of work-related duties, at any time during their employment. Refer to Categorization of Employee Exposure Risk, SM0032 and Hepatitis B Vaccination Administration, SM0035.

The Hepatitis B vaccine will be offered to all new Category I employees after completion of the Bloodborne Pathogen training program and within the first 10 days of employment.

NOTE: Category I employees must be offered the hepatitis B vaccine and the series must be initiated, if applicable, prior to beginning any work duties which could put them at risk to potential exposure.

Volunteers (interns, externs, etc.) performing Category I duties must be offered the hepatitis B vaccine free-of-charge prior to beginning any work duties which could put them at risk to potential exposure. The series shall be administered as indicated in this procedure. However, all forms must indicate that person is a volunteer. Temporary employees performing Category I duties must also be offered the hepatitis B vaccine free-of-charge. Both Category I volunteers and temporary employees must go through Manufacturing Safety and Bloodborne Pathogens training prior to receiving the vaccination. The local Hepatitis B Vaccination program administrator must be notified of any new Category I volunteers or temporary employees to ensure that they are scheduled for vaccination as appropriate.

Continued on next page

Blood Systems, Inc.

DOCUMENT	REV.	SPONSOR	
SM0034	4	RM	Page 3 of 7

Policy (continued)

§ Hepatitis B vaccination shall be made available after the employee has received training in bloodborne pathogens and within 10 working days of the initial assignment to all employees who have the risk of potential exposure. Hepatitis B vaccination for employees performing Category I duties is strongly recommended unless the employee can demonstrate documented completion of the hepatitis B vaccination series, or if the vaccine is contraindicated for medical reasons. This will be documented on the Employee Immunization/Treatment Record, BSI 134. The BSI 134 will be a permanent part of an employee's medical record. This record will be completed and filed in the employee's medical file and must be readily available for review. These records should be kept in a secure location to ensure that confidentiality is maintained at all times.

§ Employees who are felt to be at risk of occupational exposure to Hepatitis B will be asked to make the determination, based on informed consent, to accept or decline the hepatitis B vaccine. Employees shall receive Information on Hepatitis B Vaccine, located on the Informed Consent for Hepatitis B Vaccination, BSI 124. Additional information is also available in Overview of Hepatitis B Virus, Vaccination, and Immune Globulin, SM0070. Employees will be asked to agree or decline to participate in the program and to sign a BSI 124 which will be retained in the employee's medical file.

§ Employees who decline the vaccine initially may opt to request the vaccine at any time during their employment at Blood Systems, Inc., provided they continue in a position felt to be at risk for occupationally related exposure to hepatitis B virus. At that time employees will need to sign a BSI 124 indicating that they agree to participate in the hepatitis B vaccination program.

§ Employees who agree to receive hepatitis B vaccinations must be provided with a copy of the Center for Disease Control and Prevention (CDC) Hepatitis B Vaccine Information Statement (VIS) VF-053 prior to every administration of the vaccine and provision of the VIS must be documented on the Employee Immunization/Treatment Record, BSI 134.

Continued on next page

Blood Systems, Inc.

DOCUMENT	REV.	SPONSOR	
SM0034	4	RM	Page 4 of 7

Policy (continued)

§ Employees who claim to have been vaccinated at a previous place of employment must present documentation of such vaccination, including reactive anti-HBs test results. This documentation should be available for the duration of the employee's first 10 working days to ensure availability at the time of bloodborne pathogen training and the subsequent option to receive hepatitis B vaccination. This documentation must be filed in the employee's medical record. If no documentation, or if incomplete documentation, is available, the employee will be recommended to begin a full course of vaccination.

Individuals who have terminated their employment with Blood Systems, Inc., and are currently receiving hepatitis B vaccine according to an established schedule, shall be offered the opportunity to complete the vaccination series with Blood Systems at no charge to the employee. It is the employee's responsibility to follow-up and complete the series. The remaining vaccinations will be administered by a BSI employee at a BSI location. Documentation should be placed on the Informed Consent for Terminating BSI Employee to Complete the Hepatitis B Vaccination Series, BSI 124G. This form shall be retained in the terminated employee's medical record at Blood Systems, Inc. Central Offices/Human Resources.

All employees who have an exposure to blood or other body fluids will have their Employee Immunization/Treatment Record, BSI 134 reviewed. Employees should be managed as recommended in Management of Blood/Body Fluid Exposure Incidents, SM0040.

The Safety Officer or designee shall review the center employees' Employee Immunization/Treatment Record, BSI 134 forms on a regular basis to ensure that records are complete and current, and that any required follow-up is completed on schedule.

NOTE: Each center is responsible for determining an effective system for documenting and tracking the Hepatitis B Vaccination Program. The program must be implemented and maintained in such a way that it is consistent for all Hepatitis B Vaccination Program participants.

§ Upon completion of the vaccine series administered by Blood Systems, the employee will be tested for anti-HBs antibodies to determine seroconversion. Request testing on Special Testing Request Form – Hepatitis Related Testing, BSI 123H.

Continued on next page

Blood Systems, Inc.

DOCUMENT	REV.	SPONSOR	
SM0034	4	RM	**Page 5 of 7**

Policy (continued)

- § If adequate antibody levels are present, enter the information on the BSI 134 and notify the employee. No further action is required.
- § If inadequate (nonreactive or borderline) antibody levels are determined, the employee will be notified and a second vaccination series, including repeat testing for anti-HBs antibodies, will be recommended.
  - § This information will be entered on the BSI 134.
- § Upon completion of the second vaccine series administered by Blood Systems, the employee will be tested for anti-HBs antibodies to determine seroconversion. Request testing on Special Testing Request Form – Hepatitis Related Testing, BSI 123H.
- § If adequate antibody levels are present, notify the employee and enter the information on Employee Immunization/Treatment Record, BSI 134. No further action is required.
- § If levels of protective antibodies are still inadequate (nonreactive or borderline) after a second course of vaccination, the employee is considered to be a non-responder to vaccination. The employee should be counseled that they remain at risk of infection through exposure to blood or bodily fluids since they have not developed protective antibodies against hepatitis B virus. The employee should also be counseled that one reason some people do not respond to vaccination is current infection with hepatitis B virus, and they should consider following up with their physician. Document counseling on the BSI 116. Employees should receive also receive a copy of Notification Letter for 2x Anti-HBs Non-Responders, BS 197.

Medical Recordkeeping

Each center/facility within Blood Systems shall establish and maintain an accurate, up to date, and readily available medical record for each employee with an occupational exposure. These medical records shall be kept confidential and not disclosed or reported to any person without the employee's express written consent.

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Medical Recordkeeping (continued)

This record may include but not be limited to:

- A copy of Employee Immunization/Treatment Record, BSI 134
- Informed Consent for Hepatitis B Vaccination, BSI 124, including information on hepatitis B vaccine
- Counseling for HBV vaccine Non-responder, BSI 116
- Informed Consent for Terminating BSI Employee to Complete Hepatitis B Vaccination, BSI 124G
- Forms generated as a result of an occupational exposure:
  - Informed Consent for Hepatitis B Immune Globulin, BSI 124B
  - Release of Medical Records, BSI 124C
  - Informed Consent for Post Exposure Testing, BSI 124D
  - Source Informed Consent for Testing, BSI 124E
  - Informed Consent for Terminating BSI Employee to Complete Post Exposure, BSI 124F
  - Employee/Source Material Test Result Record, BSI 135
  - Medical Incident or Report of Occupational Exposure Form, BSI 149
  - Post Exposure Evaluation – Licensed Medical Professional's Written Opinion, BS 573
  - Disqualified Donor Maintenance (DDD), BS 315 or SafeTrace equivalent

- Each center/facility within Blood Systems, Inc. shall maintain these medical records for the duration of the employee's employment, plus 30 years in accordance with 29 CFR 1910.1020.
- Upon termination of employment, the employee's records should be sent to Blood Systems, Central Offices/Human Resources. Medical records shall be available to the employee upon request for review and copying purposes.
  - Refer to the appropriate Record Retention Schedule in the Records Management Manual.

References

§ CDC. Updated U. S. Public Health Service Guidelines for the Management of Occupational Exposures to HBV, HCV, and HIV and Recommendations for Postexposure Prophylaxis. MMWWR 2001; 50(no. RR-11).
§ CDC. A Comprehensive Immunization Strategy to Eliminate Transmission of Hepatitis B Virus Infection in the United States. Recommendations of the Advisory Committee on Immunization Practice (ACIP). Part 1: Immunization of Infants, Children, and Adolescents. MMWR 2005; 54 (no. RR-16).
§ CDC. A Comprehensive Immunization Strategy to Eliminate Transmission of Hepatitis B Virus Infection in the United States. Recommendations of the Advisory Committee on Immunization Practice (ACIP). Part 2: Immunization of Adults. MMWR 2006; 55(no. RR-16).

Revision History

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REVISION	ISSUED	IMPLEMENTED	REASON
4	ACR-09-026	01/04/10	§ Changed title of BSI 149.
3	ACR-09-017	07/06/09	§ Changed HBV vaccination policies and procedures. § Added references to new and modified forms. § Added references for SOPs.
2	COD-010-07-RM	04/02/07	§ Changed title. § Corrected form name. § Changed form name, BSI 124. § Added to Materials block. § Added to Related Documents block. § Clarified retention of BS 572. § Clarified Policy block. § Added Safety Officer or designee as acceptable reviewer.
1	COD-150-05-RM	8/15/05	§ Initial release under Document Control.

Blood Systems, Inc.

DOCUMENT	REV.	SPONSOR	
SM0022	5	RM	Page 1 of 5

Personal Protective Equipment

Purpose

To identify new and important requirements relating to basic safety and health programs imposed by OSHA's final standard on personal protective equipment, 29 CFR 1910 132, Subpart I (hereafter referred to as the standard).

Responsibility

The Safety Officer shall determine a need for Personal Protective Equipment (PPE), monitor its effectiveness, train employees, and monitor and enforce its proper use.

At Blood Systems, the Risk Management department may be contacted to assist departments with their hazard assessment and the required PPE training.

Definition

PPE is specialized clothing or equipment worn by an employee for protection against a hazard. OSHA defines PPE as “appropriate” only if it does not permit blood or Other Potentially Infectious Material (OPIM) to pass through to employee’s work clothes, undergarments, skin, eyes, mouth or other mucous membranes under normal conditions of use and for the duration of time which the protective equipment will be used.

Materials

- Hazard Assessment Form, BS 528
- Personal Protective Equipment Standard Training Certification, BS 529
- Material Safety Data Sheets (MSDS)
- Guidelines for the Selection of Chemical Protective Clothing published by the American Conference of Governmental Industrial Hygienists (ACGIH)

Related Documents

- Hazard Analysis, SM0083
- General Safety Rules, SM0016
- Infectious Biohazardous Waste Handling Policy, SM0063

Requirements

OSHA's standard states general requirements for the selection and use of PPE. Included in these requirements are the following:

- Employers must conduct a hazard assessment to determine if hazards present necessitate the use of PPE.
  - Refer to Hazard Analysis, SM0083.
- Employers must certify on the Hazard Assessment Form, BS 528 the hazard assessment was conducted.
- PPE selection must be made on the basis of hazard assessment and affected workers being properly trained to use it.
- Defective or damaged PPE must not be used.
- Employer must certify on the Personal Protective Equipment Standard Training Certification, BS 529 that training programs were provided and understood.

Practices

To ensure that PPE is not contaminated and is in appropriate condition to protect employees from potential exposure, each center/facility within Blood Systems, Inc. shall adhere to the following practices:

- All PPE is inspected, periodically cleaned, and repaired as needed to maintain its effectiveness.
- Reusable PPE or equipment that cannot, for whatever reason, be decontaminated is disposed of in accordance with the Infectious Biohazardous Waste Handling Policy, SM0063.
- Each facility shall have a supply of disposable garments available in case an employee needs to remove contaminated clothing/uniform.
- To make sure that PPE is used as effectively as possible, employees shall adhere to the following practice when using their PPE:
  - Any garments penetrated by blood or other potentially infectious materials are removed immediately, or as soon as feasible.
  - **All PPE is removed prior to leaving a work area.**

Eye Protection

- Eye protection such as goggles, glasses, and face shields will be made available for all employees and visitors who wish to wear them.
- Eye protection must be worn at all times in designated areas.

United Blood Services Laboratory Staff Only - Face Shields

Per OSHA standard 1910.1030(d)(3)(x): *Masks in combination with eye protection devices, such as goggles or glasses with solid side shields, or chin-length face shields, shall be worn whenever splashes, spray, spatter, or droplets of blood or other potentially infectious materials may be generated and eye, nose or mouth contamination can be reasonably anticipated.*

Face, eye, and mouth protection is mandatory for all activities which could result in a splash exposure.

These activities include:
- § Segmenting
- § Breaking a Seal
- § Sterile Connecting Device (SCD)
- § Heat Sealing
- § Cutting Segments for QC Testing
- § Manual De-Capping of Specimen Tubes
- § All other procedures with risk of splash exposures

The recommended face shield would be considered a full face shield (Chin Length – minimum 7 ½" long). Alternatives to the face shield are surgical masks in combination with eye protection devices such as goggles or glasses with solid side shields.

NOTE: Centers should work with Purchasing to order face shields that meet the above recommendations if they want to order something not already found in iProcurement

Clothing

Appropriate protective clothing such as, but not limited to, gowns, aprons, lab coats, or similar outer garments must be worn when it can be reasonably anticipated that the employee may have contact with untested, known positive blood or other potentially infectious material and when handling or touching contaminated items or surfaces.

- § Lab coats should be provided for protection and convenience and should be worn at all times in designated areas.
- § Lab coats are to be completely fastened closed with the sleeves down while working and removed prior to exiting potentially hazardous (dirty) areas.

Continued on next page

Blood Systems, Inc.

DOCUMENT	REV.	SPONSOR	
SM0022	5	RM	Page 4 of 5

Clothing (continued)

- § Proper footwear and clothing is required and must be worn at all times in all work areas.
  - § Refer to General Safety Rules, SM0016
- § Consult the Material Safety Data Sheets (MSDS) to determine recommended clothing or PPE for a particular chemical. Examples are latex, nitrile or PVC gloves, or aprons.

Gloves

Gloves must be worn when it can be reasonably anticipated that the employee may have hand contact with untested, known positive blood or other potentially infectious material and when handling or touching contaminated items or surfaces.

Disposable (single use) gloves, such as surgical or examination gloves shall be replaced as soon as practical when contaminated, or as soon as feasible if they are torn, punctured, or when their ability to function as a barrier is compromised.

Disposable (single use) gloves shall not be washed or decontaminated for re-use.

Utility gloves may be decontaminated for re-use if the integrity of the glove is not compromised. However, they must be discarded if they are cracked, peeling, torn, punctured, or exhibit other signs of deterioration or when their ability to function as a barrier is compromised.

Gloves of various types are available and should be chosen for each specific job for compatibility and breakthrough characteristics. An excellent information guide is the Guidelines for the Selection of Chemical Protective Clothing published by the American Conference of Governmental Industrial Hygienists (ACGIH) or information provided by glove manufacturers.

NOTE: Hypoallergenic gloves, glove liners and other similar alternatives are readily available to employees who are allergic to the gloves in general use within each center/facility of BSI. The Safety Officer, in conjunction with the department director, manager or supervisor, is responsible for ensuring that all departments and work areas have appropriate PPE available to employees.

Blood Systems, Inc.

Revision History

The following table represents the revision history of this document.

REVISION	ISSUED	IMPLEMENTED	REASON
5	ACR-09-017	07/06/09	§ Added Definition block. § Added Face Protection block for UBS Laboratory staff.
4	ACR-08-031	12/29/08	§ Clarified Clothing block.
3	COD-147-07-RM	10/22/07	§ Added instructions that in areas where the potential for an exposure exists, no skin is to be exposed. § Updated information about lab coats. § Updated information about footwear.
2	COD-010-07-RM	04/02/07	§ Corrected form name. § Added to Materials block. § Added Related Documents block. § Changed block title. § Added reference to Hazard Analysis. § Added Practices block. § Revised Eye Protection, Clothing and Gloves blocks. § Added References block.
1	COD-150-05-RM	8/15/05	§ Initial release under Document Control.

Blood Systems, Inc.

DOCUMENT	REV.	SPONSOR	
SM0040	6	RM	Page 1 of 9

Management of Post Exposure Incidents

Purpose

To provide instructions for incidents involving exposure or potential exposure to blood, body fluid, or other potentially infectious materials.

Policy

An affected employee shall be offered guidance and indicated medical assistance following an exposure to blood, body fluids or other potentially infectious materials.

Volunteers and Temporary employees who sustain work-related exposure to blood and other potentially infectious materials must be managed the same way as a regular employee of the company. All required forms must indicate that person is a volunteer/temporary employee.

Definition

Exposure Incident - An exposure incident/occupational exposure is identified as a specific eye, mouth, or other mucous membrane, non-intact skin, or parenteral contact with blood or other potentially infectious materials that result from the performance of an employee's duties.

Scope

Post-exposure and follow up procedures apply to any employee, temporary employee or volunteer that encounters an occupational exposure at each center or facility of Blood Systems, Inc., including subcenters and mobile units.

Additional Resources

National Clinicians' Post-Exposure Prophylaxis Hotline (PEPline)1-888-448-4911, www.nccc.ucsf.edu

Materials

- § Medical Incident or Report of Occupational Exposure Form, BSI 149
- § Special Testing Request Form – Panels, BSI 123P
- § Source Informed Consent for Testing, BSI 124E
- § Employee Immunization/Treatment Record, BSI 134
- § Employee/Source Material Test Result Record, BSI 135
- § Informed Consent for Post Exposure Testing, BSI 124D
- § Post Exposure Evaluation – Licensed Medical Professional's Written Opinion, BS 573
- § Disqualified Donor Maintenance (DDD), BS 315 or SafeTrace equivalent
- § Informed Consent for Hepatitis B Vaccination, BSI 124
- § Release of Medical Records, BSI 124C, if applicable
- § Informed Consent for Terminating BSI Employee to Complete Post Exposure Testing BSI 124F, if applicable
- § Sharps Injury Log, BS 190
- § California Sharps Injury Form, BS 190CA

Related Documents

- § Counseling for Exposed Persons, SM0041
- § Workers' Compensation Reporting Procedures, SM0005
- § Post-Exposure Prophylaxis (PEP), SM0042
- § Testing of Persons Exposed to Blood/Body Fluids, SM0043
- § Methods of Compliance with the Exposure Control Plan, SM0033
- § Hepatitis B Vaccination Program, SM0034
- § Employees with Serious Health Conditions, HRG0020

Responsibility

- § The Employee is responsible for reporting the incident and contacting their Supervisor/Manager immediately.
- § The Supervisor/Manager is responsible for evaluating whether the incident represents an exposure, performing baseline testing of exposed person and exposure source, and reporting all exposures to the Licensed Medical Professional or Center Medical Director no later than 1 hour after the exposure.
- § The Licensed Medical Professional or Center Medical Director is responsible for evaluating whether additional postexposure management is needed, and if so, for directing the postexposure management.

Blood Systems, Inc.

DOCUMENT	REV.	SPONSOR	
SM0040	6	RM	Page 3 of 9

Immediate Care of Potential Exposure Site

Provide immediate care to the potential exposure site using the following steps:

- § Wash exposed area with antibacterial soap and water, and/or
- § Flush mucous membranes with water.
- § If clothing is contaminated with OPIM, refer to Methods of Compliance with the Exposure Control Plan, SM0033 (Contaminated Laundry).

Immediately notify Supervisor/Manager about the incident.

Initial Documentation of an Incident

§ Employee completes the Medical Incident or Report of Occupational Exposure Form, BSI 149

- § Supervisor/Manager will complete "Supervisor/Manager" section of Employee/Source Material Test Result Record, BSI 135
  - § Include a detailed description of incident (e.g., task being performed at time of incident, amount of blood or material involved, severity of exposure, and condition of skin, if applicable).
  - § Pertinent information about the incident will determine if incident qualifies as an exposure. See Determining if Incident Qualifies as an Exposure Incident block.
  - § Information about the source, including donation number and identification number must be completed. If source identity is unknown, indicate so.
  - § Document pertinent medical history, specifically history of HBV, HCV, or HIV and whether source is an autologous donor.
- § If exposure involved a sharp or needlestick, Supervisor/Manager completes the Sharps Injury Log, BS 190. (**California only** – complete BS 190CA instead). See Methods of Compliance with the Exposure Control Plan, SM0033 (Sharps Injury Log Recordkeeping).
- § Supervisor/Manager will report incident to Workers' Compensation Carrier as a Notice Only Claim per Workers' Compensation Reporting Procedures, SM0005

NOTE: All incidents involving blood or other potentially infectious material (OPIM) should be recorded on the Employee/Source Material Test Result Record, BSI 135 regardless of whether it qualifies as an exposure incident or not.

Determining if Incident Qualifies as an Exposure Incident

The Supervisor/Manager should determine if the incident qualifies as an exposure using the following criteria:

- Significant exposures to any of the following may require further evaluation:
  - Blood or blood product
  - Semen, vaginal secretions, cerebrospinal fluid, synovial fluid, peritoneal fluid, pericardial fluid, or amniotic fluid.
- The following body fluids do NOT pose a risk of bloodborne pathogen transmission unless visibly contaminated with blood:
  - Urine, saliva, nonpurulent sputum, stool, emesis, nasal discharge, tears, and sweat.
- Method of exposure was one of the following:
  - Percutaneous injury (e.g., needle stick , cut with a sharp object)
  - Contact with mucous membrane (e.g., eyes, mouth, nose)
  - Contact with non-intact skin (e.g., dermatitis, abrasion, or open wound)
  - Bites resulting in blood exposure to either person involved.

Use the information about the incident to determine if incident qualifies as an exposure. Document on BSI 135 whether incident qualifies as an exposure incident or not.

- If incident is not an exposure incident, no further workup is necessary.
- If incident does qualify as an exposure, continue to next block.

If an Incident Qualifies as an Exposure

- If the incident qualifies as an exposure, Supervisor/Manager must contact the Licensed Medical Professional or Center Medical Director and perform postexposure testing.
- Perform testing for exposed person and source as described in Testing of Persons Exposed to Blood/Body Fluids, SM0043.
- Contact the Licensed Medical Professional or Center Medical Director as soon as possible, no later than 1 hour after the exposure. Central Office Medical Affairs Hotline may also be contacted.
  - This is critical in order to maximize efficacy of any required post-exposure prophylaxis.
  - Document contact with Licensed Medical Professional or Center Medical Director on BSI 135.
  - If an on-call Licensed Medical Professional or Center Medical Director is not available to evaluate the exposure, the employee should be referred to an urgent medical care facility.
    - If employee is referred to an outside facility, treating facility should be effectively alerted that an employee has been exposed to potentially infectious material and that the employee is en route to the facility for treatment.

Overview of Initial Evaluation by Licensed Medical Professional or Center Medical Director

- Licensed Medical Professional or Center Medical Director will review information provided by Supervisor/Manager and documented on BSI 135 to determine if there are strong risk factors or known history of HIV.
- **If there are strong risk factors or known history of HIV**, exposed person:
  - Should be counseled about transmission risks and other issues per Counseling for Exposed Persons, SM0041.
  - Should be referred immediately to urgent care facility for post-exposure prophylaxis as described in Post-Exposure Prophylaxis, SM0042.
- Using information provided by Supervisor/Manager and documented on BSI 135 as well as information documented in Employee Immunization/ Treatment Record, BSI 134, Licensed Medical Professional or Center Medical Director will:
  - Counsel exposed person about transmission risks of other infectious diseases per Counseling for Exposed Persons, SM0041.
  - Offer post-exposure prophylaxis as per Post-Exposure Prophylaxis, SM0042.
- Licensed Medical Professional or Center Medical Director will document evaluation of exposed employee after initial exposure and during follow-up testing periods using Employee/Source Material Tested Result Record, BSI 135.
  - The BSI 135 shall be retained within the employee's medical file.
  - Results of all testing should be documented on BSI 135.
  - Update BSI 135 as additional information becomes available.
- Employees sustaining a documented exposure shall be deferred as potential donors for a period of 12 months following the exposure.
  - Complete a Disqualified Donor Maintenance (DDD), BS 315 or SafeTrace equivalent on exposed employee and submit to appropriate personnel for DDD maintenance within 2 business days.
- Licensed Medical Professional or Center Medical Director will direct follow-up testing, if indicated, as per Testing of Persons Exposed to Blood/Body fluids, SM0043.

Blood Systems, Inc.

DOCUMENT	REV.	SPONSOR	
SM0040	6	RM	Page 6 of 9

California Centers Only

Post-Exposure Evaluation & Follow-up

- Following an exposure incident, California employees must be advised that they may refuse to consent to post-exposure evaluation and follow-up from the Blood Systems' licensed medical professional(s). If consent is refused, a confidential medical evaluation and follow-up from a healthcare professional outside of Blood Systems' Inc must immediately be made available. T8 CCR 5193(f)(1)(A).
- This outside evaluation should be free of charge to the employee.
- Each Center must identify and establish a documented agreement with an outside healthcare professional or medical facility that would be available 24 hours a day to provide post-exposure evaluation and follow-up.
- If post-exposure prophylaxis treatment is indicated (either by BSI health care provider or the designated outside healthcare professional) and an employee is referred to an Emergency Room or other treating facility for immediate prophylaxis treatment, a procedure must be in place that ensures that the treating facility is effectively alerted that an employee has been exposed to potentially infectious material and that the employee is en route to the facility for treatment.

Management if Exposed Person is or Becomes Positive for Infectious Disease Marker

If exposed person is confirmed positive for an infectious disease during baseline or follow-up testing :

- Contact the Center Medical Director for further management.
- Medical Affairs, Donor Counseling Notification Services (DCNS), may be contacted to assist in notification and counseling of the employee, except in the case of HIV positive results.
  - HIV-positive employees will be counseled by the Center Medical Director. Medical Affairs, DCNS, assists with the notification letter to the employee.
  - The Center Medical Director must provide the employee with a copy of Employees with Serious Health Conditions, HRG0020 and counsel them on the policy. Document this counseling on the Employee/ Source Material Test Result Record, BSI 135.
  - Employee should be referred to an outside physician who specializes in managing the infectious disease in question for treatment, counseling, and medical management.

Continued on next page

Blood Systems, Inc.

DOCUMENT	REV.	SPONSOR	
SM0040	6	RM	Page 7 of 9

Management if Exposed Person is or Becomes Positive for Infectious Disease Marker (continued)

§ Update the Disqualified Donor Maintenance (DDD), BS 315 or SafeTrace equivalent to reflect appropriate deferral for the positive infectious and submit to appropriate personnel for DDD maintenance within 2 business days.

Document each follow-up visit on Employee/Source Material Test Result Record, BSI 135.

Terminating BSI Employee Completion of Post-Exposure Evaluation

§ Individuals who have terminated their employment with Blood Systems, Inc., and are currently participating in the Post Exposure Program shall be offered the opportunity to complete the post exposure testing and evaluation at no charge to the employee.
§ The remaining testing will be performed by a BSI employee at a BSI location.
§ Documentation will be placed on the Informed Consent for Terminating BSI Employee to Complete Post Exposure Testing, BSI 124F. This form shall be retained in the terminated employee's medical record at Blood Systems, Inc. Central Offices/Human Resources.

Completion of Post-Exposure Evaluation

§ Upon completion of all management steps, the Summary of Post-Exposure Evaluation on BSI 135 must be completed and Center Medical Director must review and sign.
  § Summary may contain the initial assessment, the management steps, and the final disposition including any treatment and/or referrals made to an outside health care facility.
§ Employees must be informed of the results by the operating unit.

Written Medical Opinion of Post Exposure Evaluation

§ The Post Exposure Evaluation – Licensed Medical Professional's Written Opinion, BS 573 is reviewed for completeness and signed by the Licensed Medical Professional or Center Medical Director.
§ The Licensed Medical Professional or Center Medical Director must provide employee with a completed and signed copy of the Post Exposure Evaluation – Licensed Medical Professional's Written Opinion, BS 573 within 15 days of completion of the evaluation.

Medical Recordkeeping

Each center/facility within Blood Systems shall establish and maintain an accurate, up to date, and readily available medical record for each employee with an occupational exposure. These medical records shall be kept confidential and not disclosed or reported to any person without the employee's express written consent.

Refer to Hepatitis B Vaccination Program, SM0034 for additional information on record retention items.

References

§ CDC. Updated U. S. Public Health Service Guidelines for the Management of Occupational Exposures to HBV, HCV, and HIV and Recommendations for Postexposure Prophylaxis. MMWWR 2001; 50(no. RR-11).
§ CDC. Updated U. S. Public Health Service Guidelines for the Management of Occupational Exposures to HIV and Recommendations for Postexposure Prophylaxis. MMWWR 2005; 54 (no. RR-9).
§ CDC. A Comprehensive Immunization Strategy to Eliminate Transmission of Hepatitis B Virus Infection in the United States. Recommendations of the Advisory Committee on Immunization Practice (ACIP). Part 1: Immunization of Infants, Children, and Adolescents. MMWR 2005; 54 (no. RR-16).
§ CDC. A Comprehensive Immunization Strategy to Eliminate Transmission of Hepatitis B Virus Infection in the United States. Recommendations of the Advisory Committee on Immunization Practice (ACIP). Part 2: Immunization of Adults. MMWR 2006; 55 (no. RR-16).
§ *PEP Steps. A Quick Guide to Postexposure Prophylaxis in the Health Care Setting.* Denver, CO: Mountain Plains AIDS Education and Training Center, in Consultation with National Clinicians' Postexposure Prophylaxis (PEP) Hotline. Last updated April, 2006.
§ Management of Occupational Exposure to HBV, HCV, HIV. Centers for Disease Control and National Clinicians' Postexposure Prophylaxis Hotline. Postexposure Prophylaxis Management website: www.nccc.ucsf.edu (1-888-448-4911).

Revision History The following table represents the revision history of this document.

REVISION	ISSUED	IMPLEMENTED	REASON
6	ACR-09-026	01/04/10	§ Changed title of BSI 149.
5	ACR-09-017	07/06/09	§ Changed title of document. § Merged with SM0036.
4	ACR-08-031	12/29/08	§ Corrected form name.
3	MCO-07-20	12/03/07	§ Changed Donor Panel to BSI Post Exposure Panel.
2	COD-010-07-RM	04/02/07	§ Added to Materials block. § Corrected form names. § Changed form name of BSI 124 series and BS 573. § Added to Responsibility block. § Added report requirement to Immediate Care block. § Reworked and renamed Determining Risk, Evaluating Exposure Source and Evaluating Exposed Person blocks. § Added Initial Evaluation of an Incident/ Initial Action, Determining if Incident Qualifies as an Exposure Incident, Evaluation of an Exposure Incident and Management of Exposed Person blocks.

Blood Systems, Inc.

DOCUMENT	REV.	SPONSOR	
TE0008	13	T&E	Page 1 of 9

Training Documentation

Purpose

To establish the general requirements for training documentation, ensure compliance with CFR standards and meet Blood Systems goals for complete, accurate and current training documentation.

Scope

Employee training, education and development documentation at Blood Systems includes:

- § Employee hard copy training files
- § Attendance Documentation
- § Competency Documentation
- § Program Documentation
- § Network training for Learning Management System (LMS)

Related Documents

On Track Database, TE0010
Competency Assessment, TE0013

Blood Systems, Inc.

Employee Training Files

Individual hard copy training files must be maintained for all employees in a lockable room or cabinet with limited access.

Minimal requirements for each file	Optional (if applicable to employee's position)	Prohibited Items
§ Current Education & Experience Summary, TED 101, STD 101 § General Development (Technical or Non-Technical) Training Profile, TED 152 or TED 152T, TED 152B	§ Copies of certificates of attendance (internal and external, conferences, vendor training, etc.) § Competency Documentation (Trainer Evaluation/Certification (TEC), Training and Competency Records (TCRs), Competency Assessment Record (CARs), etc.) § Other records or certifications (e.g., CLIA, CPR, Language required)	Employee training files must ***not*** contain: § Salary or raise information § Corrective (disciplinary) action documentation § Job performance evaluations § Personnel action § Demographic data § Reports of medical conditions § Tests or answer sheets (unless specifically instructed) § Training Event Attendance Records, TED 100 or copies § BSI Computer Security Employee Responsibility BSI 436 dated after 07/02 **NOTE: Any documentation that contains prohibited information must be blackened out.**

Continued on next page

Training Documentation

Attendance Documentation		
Form	**Use**	**Disposition**
Training Event Attendance Record, TED 100	§ Documents employee attendance of training programs for internal training events, courses, workshops, self-study programs, etc. including completion of ***knowledge-based*** tasks. § Employees must attend the entire training event to sign the TED 100. § This may also include required training provided by vendors. § All manufacturing related SOP revision training, including "Read Only", will be documented on a TED 100, with the possible exception of Center Specific or Center Internal Procedures (CSPs or CIPs)	Completed TED 100s are entered into the LMS and maintained by the Training Specialist (TS). The documents may be maintained at the TS location and not necessarily at the main center.
Sign-Off Record, TED 103	Documents communication of information in read only Correspondence memo and other document(s). Typically used for clarification of regulatory issues. Signatures indicate employees have read required information and have had any questions answered by the supervisor and/or CO.	§ Completed TED 103s can be maintained by the TS or Department Heads. § TED 103s do not get entered into LMS, except for the following. § If TED 103s are used for documenting training of CSPs or CIPs the center can elect to enter the TED 103 into the LMS ad hoc.
Agent Training History TED 105A	Optional form documents Agent training if not documented on a TED 100. **NOTE: Agents are volunteers, temporary employees or contractors working in non-regulated areas over 80 hours per year. Refer to TED 161 for training requirements.**	Completed TED 105s are maintained in employee training files or designated agent file.

Continued on next page

Training Documentation (continued)

<table>
<tr><th colspan="4">Competency Documentation</th></tr>
<tr><th>Form</th><th colspan="2">Use</th><th>Disposition</th></tr>
<tr><td rowspan="8">Training & Competency Record (TCR)

and

Competency Assessment Checklist (CAC)</td><td colspan="2">Documents the completion of a skill-based task. The TCR or CAC documents both the completion of training and the competency evaluation (observation).

NOTE: Use of “N/A” is not allowed on TCRs or CACs. Approved alternate documentation is listed in Competency Assessment, TE0013.</td><td>Completed TCRs and CACs are entered into the LMS and maintained in the employee training files.</td></tr>
<tr><td>If</td><td>And Employee</td><td>Then</td></tr>
<tr><td rowspan="2">Initial Assessment</td><td>PASSES</td><td>File signed TCR or CAC in employee’s training file.</td></tr>
<tr><td>Does NOT PASS</td><td>Retrain employee, dispose of TCR, CAC and repeat initial assessment with new TCR or CAC.</td></tr>
<tr><td rowspan="2">Annual Assessment (if applicable)</td><td>PASSES</td><td>File signed TCR or CAC in employee’s training file.</td></tr>
<tr><td>Does NOT Pass</td><td>§ Mark “NO” on the TCR or CAC for that function and retain in the employee’s training file.
§ Remove the individual from performing that task.
§ Retrain the individual.
§ Repeat assessment</td></tr>
<tr><td rowspan="2">“Other” Assessment (e.g. retraining, rehired staff, or responses to corrective actions)</td><td>PASSES</td><td>File signed TCR in the employee’s training file with an explanation for “Other”.</td></tr>
<tr><td>Does NOT Pass</td><td>§ Mark “NO” on the TCR for that function and retain TCR in the employee’s training file.
§ Remove the individual from independent performance of the task.
§ Determine best appropriate corrective action.</td></tr>
</table>

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Training Documentation (continued)

Competency Documentation (continued)		
Form	**Use**	**Disposition**
Competency Record, TED 102 (Optional)	Form can be used as a running log of competency completion.	Completed forms may be filed in the employee training file as a coversheet for TCRS, or in a manager's file.

Management Oversight

For areas that require task training, management is required to have a system in place that lists, by employee, those tasks the employee is qualified to perform. (e.g. LMS reports, TED 102s, excel spreadsheets, etc.).

Program Documentation

Program documentation must be maintained for all training events documented on a TED 100.

Program Documentation	
Examples	**Filing**
Program Documentation is defined as training program outlines, lesson plans, handouts, checklists, copies of overheads, competency assessments, SOPs, forms, etc. must be maintained (in ring binders, folders, electronically, etc.) separate from the employee training file. § Documentation for standardized training programs will be maintained by Central Office Training & Education Department. § Documentation for center developed training programs must be kept at the center level.	Program documentation must be filed in a manner that allows easy accessibility. Program documentation and TED 100s may be filed together or separately.

Special Situations

UBS and UBS CC Terminated Employee Files	Upon termination or resignation of an employee, center or department administrative personnel should forward the training file with the Terminated Student History training database printout (refer to Training & Education Database, TE0010) and the employment file to the Human Resources department at Blood Systems, per the established schedule.	
Transferred Employee Training Files (Does not apply to BCP)	Upon transfer to a different center, employee training file will be promptly forwarded to the new center Training Specialist in its entirety.	
CO Quality Staff	CO Quality Staff located in the field at centers are supported by the TS at the center and will have their training documentation and files maintained at the center.	
Missing or Lost Training Documentation	In the event that hard copy training documentation cannot be located, follow one of the options below.	
	If the trainer	**Then**
	Can attest to the participant's attendance at the training	A new record (e.g. TED 100 or TCR) may be recreated. § The trainer completes a new record using the date of the original training and has class participants sign. § The trainer notes the following in the Comment section of the record and EC and date comment. § Misplaced/Lost Original: Document recreated on (current date)
	Is unsure of a participant's attendance at the training	Conduct subsequent retraining. § Use a new TED 100 or TCR/CAC to document participation.

Continued on next page

Special Situations (continued)

Remote Training Documentation	**Definition:** § When synchronous (live, real time) training is provided and the trainer is physically at another location. § Examples include Live Meeting teleconferences, videoconferences, etc. **Procedure:** All remote training must be documented on a Training Event Attendance Record, TED 100. Each remote location must designate a training monitor for each remote training session who will have the following responsibilities: § Ensure that the remote training is documented accurately at the center. § Confirm attendance and present the TED 100 for signatures of center attendees. § Sign the Signature of Trainer line on the TED 100 and indicate Monitor for remote training session – Delivered by _____ (print name and title of trainer). **NOTE: The Monitor may also be a participant of the training session and sign the TED 100 as both a participant and monitor.**
Self-Study Documentation	**Definition:** When asynchronous, self-directed, training is completed. Examples: § Recorded web conferences, online training courses, self study modules, “Read Only” SOP revision training, etc. **Procedure:** The attendee will sign a Training Event Attendance Record, TED 100, per procedure and will write “Self-Study” on the signature line of the trainer to indicate the training was conducted as a self-study.
Trainer Credit	All Trainers should sign the TED 100 as the Trainer and as an Attendee the first time he or she delivers the training. This will ensure the trainers’ name will be entered into the LMS.

Continued on next page

Special Situations Trainer Credit (continued)

	If	Then
Manufacturing SOP Revision Training	TED 100 documents the completion of SOP revision training which includes completion of training (slide presentation) and "Read Only" SOPS	Use the date of completion of training (slide presentation) on the TED 100 and ensure that employees read all of the required SOPs prior to viewing.
	TED 100 documents completion of SOP revision training for just "Read Only" SOPs	Use the SOP revision implementation date on the TED 100. Each employee signature indicates that they have completed the required reading prior to the implementation.
	The training is "Read Only"	No trainer signature is required on the TED 100
Disaster/ Volunteer Training	Emergency use of volunteers (working less than 80 hours per year) may be implemented to perform specific regulated functions with minimal training documentation. These volunteers will not be subject to the Agent training requirements. § Refer to the Disaster Recovery Plan Manual for specific tasks/training and documentation requirements.	

Revision History

The following table represents the revision history of this document.

REVISION	ISSUED	IMPLEMENTED	REASON
13	ACR-09-036	01/04/10	§ Changed title of personnel file.
12	ACR-09-025	08/03/09	§ Added clarification to Training Event Attendance Record, TED 100. § Removed verbiage From Special Situations. § No trainer signature is required on the TED 100 if training is "Read Only."

Continued on next page

Blood Systems, Inc.

Revision History (continued)

REVISION	ISSUED	IMPLEMENTED	REASON
11	ACR-08-045	12/29/08	§ Clarified "Read Only" manufacturing training documentation. § Added Management Oversight block. § Clarified missing or lost training documentation. § Clarified Manufacturing SOP Revision Training.
10	MCO-07-20	12/3/07	§ Clarified documentation requirements and LMS entries for TED 100s and TED 103s.
9	COD-067-07-TR	05/14/07	§ Changed Training Facilitator (TF) to Training Specialist (TS). § Added comment concerning implementation date in LMS for TED 103s. § Changed TCBB to UBS CC.

Blood Systems, Inc.

DOCUMENT	REV.	SPONSOR	
DN0193	6	DN	**Page 1 of 3**

Nucleic Acid Amplification Technology (NAT) Testing for West Nile Virus (WNV)

Overview

In addition to the routine screening tests currently performed on each blood donation, effective July 1, 2003, Blood Systems, Inc. implemented Nucleic Acid Amplification Technology (NAT) testing for the West Nile Virus (WNV) as part of an experimental protocol. A WNV test assay was licensed by the Food and Drug Administration (FDA) on December 1, 2005. Blood Systems Laboratories implemented the licensed assay on May 1, 2006.

Related Documents

Comments and Deferrals in Donor Demographics, COL0027

Notification

Donors with reactive WNV IDT NAT Assay results are deferred and notified.

If	Then
The individual specimen tests positive for WNV by Procleix® WNV NAT Assay	Send a donor notification letter as confirmation of testing results when complete results are available.
Others markers are positive	Proceed as above.

- § Donor Counseling & Notification Services sends the Notification Letter, Laboratory Findings, and West Nile Virus (WNV) Information Sheet, BS 984B to all donors testing positive for WNV when complete test results are available.
- § Centers notify physicians of autologous and hereditary hemochromatosis (HH) donors using Letters J-23 and J-23 (HH).

Donors NOT Enrolled in Study

Donors not enrolled in the Virology and Immunology WNV Study will be deferred for 120 days from the reactive test result.

Blood Systems, Inc.

DOCUMENT	REV.	SPONSOR	
DN0193	6	DN	**Page 2 of 3**

Virology & Immunology WNV Study

Donors enrolled in the Virology & Immunology WNV Study will be identified by Medical Affairs/DCNS based on the S/CO ratio of the IDT WNV NAT positive index donation.

- § MA/DCNS staff will contact the donor for enrollment in the study.
- § Medical Affairs will enter an 18 month deferral for enrolled donors when complete test results are available, following procedural steps in Comments and Deferrals in Donor Demographics, COL0027.
- § Samples will be collected from the donor per BSRI schedule.
  - § First sample to be collected ASAP.
- § Sample collections are to be coordinated by the local blood center.
- § These samples will be shipped to Blood Systems Research Institute (BSRI) for testing.
- § Donors enrolled in Virology & Immunology WNV Study will be re-entered 120 days from the date of the most recent WNV sample collection.

Notification Summary

Refer to the following table to determine what letter is required for a specific scenario.

Letters	WNV NAT Pool	WNV NAT Individual	Retest WNV NAT Individual	WNV IgM	WNV IgG
W-1	Reactive	Reactive	R/NR/QNS	Pos/Neg/Equiv	Pos/Neg/Equiv
W-2 V & I Study	Reactive	Reactive	R/NR/QNS	Pos/Neg/Equiv	Pos/Neg/Equiv
W-4 WNV Re-entry letter					

Notification Files

Donor Counseling and Notification Services maintains files on all WNV positive donors that contain:

- § Progesa reports
- § Laboratory results
- § Copies of notification letters
- § Deferral records

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DOCUMENT	REV.	SPONSOR	
DN0193	6	DN	**Page 3 of 3**

References

- § FDA Approves First Test to Screen for West Nile Virus in Donors of Blood, Organs, Cells and Tissues – FDA News Release – P05-93, December 1, 2005.
- § FDA Guidance: Assessing Donor Suitability and Blood and BIood Product Safety in Cases of Known or Suspected West Nile Virus Infection, June 2005.

Revision History

The following table represents the revision history of this document.

REVISION	ISSUED	IMPLEMENTED	REASON
6	MCR-09-013	08/03/09	§ Updated deferral and re-entry information.
5	MCO-07-18	08/27/07	§ Updated to reflect 2007 WNV Research Study.
4	MCO-06-15	06/12/06	§ Updated to reflect FDA licensing of WNV Assay and 120 day deferral.
3	MCO-05-23	06/06/05	§ Updated to reflect new IND algorithm and FDA draft guidance.
2	MCO-04-38	1/24/05	§ Updated to reflect new IND algorithm. § Changed timeframe to 56 days post index donation.

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DOCUMENT	REV.	SPONSOR	
TE0010	10	T&E	**Page 1 of 6**

OnTrack Database

Purpose

§ To provide a networked, windows-based environment to record, track and ensure required training is conducted at Blood Systems.

NOTE: Data entry was suspended on January 25, 2007 in preparation for migration to the LearnCenter Learning Management System but was resumed on May 23, 2007.

§ To provide Central Office access to system wide training data.
§ To provide back up documentation for the Records Management System.

Policy

§ This database does not replace hard copy original source documents.
§ Refer to the original source training documentation for proof of training and/or competency.
§ All data entered/updated in the database must be completed within four weeks of the event.
§ Centers conducting or hosting training events are responsible for the data entry of the event, regardless of the division code of the attendee.

Related Documents

OnTrack Users Guide

Access & Security

Central Office Training & Education OnTrack Application Administrator and the Security Officer will issue security clearances and access privileges for all users.

§ A Computer System Security Authorization, BS 441 must be completed and sent to the Security Officer for access privileges.

OnTrack Security Levels:

§ View Only – Access to view records and pull reports
§ Level Two - Access to enter certain student training events
§ Administrator – System Administrator and Application Administrator

The Training & Education OnTrack Application Administrator maintains security documentation.

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DOCUMENT	REV.	SPONSOR	
TE0010	10	T&E	**Page 2 of 6**

Required Training

Access to OnTrack will be granted after training has been conducted using the Evaluation Copy (training environment) of OnTrack for Training, along with the OnTrack Users Guide. Contact the Training Specialist or designee at the center.

Document training on a TED 100 titled "OnTrack Training, View Only (or Level Two)." A copy of the TED 100 shall be sent to the OnTrack Application Administrator, CO Training & Education Department, and a new user account will be created.

§ Document the applicable security level (not both levels) on TED 100.

View Only Training

Training must be conducted on the following OnTrack Job Aids in the OnTrack Users Guide to receive a View Only security clearance:

§ OT0002 – General Introduction to OnTrack
§ OT0004 – Logging into OnTrack
§ OT0006 – Maintain Menus
§ OT0008 – Changing Passwords
§ OT0010 – Customize Student Screen
§ OT0040 – Student Training History (reports)

Level Two Training

Training must be conducted on the following OnTrack Job Aids in the OnTrack Users Guide to receive a Level Two security clearance:

- OT0002 – General Introduction to OnTrack
- OT0026 - OnTrack for Training Process Flow
- OT0004 – Logging into OnTrack
- OT0006 – Maintain Menus
- OT0008 – Changing Passwords
- OT0009 - User Screen Options
- OT0010 – Customize Student Screen
- OT0011 - Customizing Class Schedule and Classes Held Screens
- OT0015 - Adding Instructors
- OT0017 - New/Update Student Detail
- OT0018 - Quick Scheduling Using the Instructor Led Screen
- OT0024 - Quick Registration from Class Schedule and Classes Held Screens
- OT0028 - Registering Students from a Different Center
- OT0029 - Transferring Process
- OT0030 - Transferring a Class to Held from Class Schedule Screen
- OT0032 - Adding Self-Study Activity
- OT0033 - Adding ADHOC Classes
- OT0034 - Entering Evaluations
- Entire Reports Section, OT0036 - OT0042
- Entire Exporting Date Section, OT0043 and OT0044

Data Retention & Back Up

- Hard copies of all data entered into the system by Training Specialists or designees must be retained in a "hold" file for a minimum of two business days.
  - This policy ensures availability of the data in case of database failure.
- Completed Training Event Attendance Records, TED 100s, Training & Competency Records (TCRs, CARs, CACs, and CBT certificates) will be maintained for hard copy documentation.
  - The database is incrementally backed up nightly.
  - Each week, the entire database will be backed up and retained for five weeks.

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DOCUMENT	REV.	SPONSOR	
TE0010	10	T&E	**Page 4 of 6**

Protected Data

The following data is protected from unauthorized additions, modifications and deletion:

- § Job Title Table/Required Skills
- § Instructor Led Courses
- § Self Study Courses

Student Data

Training Specialists or designees will update new or transferred/promoted employee(s) and will remove non-required courses that have been posted to the student's record and have not yet been completed when updating student data at their center. This shall be completed within four weeks of the weekly Information Technology download from the Human Resources database.

- § Refer to New/Update Student Detail, OT0017 for instructions

Attendance Data

- § Centers conducting or hosting training events are responsible for the data entry of the event, *regardless of the division code of the attendee*
- § Source documents for the OnTrack database data entry are:
- § TED 100s
- § Certificates
- § Training & Competency Records (TCRs, STDs, CARs and CACs)
- § TED 150 or BSI 193

NOTE: Sign-off Records, TED 103s, shall only be entered into the OnTrack database if so indicated by LMS ID/Title information in a COD, Release Memo, etc. If no LMS ID/Title information is present, TED 103s *shall not* be entered into the OnTrack database.

Class Evaluation Data

- § Evaluation data will be entered using the standard evaluation sets established by Central Office Training & Education.
- § Evaluation sheets may be discarded after data entry or appropriate action has been taken.

Report Requirements

The table below describes the required reports. For further information regarding other types of reports, refer to the OnTrack Users Guide.

Report	Explanation
System Training Report	§ This report is printed by course, listing those employees who are required to attend but have not yet completed the training courses. § Required courses - this report is printed for all courses listed on TEDs and STDs 152, 152T, 153 and 153T and forwarded to center management. § Required timeframe - twice annually, at a minimum **NOTE: This report can be a Quick Activity Query or a Quick Report in OnTrack.**
Employee Termination Report	§ This report shall be printed within four weeks of termination. § A Student Training History shall be printed/run and placed in the training file. § This report contains information regarding training/course activities completed by the employee during their employment with Blood Systems.

Center Specific Courses

To request the addition of a continuous center-specific course:

- § Send an email request to the Central Office Training & Education OnTrack Application Administrator.
- § CO T&E will evaluate the request, contact the center for clarification and add approved courses within one week of receipt.
- § ADHOC entry is the preferred method for data entry of one-time-only and outside vendor training courses.

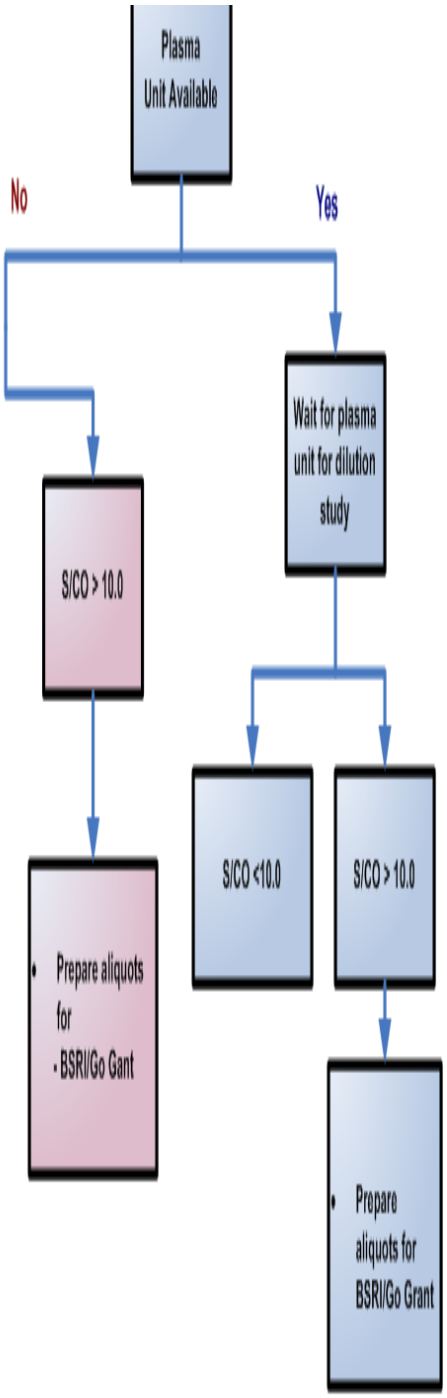
OnTrack for Training Assistance

- § CO Training & Education OnTrack Application Administrator is available for assistance and troubleshooting.
- § Report difficulties with the network or hardware through the Blood Systems IT Support Center.
- § Any updates to the software must be cleared through the Computer Change Control process.

Revision History

The following table represents the revision history of this document.

REVISION	ISSUED	IMPLEMENTED	REASON
10	ACR-09-025	08/03/09	§ Changed requirement of original to copy of TED 100 as proof of training. § Clarified when new user account is created. § Changed method of request for the addition of a continuous center-specific course.
9	ACR-09-001	02/09/09	§ Clerical corrections. § Removed references to use of the skills side of OnTrack.
8	COD-174-07-TR	11/26/07	§ Reflects 05/23/07 recommencement of data entry into OnTrack due to delay in LMS implementation.
7	COD-067-07-TR	03/14/07	§ Changed title of document. § Reflects 01/25/07 freeze of data entry into OnTrack and retention of OnTrack database for lookback purposes only.
6	COD-054-04-TR	4/29/04	§ Added the requirement to remove skills from the training database. § Added Quick Report to Report Requirements block.


Plasma Unit Available
No
Yes
Wait for plasma unit for dilution study
S/CO > 10.0
S/CO <10.0
S/CO > 10.0
Prepare aliquots for
- BSRI/Go Gant
Prepare aliquots for BSRI/Go Grant

June 12, 2012

Creative Testing Solutions

DOCUMENT	REV.	
CTS-00408-SOP	1	Page 1 of 3

Unlinking Samples for Clinical Trial or Research Testing

Purpose

To provide instructions for unlinking samples for use with clinical trials or research testing. Samples are unlinked from donor identifiers prior to any testing for the clinical trial. Following completion of the unlinking process, samples may be used for clinical trial or research testing purposes.

Related Documents

Clinical Trial Management Procedure, CTS-00407-SOP

Materials

- Adhesive labels or Validation labels
- Labeling System (e.g. Zebra label printer, Smart Label Printer, etc.)
- Tubes and caps for aliquoting
- Black marker
- Samples as required according to the clinical trial

Determine Samples Required

Identify appropriate sample groups required according to the clinical trial protocol.

Samples may remain linked to donor identifiers.

- Refer to Clinical Trial Management Procedure, CTS-00407-SOP for management of linked samples.

Samples may be unlinked from donor identifiers.

- Test results generated prior to unlinking the samples may not be used for clinical trials intended for FDA submission.
- Non-identifying donor demographics (e.g., gender, race, ethnicity, approximate age, clinical symptoms) and/or test results may be provided for research testing of unlinked samples.

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Determine Validation Numbers Required

Identify appropriate validation numbers required according to the clinical trial protocol.

- Examples include Validation barcodes, sequential number series or random number series.

NOTE: Validation numbers are determined by the applicable protocol.

Unlinking Pilot Tube Samples

Perform the following steps for samples requiring pilot tubes.

Step	Action
1	Pull samples required for clinical trial or research.
2	Completely obliterate both the barcode number and the eye readable number with a black marker or completely remove the barcode number from the sample. ▪ Ensure both numbers are completely obliterated or completely removed.
3	Place validation number/barcode over the obliterated label or tube(if barcode is removed).
4	Route samples for appropriate testing.

Unlinking Aliquot Tube Samples

Perform the following steps for samples requiring aliquot tubes.

NOTE: If non-identifying donor demographics and/or test results are required for research purposes, refer to the applicable instruction block below.

Step	Action
1	Pull samples required for clinical trial or research.
2	Aliquot samples one at a time into the appropriate aliquot tube(s). ▪ It is acceptable to prelabel aliquot tubes. ▪ Ensure only one sample tube and its corresponding aliquot tube(s) are open during aliquoting. ▪ Maintain caps on all other tubes during this process or maintain other tubes in a separate work area protected from contamination.

Continued on next page

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Unlinking Aliquot Tube Samples (continued)

Step	Action
3	Verify aliquot tubes have the appropriate validation number/barcode.
4	Route samples for appropriate testing.

Providing Non-Identifying Information and/or Test Results for Research

Perform the following steps.

Step	Action
1	Generate a table containing the required non-identifying information and/or test results to be used for research.
2	Assign applicable validation or research numbers to each sample in the table.
3	Prepare applicable aliquots of each sample using the assigned validation or research number as described above.
4	Remove any identifying information from the table generated in Step 1 above.
5	Route samples, non-identifying information and/or test results as applicable.

Revision History

The following table represents the revision history of this document.

Revision	Reason
1	▪ Initial release into SmartSolve.

WNV Aliquoting IP-126

Date Posted: _____ EC: _____

Sample Type	Test Results Are	Aliquot Information **Verify all testing is complete in DTL**
UBS Center/BCP sample Donation **does not** have a plasma unit	S/CO ≥ 10.0	• Prepare plasma aliquots from the 2nd WNV tube •
UBS Center/BCP sample Donation **does** have a plasma unit	S/CO ≥ 10.0	• Prepare plasma aliquots from the 2nd WNV tube • Plasma Unit • Prepare plasma aliquots for GO grant

West Nile Virus Reactive Donations

Step	Task	DCNS Manager/ Designee	DCNS Counselor	Notif. Spec.	AA II
1	Receive WNV Alert and assign	*****			
2	Enter Reactive donation in Database on day case assigned; forward file to specified counselor			*****	
3	Send Email to MDs, etc., on day case assigned		*****		
4	*If S/CO >10, contact the donor (within 1 day of case assignment) to notify of the test result and recruit for enrollment in the study. **Use the Research Subject Information and Consent Form to document verbal consent. Touch on each subject in the form. You do not have to read it word for word. If the donor agrees, print the donor's name in the subject line on page 5 and sign and date on the line below.** Administer WNV Questionnaire A. Update database. If S/CO <10, go to Step 10		***** (see step # 7 if unable to contact donor by telephone)		
5	Forward questionnaire to manager or a counselor for review by 2nd day after case assigned. If not completed, advise manager or designee		*****		
6	Review Questionnaire A, database entry and update questionnaire status, as applicable, within 1 day of receipt. Update database. Forward questionnaire to AAII.	*****	******		
7	If unable to contact donor by telephone, send a pending WNV letter with instructions for the donor to call DCNS counselor.		*****		

Step	Task	DCNS Manager/ Designee	DCNS Counselor	Notif. Spec.	AA II
8	After successful donor contact • Email the center and BSRI regarding enrollment in study • Include any updated donor contact information and center contact information .		*****		
9	Copy Questionnaire A and the Consent Form and send originals to BSRI via FedEx on a weekly basis. Return a copy of each to Counselor.				*****
10	Receive Supplemental Results from BSL (WNV Retest, IgG, IgM)and forward to Manager or designee			*****	*****
11	Enter Supplemental results in Database on date of receipt	*****			
12	Forward Supplemental results to Counselor for review and Notification Specialist for donor file, as applicable	*****			
13	Review database entry of Supplemental Results, within 1 day of receipt **Update the donor's WNV deferral in Progesa to an 18 month deferral, per current guidelines and SOPs (TA0027) if enrolled in the study and donation has archived.**		*****		
14	Notify local Health Department via Email. FAX or telephone if retest is reactive or IgG or IgM reactive, within 1 day of receipt			*****	
15	Prepare Donor Notification Letter when Progesa Report is received, or within 1 week of complete Supplemental results, and forward for review.			*****	
16	Review Notification letter and return to Notification Specialist within 1 day.		*****		
17	Mail notification letter, per DCNS mailing schedule			*****	*****
18	Administer Questionnaire B, 2 weeks after index donation date and update database. If unable to contact after 3 telephone attempts, send a letter requesting the donor call DCNS.		*****		
19	Review Questionnaire B and update database.	*****			

Step	Task	DCNS Manager/ Designee	DCNS Counselor	Notif. Spec.	AA II
20	Copy Questionnaire B and send original to BSRI via FedEx on a weekly basis. Return copy to Counselor.				*****
21	Copies of Q'aire A & B will be filed with the donor's notification letter.		*****		
22	Disposition of WNV Notification Files – File with monthly Center letters.			*****	
23	**When notified by BSRI that the donor has had his/her last sample collected, or is lost to follow-up per the center or BSRI:** • **Update the donor's WNV deferral to 120 days after the last sample collection, per current guidelines and SOPs (TA0027).** • Re-entry file/letter is not required. • For SafeTrace centers, notify designee of study completion or discontinued sample and designee will update the SafeTrace deferral record		*****		

* Explain sample collection for the study.
(Refer to BSRI documents for specimen collection schedule.)
Inform enrollee of current payment per sample for time and travel, and current payment schedule, as per the Consent Form.
Instruct the donor to contact his/her blood center to schedule sample collection, with the first sample to be collected within the week of donation.

Nlk 06/16/2011

West Nile Virus Reactive Donations

Step	Task	DCNS Manager/ Designee	DCNS Counselor	Notif. Spec.	AA II
1	Receive WNV Alert and assign	*****			
2	Enter Reactive donation in Database on day case assigned; forward file to specified counselor			*****	
3	Send Email to MDs, etc., on day case assigned		*****		
4	*If S/CO >10, contact the donor (within 1 day of case assignment) to notify of the test result and recruit for enrollment in the study. **Use the Research Subject Information and Consent Form to document verbal consent. Touch on each subject in the form. You do not have to read it word for word. If the donor agrees, print the donor's name in the subject line on page 5 and sign and date on the line below.** Administer WNV Questionnaire A. Update database. If S/CO <10, go to Step 10		***** (see step # 7 if unable to contact donor by telephone)		
5	Forward questionnaire to manager or a counselor for review by 2nd day after case assigned. If not completed, advise manager or designee		*****		
6	Review Questionnaire A, database entry and update questionnaire status, as applicable, within 1 day of receipt. Update database. Forward questionnaire to AAII.	*****	******		
7	If unable to contact donor by telephone, send a pending WNV letter with instructions for the donor to call DCNS counselor.		*****		

Step	Task	DCNS Manager/ Designee	DCNS Counselor	Notif. Spec.	AA II
8	After successful donor contact • Email the center and BSRI regarding enrollment in study • Include any updated donor contact information and center contact information .		*****		
9	Copy Questionnaire A and the Consent Form and send originals to BSRI via FedEx on a weekly basis. Return a copy of each to Counselor.				*****
10	Receive Supplemental Results from BSL (WNV Retest, IgG, IgM)and forward to Manager or designee			*****	*****
11	Enter Supplemental results in Database on date of receipt	*****			
12	Forward Supplemental results to Counselor for review and Notification Specialist for donor file, as applicable	*****			
13	Review database entry of Supplemental Results, within 1 day of receipt **Update the donor's WNV deferral in Progesa to an 18 month deferral, per current guidelines and SOPs (TA0027) if enrolled in the study and donation has archived.**		*****		
14	Notify local Health Department via Email. FAX or telephone if retest is reactive or IgG or IgM reactive, within 1 day of receipt			*****	
15	Prepare Donor Notification Letter when Progesa Report is received, or within 1 week of complete Supplemental results, and forward for review.			*****	
16	Review Notification letter and return to Notification Specialist within 1 day.		*****		
17	Mail notification letter, per DCNS mailing schedule			*****	*****
18	Administer Questionnaire B, 2 weeks after index donation date and update database. If unable to contact after 3 telephone attempts, send a letter requesting the donor call DCNS.		*****		
19	Review Questionnaire B and update database.	*****			

Step	Task	DCNS Manager/ Designee	DCNS Counselor	Notif. Spec.	AA II
20	Copy Questionnaire B and send original to BSRI via FedEx on a weekly basis. Return copy to Counselor.				*****
21	Copies of Q'aire A & B will be filed with the donor's notification letter.		*****		
22	Disposition of WNV Notification Files – File with monthly Center letters.			*****	
23	**When notified by BSRI that the donor has had his/her last sample collected, or is lost to follow-up per the center or BSRI:** • **Update the donor's WNV deferral to 120 days after the last sample collection, per current guidelines and SOPs (TA0027).** • Re-entry file/letter is not required. • For SafeTrace centers, notify designee of study completion or discontinued sample and designee will update the SafeTrace deferral record		*****		

* Explain sample collection for the study.
(Refer to BSRI documents for specimen collection schedule.)
Inform enrollee of current payment per sample for time and travel, and current payment schedule, as per the Consent Form.
Instruct the donor to contact his/her blood center to schedule sample collection, with the first sample to be collected within the week of donation.

Nlk 06/16/2011

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Title: Preparation of WNV "Ready-to-go-shippers" for the Natural history and pathogenesis of WNV in viremic donor study.				Page 1 of 2	
Doc. #:	**VRLRC 0001**	**Revision:**		**Effective date:**	**11/02/09**

1	**Purpose**		To outline the responsibilities and to define the steps to be followed for the pre-stocking of "ready-to-go-shippers" used for the Natural history and pathogenesis of WNV in viremic donors study.
2	**Scope**		This procedure is used to ensure the consistency of each "ready-to-go-shippers" and that each UBS Blood Center is pre-stocked with "ready-to-go-shippers".
3	**Responsibility**	3.1 3.2 3.3	It is the responsibility of the Supervisor to ensure that laboratory personnel have been trained in accordance with this procedure before handling any "ready-to-go-shippers" and to ensure that the training is documented. It is the responsibility of laboratory personnel to ensure that he or she understands and follows this procedure when preparing each"ready-to-go-shippers". It is the responsibility of laboratory personnel to record and notify the Supervisor of any deviations from this procedure, which are not accounted for in other procedures.
4	**Materials**	4.1 4.2 4.3 4.4 4.5 4.6 4.7 4.8 4.9 4.10	Committee for Human Research (CHR) approved number, size and type of vacutainer tubes used in the phlebotomy of study participants One Research Subject Information and Consent form One Experimental Subject's Bill of Rights (all sites). One Virology and Immunology WNV Study-Shipping List for Specimens form Phlebotomy Instructions A completed FedEx airbill for shipment to BSRI 4G outer box and polypropylene secondary container with absorbent material (red topped container) EXAKT-PAK overpack 20x15x15 ULINE secondary overpack ULINE Industrial tape
5	**Equipment Required**	5.1	None
6	**Solutions/Buffer required**	6.1	None

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7	**Procedure**	7.1	Insert EDTA (lavender top) tubes, PAXgene (orange top) tube inside the polypropylene secondary container (red cap canister).
		7.2	Place the red cap canister inside the 4G outer box along with: one Research Subject Information and Consent form, one Experimental Subject's Bill of Rights (all sites), one Virology and Immunology WNV Study-Shipping List for Specimens form and Phlebotomy Instructions.
		7.3	On the outside of the 4G outer box place a completed FedEx airbill for shipment to BSRI.
		7.4	Once steps 7.1 through 7.3 are completed, the 4G outer box , i.e. "ready-to-go shipper" is ready.
		7.5	Place between 8 to 10 "ready-to-go shippers" inside a 20x15x15 ULINE secondary overpack.
		7.6	Seal the 20x15x15 ULINE secondary overpack with ULINE Industrial tape and send to specific UBS Blood Center.

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Title: VRLRC WNV Study "ready-to-go-shipper" Inventory		**Page 1 of 2**
Doc. #: VRLRC 0027	**Revision:**	**Effective date: 6/15/10**

1	**Purpose**		To outline the responsibilities and define the steps involved in maintaining a WNV Study Ready-to-go-shipper Inventory
2	**Scope**	2.1	This procedure is used for the oversight of WNV Study "ready-to-go-shippers". Oversight includes: 1) providing each "ready-to-go-shippers" with an inventory number, 2) keeping track of the expiration date of the phlebotomy tubes provided in each "ready-to-go-shipper", 3) keeping track of the date each "ready-to-go-shipper" is shipped to a phlebotomy site, 4) keeping track of the phlebotomy site to which each "ready-to-go-shipper" is shipped and 5) keeping track of when each "ready-to-go-shipper" is received from a phlebotomy site.
3	**Responsibilities**	3.1	It is the responsibility of the Supervisor to ensure that laboratory personnel have been trained in accordance with this procedure before assuming this responsibility and to ensure that the training is documented.
		3.2	It is the responsibility of laboratory personnel to ensure he/she has read, understood, and will follow this procedure while performing daily monitoring tasks.
		3.3	It is the responsibility of VRLRC personnel to record and notify the Supervisor of any deviation from this procedure, which is not accounted for in study specific procedures.
4	**Materials Required**	4.1 4.2 4.3	4G outer box and polypropylene secondary container (red topped container) Committee for Human Research (CHR) approved number, size and type of vacutainer tubes used in the phlebotomy of study participants. "Ready-to-go-shipper" Inventory form (Attachment 1)
5	**Procedures**	5.1	Each "ready-to-go-shipper" must have it's own unique identification number (ID#).
		5.2	When sending out a "ready-to-go-shipper", record on "Ready-to-go-shipper" Inventory form: ▪ "Ready-to-go-shipper" ID# ▪ Phlebotomy site to which "ready-to-go-shipper" is being sent ▪ Date of shipment ▪ Expiration date of 10mL EDTA phlebotomy tubes ▪ Expiration date of 2mL EDTA phlebotomy tubes ▪ Expiration date of phlebotomy tubes for RNA isolation
		5.3	When receiving a "ready-to-go-shipper", record the receipt date on the "Ready-to-go-shipper" form in the appropriate row.
		5.4	Recall "ready-to-go-shippers" at least two weeks before phlebotomy tubes they contain are going to expire.

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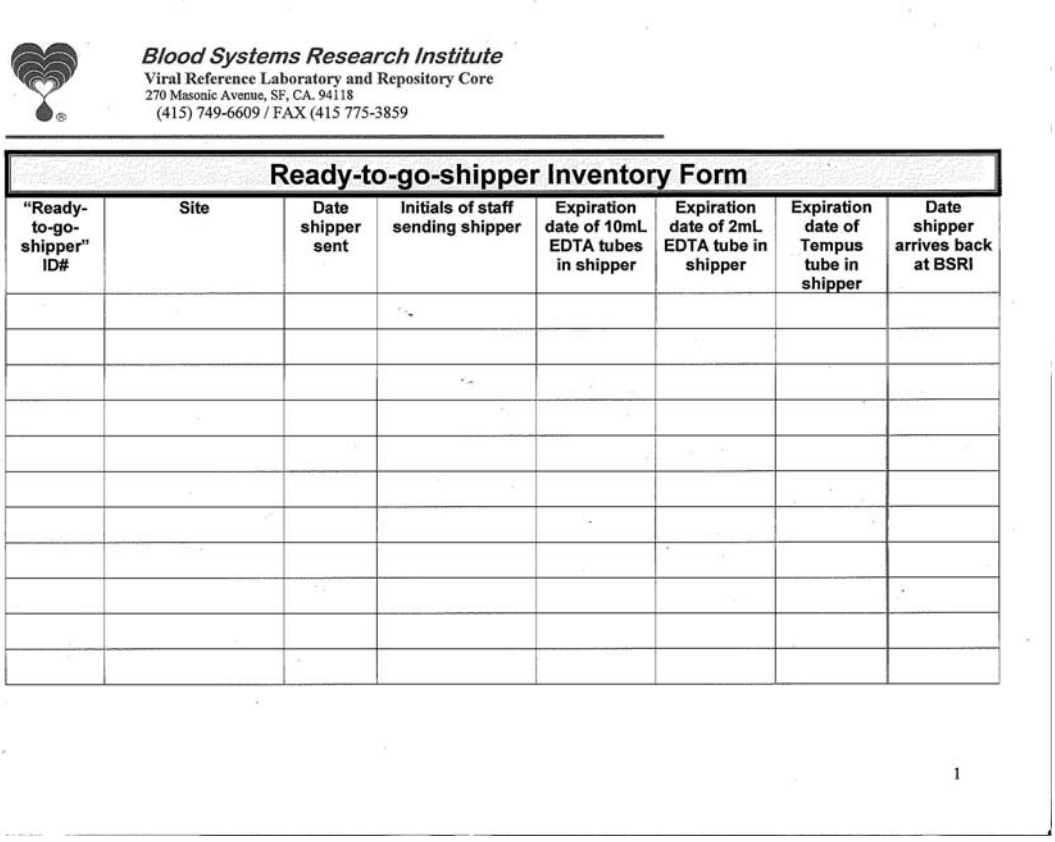
		5.5	Place completed “Ready-to-go-shipper” Inventory forms in the “Ready-to-go-shipper” Inventory forms binder.



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Ready-to-go-shipper Inventory Form

“Ready-to-go-shipper” ID#	Site	Date shipper sent	Initials of staff sending shipper	Expiration date of 10mL EDTA tubes in shipper	Expiration date of 2mL EDTA tube in shipper	Expiration date of Tempus tube in shipper	Date shipper arrives back at BSRI

1

Attachment 1

Blood Systems Research Institute
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Title: Sample Collection for the Natural history and pathogenesis of WNV in viremic donors study				Page 1 of 8	
Doc. #:		**Revision:**		**Effective date:**	

1	**Purpose**		To outline the responsibilities and to define the steps to be followed during the blood specimen collection process for the Natural history and pathogenesis of WNV in viremic donors study.
2	**Scope**	2.1	This procedure is used for specimen collection at remote blood collection sites involved in collecting blood for the Natural history and pathogenesis of WNV in viremic donors study.
		2.2	A West Nile positive blood donor is defined as a blood donor with a WNV RNA positive donation during universal WNV TMA screening.
		2.3	In general there are seven steps to collecting blood specimens from WNV positive blood donors: 1) pre-stocking blood collection sites with "ready-to-go shippers, 2) alerting a blood collection sites that they have a WNV positive donor among their donors, 3) the donor coming for his/her phlebotomy, 4) the donor signing a consent form for the study, 5) the donor being phlebotomized, 6) the blood center staff placing the blood tubes in the "ready-to-go" shipper and 7) placing the shipper for FedEx pickup.
3	**Related Documents**	3.1	Committee on Human Research approved blank consent form
		3.2	WNV Intensive Study Shipping Instruction that comply with current CHR approved version of the study.
		3.3	California Study Participant Bill of Rights (only for California residents).
		3.4	Virology and Immunology WNV- 2009 Study Shipping List for Specimens Shipping List form
4	**Responsibilities**	4.1	It is the responsibility of the Supervisor to ensure that laboratory personnel have been trained in accordance with this procedure before participating in a WNV Recall Study.
		4.2	It is the responsibility of the Viral Reference Laboratory and Repository Core (VRLRC) personnel to ensure he/she has read, understands and follows this procedure while participating in a WNV Recall Study.
		4.3	It is the responsibility of VRLRC personnel to record and notify the Supervisor of any deviation from this procedure, which are not accounted for by study specific procedures.
5	**Materials**	5.1	Ready-to-go-shipper(s) purchased from Exakt-Pak containing: 1) Committee for Human Research (CHR) approved number, size and type of vacutainer tubes used in the phlebotomy of study participants, 2) a blank consent form, 3) a California Study Bill of Rights (only for California resident), 4) phlebotomy instructions for the collection staff, 5) a Virology and Immunology WNV- 2009 Study Shipping List for Specimens, 6) and a completed Fed Ex airbill for return FedEx Priority

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			overnight shipment to Viral Reference Laboratory and Repository Core (VRLRC).

6	**Procedure**	6.1	Participating collection sites are pre-stocked with "ready-to-go" shippers prior to the onset of the WNV season.
		6.2	Each "ready-to-go" shipper contains: 1) CHR approved number, size and type of vacutainer tubes 2) Consent form 3) a California Study Bill of Rights (only for California resident) 4) Phlebotomy instructions for blood collection staff 5) a Virology and Immunology WNV- 2009 Study Shipping List for Specimens form completed Fed Ex air bill for return FedEx Priority overnight shipment to Viral Reference Laboratory and Repository Core (VRLRC)
		6.3	A WNV RNA positive donor is contacted by Blood Systems, Inc (BSI) staff from Medical Affairs and informed regarding the scope of the study and asked for a "verbal" consent.
		6.4	If the donor gives "verbal" consent, designated staff member at both the Blood Collection Site and the VRLRC department are notified by email from BSI Medical Affairs staff.
		6.5	The donor is contacted by a Blood Collection Site staff member and asked to come to the Blood Collection Site in accordance with the CHR approved study protocol.
		6.6	The phlebotomy specimens are shipped at room temperature by FedEx Priority Overnight to the VRLRC.
		6.7	Upon arrival, VRLRC staff check the specimen IDs with those on the Virology and Immunology WNV- 2009 Study Shipping List for Specimens Shipping List form included in the "ready-to-go" shipper.
		6.8	Discrepancy comments are placed in the designated area on the Virology and Immunology WNV- 2009 Study Shipping List for Specimens form that was included in the "ready-to-go" shipper on its return to VRLRC
		6.9	VRLRC staff attach a single version of the label used to process the vacutainer tubes contained the "ready-to-go" shipper is affixed to the Virology and Immunology WNV- 2009 Study Shipping List for Specimens Shipping List form.
		6.10	The Virology and Immunology WNV- 2009 Study Shipping List for Specimens Shipping List form is filed in the appropriate study binder and is retained as the study's hardcopy.
		6.11	The sample specimens are processed in **accordance the appropriate procedures** within 48 hours (preferably within 24 hours).

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7	**Special Notes**	7.1	The signed consent form **must** be included in the first ready-to-go-shipper for each study participant when it is returned to the VRLRC.
		7.2	**Do not** use the participants name anywhere on the shipping form or tubes.
		7.3	The Virology and Immunology WNV- 2009 Study Shipping List for Specimens form must be **faxed** to VRLRC on the day of phlebotomy. The original copy of this form is included in the returning ready-to-go-shipper.

Related Documents are below

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Viral Reference Laboratory and Repository Core

Virology and Immunology WNV- 2009 Study Shipping List for Specimens

Blood Center Location: _____FedEx Tracking #: _____

1. Collection center please fill in the left columns. **The study ID# for study subject can be located directly on the blood tube.**
2. Fax this form to Simon Ng (BSRI-(415)-775 3859) the same day the specimens are sent to BSRI, this alerts our lab staff of sample's arrival.
3. Include this form with shipment, fold and place inside box.
4. Shaded regions to be completed at BSRI lab upon arrival.

For Phlebotomy use only **For Lab use only**

Study ID# (Donor ID #)	Phlebotomy		Vacutainer tubes shipped to BSRI		Date tubes received	All tubes received? (Y/N)	Repository Storage			
Note: for privacy reasons **Do Not** use subject's name on this form, instead use study ID	Date	Time (24 hour clock)	Lavender Top 7 x 10mL + 1 x 4mL	PAXgene Tube (Orange Top 2.5mL draw)			#Aliquots	Box	Position	Freezer
				1						-80 °C WNV PL
							TMA			-80 °C WNV
Name of Phlebotomist							CE			$LiqN_2$ WNV CE
Signature			*Date*		*Condition of Specimens (If not satisfactory, please explain):*					

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2009 WNV Intensive Study Shipping Instructions

Dear Blood Center Staff:

For the 2009 WNV Intensive Study, please draw and ship blood samples to the attention of Simon Ng in our laboratory at 270 Masonic Ave., San Francisco. Each participant in this study is scheduled for:

Step	Action
1	**One** phlebotomy **per week** during the first four weeks following the donor's WNV positive donation, i.e. index donation (see Phlebotomy Schedule below).
2	**One** phlebotomy **during the 6th week** post-index donation
3	**One** phlebotomy **at two months** post-index donation.
4	**One** phlebotomy at **3 months** post-index donation and then an additional phlebotomies at **6, 9 and 12 months** post-index donation.

Phlebotomy Schedule		
Time Points	**Draw Number**	**Phlebotomy volume**
Week one	1st Draw	75 mL
Week two	2nd Draw	75 mL
Week three	3rd Draw	75 mL
Week four	4th Draw	75 mL
Week four	5th Draw	75 mL
Week six	6th Draw	75 mL
Month two	7th Draw	75 mL
Month three	8th Draw	75 mL
Month six	9th Draw	75 mL
Month nine	10th Draw	75 mL
Month twelve	11th Draw	75 mL

If you are reading this memo, you have received a "ready-to-go-shipper(s)" from either a UBS Blood Center or from Blood Systems Research Institute (BSRI). A number of UBS Blood Centers have been pre-stocked with "ready-to-go-shipper". This has been done so that shippers are available, allowing the Blood Center staff to immediately contact the donor once they have been notified by Medical Affairs.

The shipper contains:

Item	Description
1	7 x 10 mL plus 1 x 4 mL EDTA (lavender top) tubes. **Please note the enhanced volume of blood per phlebotomy.**
2	1 x 2.5 ml PAXgene (orange top) tube. **Note**: The blood should be drawn into the fluid in this tube. **Please note** this should be the last tube filled.

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3	Virology and Immunology WNV Study – Shipping List for Specimens form
4	Phlebotomy Instructions
5	A completed FedEx airbill for shipment to BSRI will be attached to the shipper.

Instructions for the consent form:

Step	Action
1	There needs to be one signed consent form per participant. You will receive the blank Consent Forms along with the shippers.
2	This consent form needs to be signed by the participant and a witness at the time of participant's first phlebotomy. The witness can be any staff member that watched the participant sign the consent form and identified the participant. A copy of the signed consent form should be given to the participant.
3	The original signed consent form **must** be included in the "ready-to-go-shipper" with the results of the participant's first phlebotomy.

For questions, you may contact the study coordinator, Nelly Gefter at (415) 567-6400 ext.358

Phlebotomy and Shipping Instructions

PHLEBOTOMY

Step	Action
1	Write participant's donor ID number plus the date and time of the phlebotomy on each of the tubes in the "ready-to-go-shipper".
2	Obtain blood samples by normal phlebotomy procedures.
3	During the phlebotomy procedure, invert the tubes a few times to mix the anti-coagulant.
4	The PAXgene tube should be filled last and mixed ten times.
5	**DO NOT** centrifuge or refrigerate tubes.

PREPARE SHIPPING DOCUMENTS:

Step	Action
1	If this is the participant's first phlebotomy, write the participant's Donor ID number on the signed consent form and include it in the shipper.
2	Fill out the **Shipping List for Specimens,** following instructions 1-4 on the form**. Note:** in order to protect the privacy of individuals, please **DO NOT** use their name anywhere on this form.
3	**FAX** this form to BSRI the **SAME DAY** that you ship the specimens. This alerts our laboratory staff that the shipment is coming and provides the FedEx tracking number to track the shipment during transport, if necessary.
4	Include the **original copy** of the **Shipping List for Specimens** with the shipment.

PACKAGING/SHIPPING SPECIMENS

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Step	Action
	Take the plastic container from the shipper
1	Remove cap from the plastic container
2	Insert filled lavender-top and PAXgene tubes into the holes in the gray foam insert. Fill the center holes first and work outward.
3	Replace the top gray foam disk.
4	Make sure the O-ring is seated in the groove below the threads of the white container.
5	Seal the container by screwing the cap on the container tightly. If any part of the O-ring is showing, the cap is not correctly sealed. Unscrew the cap and correctly seat it on the container.
6	Place the sealed container upright in the insulated shipper. Also, place signed consent form in shipper (if this is the first blood draw).
7	Place the top piece of white EPS foam on top of the white EPS sides. The top EPS foam **must** sit flush on top of the EPS sides.
8	Place **faxed shipping form** and **signed consent form** (only if this is the participant's first phlebotomy) on top of the white EPS foam lid.
9	Close the outer box flaps and seal the top seam of the box with postal-lock tape.
10	Using the postal-lock tape seal both side seams making sure that half the tape is on the top of the box and the other half is on the side of the box.
11	The UN3373/Diagnostic Specimen and Room Temperature labels should already be affixed to the outside of the shipper for your convenience. Please note the FedEX air-bill must NOT BE wrapped around any of the corners.
12	Ship specimens at **Room Temperature** (**DO NOT** add ice or gel packs to shipper).
13	Place shipper for Fed Ex pick-up. If you do not have regular FedEX pick-ups, call FedEX and schedule a same day pick-up.

Thank you for your help with this important research study!

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BLOOD SYSTEMS, INC.
EXPERIMENTAL SUBJECT'S
BILL OF RIGHTS

The rights below are the rights of every person who is asked to be in a research study. As an experimental subject I have the following rights:

1. To be told what the study is trying to find out,
2. To be told what will happen to me and whether any of the procedures, drugs, or devices is different from what would be used in standard practice,
3. To be told about the frequent and/or important risks, side effects, or discomforts of the things that will happen to me for research purposes,
4. To be told if I can expect any benefit from participating, and, if so, what the benefit might be,
5. To be told of the other choices I have and how they may be better or worse than being in the study,
6. To be allowed to ask any questions concerning the study both before agreeing to be involved and during the course of the study,
7. To be told what sort of medical treatment is available if any complications arise,
8. To refuse to participate at all or to change my mind about participation after the study is started. This decision will not affect my right to receive the care I would receive if I were not in the study,
9. To receive a copy of the signed and dated consent form,
10. To be free of pressure when considering whether I wish to agree to be in the study.

If I have other questions I should ask the researcher or the research assistant. In addition, I may contact the Committee on Human Research, which is concerned with protection of volunteers in research projects. I may reach the committee office by calling: (415) 476-1814 from 8:00 AM to 5:00 PM, Monday to Friday, or by writing to the Committee on Human Research, Box 0962, University of California, San Francisco, CA 94143.
Call 476-1814 for information on translations.

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Title: VRLRC procedure for receiving specimens		Page 1 of 5
Doc. #: VRLRC0003	**Revision: 8/6/10** **2nd 9/22/10**	**Effective date:** 4/26/10

1	**Purpose**		To outline the responsibilities and define the steps to be followed when receiving specimens to ensure consistency.
2	**Scope**		This procedure is applicable to all personnel within the Viral Reference Laboratory and Repository Core (VRLRC) department who are engaged in receiving specimens.
3	**Responsibility**	3.1	It is the responsibility of the Supervisor to ensure that laboratory personnel have been trained in accordance with this procedure before receiving specimens within the VRLRC department and to ensure that the training is documented.
		3.2	It is the responsibility of laboratory personnel to ensure he/she has read, understands and follows this procedure when receiving specimens within the VRLRC department.
		3.3	It is the responsibility of laboratory personnel to record and notify the Supervisor of any deviations from this procedure, which are not accounted for by another procedure.
4	**Safety**	4.1	Gloves and lab coats must be worn at all times while working in the laboratory/freezer areas.
5	**Materials**	5.1	FedEx delivered packages containing either 1) Virology and Immunology WNV Study Shipping List for Specimens or 2) another type of invoice.
		5.2	Shipping notification form
		5.3	In-house study specific log sheet
6	**Procedure**	6.1	Use Universal Safety Precautions
		6.2	Shipment notification will be received on the day a sample is shipped.
		6.3	Shipping notification will include blood center name, FedEx tracking number, phlebotomy date, number and size of tubes being shipped, and study ID#.
		6.4	Unpack the contents of each package carefully.
		6.5	When receiving a shipment note any and all deviations on the shipping notification form. Note: For WNV use the check box at the bottom of the form only if a deviation has occurred.
		6.6	For WNV shipments note occurrence of deviations in Freezerworks.
		6.7	Retain original box for reuse and return to the shipping area within the VRLRC Department.

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		6.8	Verify sample identification numbers with the invoice that arrives with the package.
		6.9	Copy specimen identification numbers along with any comments onto a VRLRC study specific log sheet.
		6.10	Date VRLRC study specific log sheet.
		6.11	Place invoice(s) and study specific log sheet(s) into the study specific log book.
		6.12	Make “in house” working labels using appropriate labeling program. Note: Labeling of tubes is study specific.

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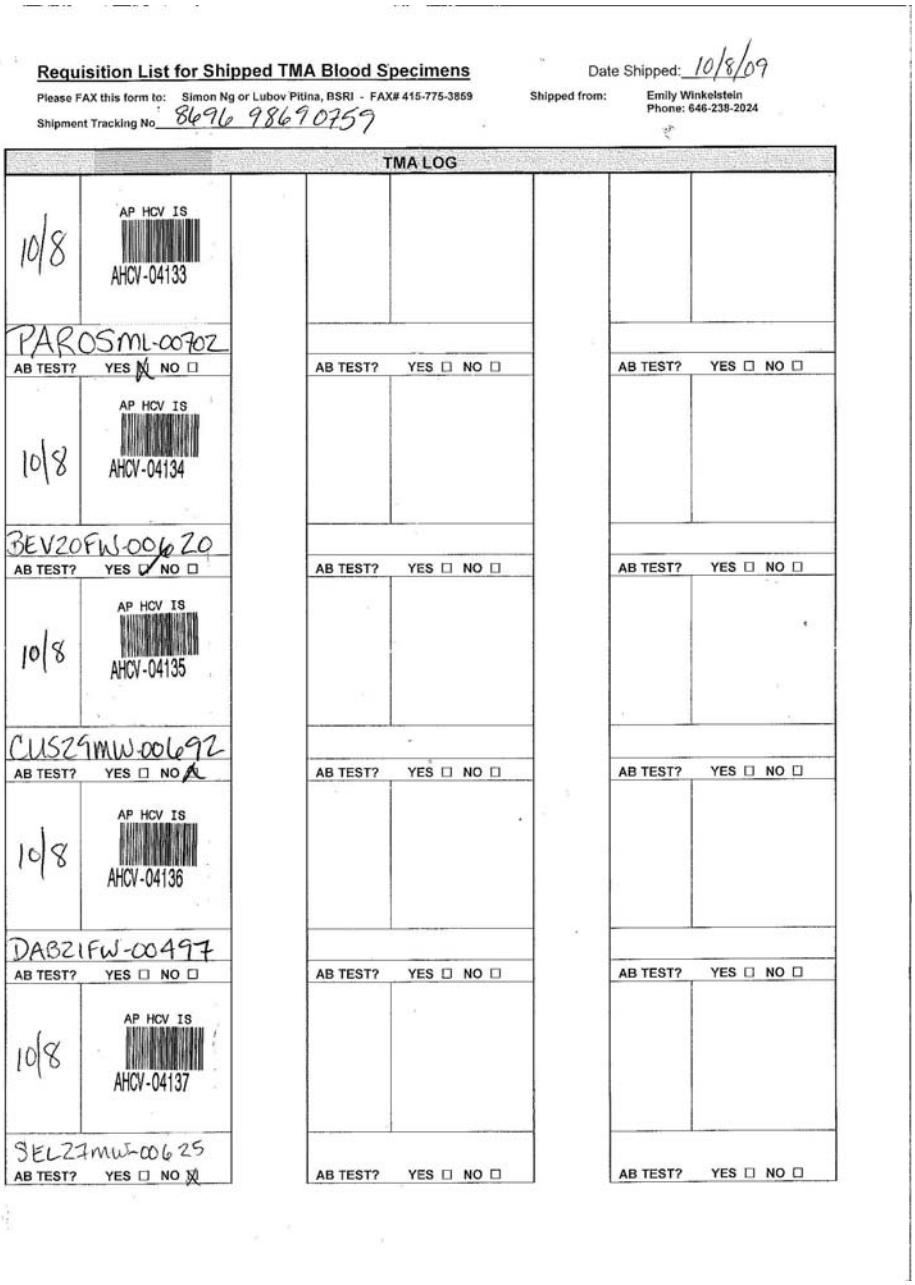
Attachments below are examples of: 1) a study specific invoice 2) shipping notification and 3) a study specific log sheet.



Requisition List for Shipped TMA Blood Specimens

Date Shipped: 10/8/09

Please FAX this form to: Simon Ng or Lubov Pitina, BSRI - FAX# 415-775-3859

Shipped from: Emily Winkelstein
Phone: 646-238-2024

Shipment Tracking No. 8696 9869 0759

TMA LOG

10/8 AP HCV IS AHCV-04133			
PAROSML-00702 AB TEST? YES ☒ NO ☐	AB TEST? YES ☐ NO ☐	AB TEST? YES ☐ NO ☐
10/8 AP HCV IS AHCV-04134			
BEV20FW-00620 AB TEST? YES ☑ NO ☐	AB TEST? YES ☐ NO ☐	AB TEST? YES ☐ NO ☐
10/8 AP HCV IS AHCV-04135			
CUS29MW-00692 AB TEST? YES ☐ NO ☒	AB TEST? YES ☐ NO ☐	AB TEST? YES ☐ NO ☐
10/8 AP HCV IS AHCV-04136			
DAB21FW-00497 AB TEST? YES ☐ NO ☐	AB TEST? YES ☐ NO ☐	AB TEST? YES ☐ NO ☐
10/8 AP HCV IS AHCV-04137			
SEL27MW-00625 AB TEST? YES ☐ NO ☒	AB TEST? YES ☐ NO ☐	AB TEST? YES ☐ NO ☐

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Please fax this form to:
(No cover sheet required)

Simon Ng, BSRI
Fax #: (415) 775-3859

Page: _____ of _____

Virology and Immunology WNV - Study Shipping List for Specimens

Blood Center Location: _____ **FedEx Tracking #:** _____

1. Collection center please fill in the left columns. **The study ID# for study subject can be located directly on the blood tube.**
2. Fax this form to Simon Ng (BSRI-(415)-775 3859) the same day the specimens are sent to BSRI, this alerts our lab staff of sample's arrival.
3. Include this form with shipment, fold and place inside box.
4. Shaded regions to be completed at BSRI lab upon arrival.

For Phlebotomy use only **For Lab use only**

Study ID# (Donor ID #)	Phlebotomy		Vacutainer tubes shipped to BSRI		Date / Time tubes received	All tubes received? (Y/N)	Repository Storage			
Note: for privacy reasons **Do Not** use subject's name on this form, instead use study ID	Date	Time (24 hour clock)	Lavender Top 7 x 10mL + 1 x 2mL	Tempus Tube (3mL)			#Aliquots	Box	Position	Freezer
							PL			-80 °C WNV PL
							TMA			-80 °C WNV
Name of Phlebotomist							CE			$LiqN_2$ WNV CE
Signature			*Date*		*Condition of Specimens (If not satisfactory, please explain):*					

Deviation from Protocol: ☐ **(Yes)**

Version 08/6/10

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SWAN (Brian Edlin) samples

Processing date: 10/09/09

#	Specimens ID	TMA	HCV EIA	Plasma	Rack	Box	Positions
1	AHCV- 04133	1	✓	1	4	45	3
2	AHCV- 04134	1	✓	2	↓	↓	4-5
3	AHCV- 04135	1		1	↓	↓	6
4	AHCV- 04136	1		1	↓	↓	7
5	AHCV- 04137	1		2	↓	↓	8-9
6	AHCV-						
7	AHCV-						
8	AHCV-						
9	AHCV-						
10	AHCV-						
11	AHCV-						
12	AHCV-						
13	AHCV-						
14	AHCV-						
15	AHCV-						
16	AHCV-						
17	AHCV-						
18	AHCV-						
19	AHCV-						
20	AHCV-						
21	AHCV-						
22	AHCV-						

Comments _____

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Title: Separation and Preservation of Plasma			Page 1 of 4
Doc. #:	**VRLRC0004**	**Revision: 10/29/10**	**Effective date:10/29/10**

1	**Purpose**		To outline the responsibilities and to define the steps to be followed for the separation of and preservation of plasma from anticoagulated blood
2	**Scope**	2.1	This procedure is used for specimens that require plasma separation by centrifugation
		2.2	Plasma is defined as the clear yellowish fluid upon separating anticoagulated whole blood into its solid and liquid components after it has been centrifuged.
		2.3	In general there are three steps in preparing plasma from anticoagulated blood: 1) centrifugation, 2) separation from the solid elements and 3) preservation.
3	**Responsibility**	3.1	It is the responsibility of the Supervisor to ensure that laboratory personnel have been trained in accordance with this procedure before making any plasma aliquots for long term preservation and to ensure that the training is documented.
		3.2	It is the responsibility of laboratory personnel to ensure he/she has read, understands and follows this procedure when preparing plasma aliquots for long term preservation.
		3.3	It is the responsibility of both the Supervisor and VRLRC personnel to ensure that plasma isolation steps are performed in a biological safety cabinet.
		3.4	It is the responsibility of laboratory personnel to record and notify the Supervisor of any deviations from this procedure, which are not accounted for in this procedure.
4	**Materials Required**	4.1	2.0 mL sterile cryovials with yellow caps (VWR)
		4.2	13 x 75mm plastic tube for TMA (VWR)
		4.3	Sterile plastic transfer pipettes (VWR)
5	**Equipment Required**	5.1	Centrifuge capable of 900 x g
		5.2	Biological safety cabinet
		5.3	-80C mechanical freezer
6	**Procedure**	6.1	Use Universal Safety precautions

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		6.2	Separate incoming tubes by identification number(s) and prepare labels using either the “study specific” or “user defined” barcode labeling program.
		6.3	Make study specific labels
			Centrifuge tube(s) at 2000 rpm (Sorvall LEGEND RT: 872 x g and Sorvall RT6000B: 827 x g) for 10 minutes (room temperature, no brake). [Sorvall LEGEND RT : Program #1]. In room 19 centrifuge tubes at 510 x g for 10 min using Program #3 (Sorvall Legend X1R).
		6.4	Label all cryovials needed. The labeling will be similar to the label placed on the study specific Shipping List for Specimens Form (Attachment 1).
		6.5	Verify all identifiers before each transfer step. You are verifying that prior to transferring the plasma from one vessel to another; identification numbers on the labeled cryovials and tube of anticoagulated blood are identical.
		6.6	In the biological safety cabinet, using a sterile transfer pipette, remove the plasma and aliquot plasma into the appropriate pre-labeled cryovials. The volume placed in each cryovial is study specific. Be careful not to disturb the red blood cells.
		6.7	One transfer pipette can be used for all blood tubes from a single study participant.
		6.8	Place used transfer pipettes into the biohazardous waste within the biological safety cabinet.
		6.9	Enter plasma aliquot(s) into Freezerworks
		6.10	Document the freezer box number and freezer box positions on the laboratory specific batch record.
		6.11	Place the study specific laboratory batch record (Attachment 2) in front of the study specific Shipping List for Specimens form in the study specific binder

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Please fax this form to: (No cover sheet required)
Simon Ng, BSRI
Fax #: (415) 775-3859
Page: _____ of _____

Virology and Immunology WNV - Study Shipping List for Specimens

Blood Center Location: _____ **FedEx Tracking #:** _____

1. Collection center please fill in the left columns. **The study ID# for study subject can be located directly on the blood tube.**
2. Fax this form to Simon Ng (BSRI-(415)-775 3859) the same day the specimens are sent to BSRI, this alerts our lab staff of sample's arrival.
3. Include this form with shipment, fold and place inside box.
4. Shaded regions to be completed at BSRI lab upon arrival.

For Phlebotomy use only **For Lab use only**

Study ID# (Donor ID #)	Phlebotomy		Vacutainer tubes shipped to BSRI		Date / Time tubes received	All tubes received? (Y/N)	Repository Storage			
Note: for privacy reasons **Do Not** use subject's name on this form, instead use study ID	Date	Time (24 hour clock)	Lavender Top 7 x 10mL + 1 x 4mL	Tempus Tube (3mL)			#Aliquots	Box	Position	Freezer
							PL			-80 °C WNV PL
							TMA			-80 °C WNV
Name of Phlebotomist							CE			$LiqN_2$ WNV CE
Signature			*Date*		*Condition of Specimens (If not satisfactory, please explain):*					

Version 03/30/10

Attachment 1

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Viral Reference Laboratory and Repository Core
Blood Systems Research Institute
San Francisco, CA 94118
(415) 749-6609

WNV Intensive Study Specimen Processing and Storage Form

Study ID	Number of Aliquots Made										
	TMA		Plasma		PBMC-One		PBMC-Two		Whole Blood		
	Box	Pos	Box	Pos	Box	Pos	Box	Pos	Box	Row	Pos

Study ID	Number of Aliquots Made										
	TMA		Plasma		PBMC-One		PBMC-Two		Whole Blood		
	Box	Pos	Box	Pos	Box	Pos	Box	Pos	Box	Row	Pos

Tech: ____ Sample Processing Date: _____ Time: _____

Tech: ____ Plasma/WB freezing -80 Date: _____ Time: _____

Tech: _____PBMC freezing: -80 Date: _____ Time: _____

Tech _____ PBMC freezing: LN2 Date_____ Time: _____

Attachment 2

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Title: Separation and Preservation of Packed Red Blood Aliquots		Page 1 of 4
Doc. #:VRLRC 0005	**Revision: 6/11/10 & 10/29/10**	**Effective date:11//02/09**

1	**Purpose**		To outline the responsibilities and to define the steps to be followed for the separation of and preservation of packed red blood cell (PRBC) aliquots from anticoagulated blood.
2	**Scope**	2.1	This procedure is used for specimens that require PRBC aliquot after removing the plasma.
		2.2	PRBC aliquots are defined as aliquots made from the buffy coat (Attachment 1) after separating anticoagulated whole blood into its solid and liquid components by centrifugation.
		2.3	In general there are six steps in preparing PRBC aliquots from anticoagulated blood: 1) centrifugation, 2) separation into the liquid and solid elements, 3) removal of most of the plasma, 4) mixing the cells of the buffy coat in among the red blood cells and 5) making PRBC aliquots and 6) preservation.
3	**Responsibility**	3.1	It is the responsibility of the Supervisor to ensure that laboratory personnel have been trained in accordance with this procedure before making PRBC aliquots for long term preservation and to ensure that the training is documented.
		3.2	It is the responsibility of laboratory personnel to ensure he/she has read, understands and follows this procedure when preparing PRBC aliquots for long term preservation.
		3.3	It is the responsibility of both the Supervisor and VRLRC personnel to ensure that the preparation of PRBC aliquots is performed in a biological safety cabinet.
		3.4	It is the responsibility of laboratory personnel to record and notify the Supervisor of any deviations from this procedure, which are not accounted for in another procedure.
4	**Materials Required**	4.1	2.0 mL sterile cryovials with red caps (VWR)
		4.2	Sterile graduated plastic transfer pipettes (VWR)
		4.3	Cryovial racks
		4.4	9 x 9 2 inch freezer boxes
5	**Equipment Required**	5.1	Centrifuge capable of 900 x g
		5.2	Biological safety cabinet
		5.3	-80C mechanical freezer

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6	**Procedure**	6.1	Use Universal Safety precautions
		6.2	Separate incoming tubes by identification number(s) and prepare labels using either the “study specific” or “user defined” barcode labeling program.
		6.3	Make study specific labels
			Centrifuge tube(s) at 2000 rpm (Sorvall LEGEND RT: 872 x g and Sorvall RT6000B: 827 x g) for 10 minutes (room temperature, no brake). [Sorvall LEGEND RT : Program #1]. In room 19 centrifuge tubes at 510 x g for 10 min using Program #3 (Sorvall Legend X1R).
		6.4	Label all cryovials needed. The labeling will be similar to the label placed on the study specific Shipping List for Specimens form (Attachment 2).
		6.5	Verify all identifiers before each transfer step. You are verifying that prior to making the PRBC aliquots; identification numbers on the labeled cryovials and tube of anticoagulated blood are identical.
		6.6	In the biological safety cabinet, using a sterile transfer pipette, remove the plasma. After most of the plasma has been removed, gently mix/resuspend the buffy coat in among the red blood cells. Re-suspension of the buffy coat (layer of white cells and platelets lying on top of the red blood cells) into the packed red blood cells is accomplished by drawing the blood up into and expelling it from the transfer pipet a minimum of 5-6 times. The volume placed in each cryovial is study specific.
		6.7	One transfer pipette can be used for all blood tubes from a single study participant.
		6.8	Place used transfer pipettes into the biohazardous waste within the biological safety cabinet.
		6.9	Enter PRBC aliquot(s) into Freezerworks
		6.10	Document the freezer box number and freezer box positions on the laboratory specific batch record.
		6.11	Place the study specific laboratory batch record (Attachment 3) in front of the study specific Shipping List for Specimens form (Attachment 2) in the study specific binder

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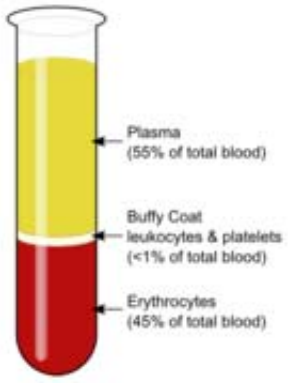

Attachment 1

Please fax this form to:
(No cover sheet required)

Simon Ng, BSRI
Fax #: (415) 775-3859

Page: _____ of _____

Virology and Immunology WNV - Study Shipping List for Specimens

Blood Center Location: _____ **FedEx Tracking #:** _____

1. Collection center please fill in the left columns. **The study ID# for study subject can be located directly on the blood tube.**
2. Fax this form to Simon Ng (BSRI-(415)-775 3859) the same day the specimens are sent to BSRI, this alerts our lab staff of sample's arrival.
3. Include this form with shipment, fold and place inside box.
4. Shaded regions to be completed at BSRI lab upon arrival.

For Phlebotomy use only **For Lab use only**

Study ID# (Donor ID #)	Phlebotomy		Vacutainer tubes shipped to BSRI		Date / Time tubes received	All tubes received? (Y/N)	Repository Storage			
Note: for privacy reasons **Do Not** use subject's name on this form, instead use study ID	Date	Time (24 hour clock)	Lavender Top 7 x 10mL + 1 x 4mL	Tempus Tube (3mL)			#Aliquots	Box	Position	Freezer
							PL			-80 °C WNV PL
							TMA			-80 °C WNV
Name of Phlebotomist							CE			$LiqN_2$ WNV CE
Signature			*Date*		*Condition of Specimens (If not satisfactory, please explain):*					

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Attachment 2

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WNV Intensive Study Specimen Processing and Storage Form

Study ID	Number of Aliquots Made										
	TMA		Plasma		PBMC-One		PBMC-Two		Whole Blood		
	Box	Pos	Box	Pos	Box	Pos	Box	Pos	Box	Row	Pos

Study ID	Number of Aliquots Made										
	TMA		Plasma		PBMC-One		PBMC-Two		Whole Blood		
	Box	Pos	Box	Pos	Box	Pos	Box	Pos	Box	Row	Pos

Tech: ____ Sample Processing Date: _____ Time: _____

Tech: ____ Plasma/WB freezing -80 Date: _____ Time: _____

Tech: ____PBMC freezing: -80 Date: _____ Time: _____

Tech _____ PBMC freezing: LN2 Date_____ Time: _____

Attachment 3

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Weekly Reagent Control Record Procedure

Title: Weekly Reagent Control Record Procedure				Page 1 of 2	
Doc. #:		**Revision:**		**Effective date:**	**10/05/09**

1	Purpose		To outline the responsibilities and to define the steps to be followed when making reagents used during the isolation of peripheral blood mononuclear cells (PBMC).
2	Scope	2.1	This procedure is used for monitoring the appropriate use, i.e. lot number and expiration date, of reagents used during PBMC isolation.
		2.2	Reagents are defined as all solutions used during the isolation of PBMCs.
		2.3	This procedure is applicable to all personnel within the Viral Reference Laboratory and Repository Core (VRLRC) who perform PBMC isolations.
3	Responsibility	3.1	It is the responsibility of the Supervisor to ensure that the laboratory personnel have been trained in accordance with this procedure before using any reagents in the isolation of PBMCs.
		3.2	It is the responsibility of the laboratory personnel to ensure he/she has read, understands and follows this procedure while using reagents in the isolation of PBMCs.
		3.3	It is the responsibility of both the Supervisor and the laboratory personnel to ensure that the Reagent Control Record is completed appropriately and placed in the Reagent Control Record binder in either laboratory in room 18 or the mezzanine laboratory.
		3.4	It is the responsibility of the laboratory personnel to record and notify the Supervisor of any deviations from this procedure, which are not accounted for in study specific procedures.
4	Material Required	4.1	Control Reagent Record form (see below)
5	Equipment Required	5.1	none
6	Solutions/Buffer Required	6.1	none
7	Procedure	7.1	At the beginning of the work week each member of the VRLRC department performing PBMC isolations must complete the attached form.

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		7.2	Place the completed, dated and initialed Control Reagent Record form in the Control Reagent Record binder.

This is a copy of the Reagent Control Record form:

Reagents used this week (_____/_____/_____) Initials_____

Freeze Media…...…….ID _____Exp_____

FBS (UCSF) ……... Lot # _____Exp _____

RPMI (UCSF) ……..Lot #_____Exp _____

DMSO (Sigma) ……...Lot #_____Exp_____

PBS (UCSF) ……...Lot # _____Exp_____

FICOLL HYPAQUE PLUS (Amersham)…Lot #_____Exp_____

LYMPHOPREP (Axis-Shield)…..……...Lot #_____Exp_____

Zap-Oglobin II Lytic (Beckman Coulter)…Lot # _____Exp_____

PARA 4 Hematology Control (Streck)…….Lot # _____Exp_____

Vi-CELL Concentration Control……..Lot # _____Exp _____

Misc. Other_____

Misc. Other_____

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Title: EDTA or ACD PBMC Cell Separation Overlay Procedure			Page 1 of 9
Doc. #:VRLRC 0008		**Revision: 5/12/10 & 5/29/10 & 10/29/10 & 07/01/11**	**Effective date: 7/01/11**
1	**Purpose**		To outline the responsibilities and to define the steps to be followed for the isolation of peripheral blood mononuclear cells (PBMC) by density gradient using the overlay technique.
2	**Scope**	2.1	This procedure is used for specimens that require PBMC isolation.
		2.2	PBMCs (lymphocytes and monocytes) are defined as those cells isolated by means of a density gradient. Optimally, this procedure will remove red blood cells, granulocytes and platelets.
		2.3	This procedure is applicable to all personnel within the VRLRC department performing PBMC isolation.
3	**Responsibility**	3.1	It is the responsibility of the Supervisor to ensure that laboratory personnel have been trained in accordance with this procedure before performing PBMC isolation.
		3.2	It is the responsibility of the Viral Reference Laboratory and Repository Core (VRLRC) personnel to ensure he/she has read, understands and follows this procedure while isolating PBMCs.
		3.3	It is the responsibility of both the Supervisor and VRLRC personnel to ensure that the PBMC isolation steps are performed in a biological safety cabinet.
		3.4	It is the responsibility of VRLRC personnel to record and notify the Supervisor of any deviation from this procedure, which is not accounted for in study specific procedures.
4	**Materials Required**	4.1	50mL or 225mL conical centrifuge tubes (VWR)
		4.2	2mL cryovials for PBMC aliquots (VWR)
		4.3	25mL,10mL, 5mL and 2mL sterile serological pipettes (USA Scientific)
		4.4	Sterile plastic transfer pipettes (VWR)
		4.5	Sterile glass Pasteur pipettes (VWR)
		4.6	20µL and 1000µL pipette tips (Rainin)
		4.7	9 x 9 freezer box with drains for LN_2 (Custom Biogenic Systems)
		4.8	Laboratory disposable gloves (E & K Scientific)
		4.9	Disposable laboratory coats (Market Lab)

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5	**Equipment Required**	5.1 5.2 5.3 5.4 5.5	Centrifuge capable of 900x g Biohazard Safety cabinet -80C mechanical freezer LN_2 freezer with LN_2 CoolCELL
6	**Solutions/Buffer Required**	6.1 6.2 6.3 6.4 6.5 6.6 6.7 6.8 6.9 6.10	Mg^{++}, Ca^{++} free phosphate buffered saline (PBS) (UCSF Cell Culture Facility) Heat inactivated Fetal bovine serum (UCSF Cell Culture Facility) Dimethyl sulfoxide, minimum 95.5% GC (Sigma/Aldrich) Ficoll-Paque Plus (Amersham) or Lymphoprep (AXIS-SHIELD PoC AS) Bleach (Blood Center Warehouse) Isoton II diluent (Beckman-Coulter™.) Zap-Oglobin II (Beckman-Coulter™) Mult-Parameter Assayed Hematology Control (Streck Laboratories, Inc., Omaha, NE) Vi-CELL Focus Control (Beckman Coulter™) Vi-CELL Concentration Control (Beckman Coulter™)
7	**Procedure**	7.1	If plasma has been removed, replace plasma volume with Ca^{++}, Mg^{++} free PBS to bring the blood volume back to its original volume. Note: subsequent dilutions of blood throughout this procedure should be performed after the blood has been returned to its original volume.
		7.2	After replacing the plasma volume, gently mix tubes by inversion and process for PBMC's as follows.
		7.3	Pour the blood from either the ACD or EDTA tubes collectively into either a 50 mL or 225mL conical collection tube. The choice of tube size is based on the amount of blood being processed.
		7.4	Wash ACD or EDTA tubes sequentially with an equal volume of PBS to obtain the blood clinging to the sides of each tube. Place the PBS wash into the collection tube holding the blood.
		7.5	The ratio should be 1 volume of Ca^{++}, Mg^{++} free PBS to 1 volume of blood + PBS.
		7.6	Gently mix the PBS – Blood mixture in either a 50mL conical or a 225mL conical tube using a sterile 25mL pipette.
		7.7	The volume of PBS-Blood will determine the number of 50mL centrifuge tubes to prepare for the overlay separation using a density gradient (see Table 1).

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		7.8	Thoroughly mix density gradient before using.
		7.9	Prepare each 50mL centrifuge tube with either 7mL or 10mL of either Lymphoprep (AXIS-SHIELD PoC AS) or Ficoll-Paque™ PLUS (Amersham Biosciences). See Table 1.
		7.10	Slowly **overlay** approximately either **20mL or 30mL of the PBS – Blood solution (must always be 3 parts blood+PBS:1 part density gradient)** into each tube with density gradient (see Figure 1)
		7.11	Centrifuge the tube(s), **no brake at room temperature** for 45 minutes using either the Sorvall RT6000B Refrigerated Centrifuge at 1400 rpm (400g) or the Sorvall Legend RT at 1355 rpm (400g). In room 19 centrifuge the tubes for 45 min at 400 x g, no brake at RT, using Program #5 (Sorvall Legend X1R).
		7.12	Once centrifugation is done, do not leave tubes in the centrifuge for an extended period of time. **The density gradient is toxic to the lymphocytes.**
		7.13	Very carefully aspirate off (with vacuum pump and glass Pasteur pipette) the upper layer (plasma+ PBS layer) leaving the lymphocyte layer undisturbed at the interface (see Figure 2).
		7.14	Collect the PBMC interface layer from each tube and transfer into a fresh sterile conical 50 mL centrifuge tube. Note: **Including excess Ficoll-Paque PLUS causes granulocyte contamination; Including excess supernatant results in platelet contamination.** Add Ca^{++}, Mg^{++} free PBS wash media up to the 50mL mark. (1^{st} wash)
		7.15	Centrifuge for 10 minutes at 1070 rpm (250g) when using the Sorvall RT6000B Refrigerated Centrifuge. Or when using the Sorvall Legend RT spin for 10 minutes at 1070 rpm (250g). Centrifuge tubes, no brake at RT, for 10 min at 250 x g (Program #4) on the Sorvall Legend X1R in room 19. This step removes platelets that are in the lymphocyte layer.
		7.16	Aspirate off Ca^{++}, Mg^{++} free PBS wash media and re-suspend the pellet in 25 mL of Ca^{++}, Mg^{++} free PBS. (2^{nd} wash).
		7.17	Centrifuge. When using the Sorvall RT6000B Refrigerated Centrifuge, spin for 10 minutes at 1070 rpm or 250g. When using the Sorvall Legend RT, spin for 10 minutes at 1070 rpm or 250g. Sorvall Legend X1R use program #4 (10 min at 250 x g).
		7.18	Aspirate off Ca^{++}, Mg^{++} free PBS wash media and re-suspend the pellet in 25 mL of Ca^{++}, Mg^{++} free PBS. (3^{rd} wash).
		7.19	Centrifuge. When using the Sorvall RT6000B Refrigerated Centrifuge, spin for 10 minutes at 1070 rpm or 250g. When using the Sorvall Legend RT, spin for 10 minutes at 1070 rpm or 250g. Sorvall Legend X1R use program #4 (10 min at 250 x g).

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		7.20	Aspirate off PBS wash media and gently re-suspend the pellet in Ca^{++}, Mg^{++} free PBS media according to Table 2.
8	**Cell count using Coulter Counter**	8.1	From this cell-PBS suspension, take 10µL and add to the labeled Coulter counting vial containing 10 mL of Isoton solution (add 3 drops of Zap-oglobin™ II Lytic Reagent to remove red cells – Coulter only).
		8.2	Follow the Coulter instrument procedure to count cells; apply raw counts, and dilution factors to get total cells in volume of PBS used to resuspend cells after 3rd wash.
		8.3	Divide the total number of cells by the number of cells needed for each aliquot to get the number of cryovials that can be frozen down.
		8.4	Centrifuge as described in step 7.15.
		8.5	Gently remove Ca^{++}, Mg^{++} free PBS without disturbing the pellet.
9	**Preparing PBMCs for Liquid Nitrogen Storage**	9.1	Add Freeze Media dropwise for the first 2 to 3 mL and then gently add the remaining volume.
		9.2	Gently resuspend the cells in the freeze medium.
		9.3	Aliquot into the proper number of labeled cryovials and freeze cells in accordance with the CoolCell procedure. **The use of the CoolCell allows for optimal freezing at 1° per hour.**
		9.4	Document the freezer box number and freezer box positions on the laboratory specific batch record.
		9.5	Store the laboratory specific batch record in front of the Virology and Immunology WNV- 2009 Study Shipping List for Specimens Shipping List form in the study specific binder.

10	**Special Note**	10.1	Use and amount of FBS used through out the procedure is study specific.
		10.2	Final concentration of PBMCs/mL is study specific.
		10.3	Therefore, number of cryovials made is study specific

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Table 1

Blood / PBS solution	Mixing tubes	PBS volume (total)	Total Volume	Density Gradient per 50mL tube	Overlay volume of blood + PBS	Number of 50 mL tubes for overlay
20 mL	50 mL tube	~20 mL	~40 mL	7 mL	20 mL	2
40 mL	225 mL tube	~40 mL	~80 mL	7 mL	20 mL	4
60 mL	225 mL tube	~60 mL	~120 mL	10 mL	30 mL	4
80 mL	225 mL tube	~80 mL	~160 mL	10 mL	30 mL	5
100 mL	225 mL tube	~100 mL	~200 mL	10 mL	30 mL	6

Table 2

Number and size of Starting EDTA tubes	Amount of Ca^{++}, Mg^{++} free PBS used to resuspend PBMC pellet after 2nd wash
7 x 10mL EDTA tubes	5mL
1 x 10mL EDTA tube	1mL
2 x 10mL EDTA tubes	2mL
3 x 10mL EDTA tubes	3mL
4 x 10mL EDTA tubes	4mL

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Figure 1:

Note: Ratio of Blood: Ficoll-Paque PLUS is not correct

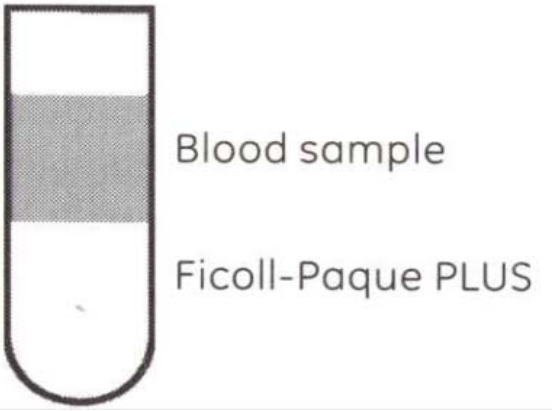
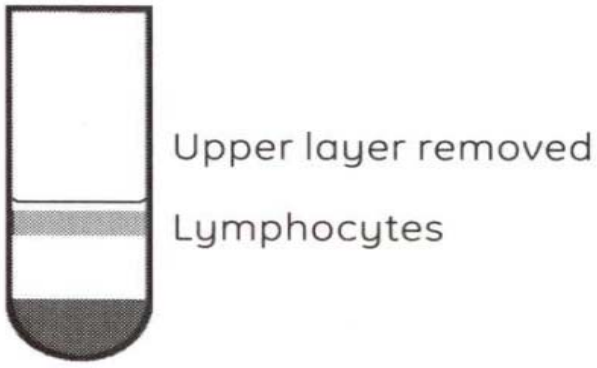


Figure 2:



Media Preparation: Use .22 µ filters for media filtration.

1. Wash media – PBS (Ca^{++}, Mg^{++} free) only, RT
2. Freeze Media – 45 mL FBS, 5 mL DMSO, 4°C

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Brief Summation of Process: PBMC Cell Separation Procedure

1. QC Samples
2. Spin blood and remove plasma
3. Replace removed plasma with equal volume with Ca^{++}, Mg^{++} free PBS
4. Dilute Blood + PBS with Ca^{++}, Mg^{++} free PBS (1 part Blood + PBS to 1part Ca^{++}, Mg^{++} free PBS)
5. Add ~7mL of density gradient to a 50mL conical centrifuge tube.
6. Overlay density gradients with ~20mL of Ca^{++}, Mg^{++} free PBS Blood mixture.
7. Centrifuge at 1400 rpm (400g), **no brake**, RT, 45 minutes. When using the Legend XIR centrifuge at 400 x g for 45 minutes with no brake.
8. Draw off upper layer using clean pipette, leaving lymphocyte layer undisturbed
9. Collect PBMC "interface" layer into a 50mL conical tube
10. Add at least 40mL of Ca^{++}, Mg^{++} free PBS (1^{st} wash)
11. Centrifuge at 1400 rpm (400g), **no brake**, RT, 10 minutes. When using the Legend XIR, centrifuge at 250 x g for 45 minutes with no brake.
12. Remove Ca^{++}, Mg^{++} free PBS
13. Wash with 25mL of Ca^{++}, Mg^{++} free PBS (2^{nd} wash)
14. Centrifuge 10 minutes at 1000 rpm, RT to remove platelets. When using the Legend XIR, centrifuge at 250 x g for 45 minutes with no brake.
15. Wash with 25mL of Ca^{++}, Mg^{++} free PBS (3^{rd} wash)
16. Centrifuge 10 minutes at 1000 rpm, RT to remove platelets. When using the Legend XIR, centrifuge at 250 x g for 45 minutes with no brake.
17. Gently remove PBS without disturbing cell pellet at the bottom of the tube
18. Resuspend PBMC pellet according to Table 2 with Ca^{++}, Mg^{++} free PBS
19. Add 10µL of cell-PBS suspension to the labeled Coulter counting vial containing 10 mL of Isoton solution (add 3 drops of Zap-oglobin™ II Lytic Reagent to remove red cells – Coulter only).
20. Calculate total cells collected using a Coulter Counter .
21. Based on the # of cells needed per cryovial determine the number of cryovials to be labeled.
22. Centrifuge 10 minutes at 1000 rpm, RT. When using the Legend XIR, centrifuge at 250 x g for 45 minutes with no brake.
23. Gently remove Ca^{++}, Mg^{++} free PBS and resuspend into appropriate volume of Freeze Medium to obtain proper # cells/mL per cryovial.
24. Freeze cells in accordance with the CoolCell procedure. **The use of the CoolCell allows for optimal freezing at 1° per hour.**

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WNV Intensive Study Specimen Processing and Storage Form

Study ID	Number of Aliquots Made										
	TMA		Plasma		PBMC-One		PBMC-Two		Whole Blood		
	Box	Pos	Box	Pos	Box	Pos	Box	Pos	Box	Row	Pos

Study ID	Number of Aliquots Made										
	TMA		Plasma		PBMC-One		PBMC-Two		Whole Blood		
	Box	Pos	Box	Pos	Box	Pos	Box	Pos	Box	Row	Pos

Tech: ____ Sample Processing Date: _____ Time: _____

Tech: ____ Plasma/WB freezing -80 Date: _____ Time: _____

Tech: _____PBMC freezing: -80 Date: _____ Time: _____

Tech _____ PBMC freezing: LN2 Date_____ Time: _____

Attachment 1

Blood Systems Research Institute
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Please fax this form to: (No cover sheet required)

Simon Ng, BSRI
Fax #: (415) 775-3859

Page: _____ of _____

Virology and Immunology WNV - Study Shipping List for Specimens

Blood Center Location: _____ **FedEx Tracking #:** _____

1. Collection center please fill in the left columns. **The study ID# for study subject can be located directly on the blood tube.**
2. Fax this form to Simon Ng (BSRI-(415)-775 3859) the same day the specimens are sent to BSRI, this alerts our lab staff of sample's arrival.
3. Include this form with shipment, fold and place inside box.
4. Shaded regions to be completed at BSRI lab upon arrival.

For Phlebotomy use only **For Lab use only**

Study ID# (Donor ID #)	Phlebotomy		Vacutainer tubes shipped to BSRI		Date / Time tubes received	All tubes received? (Y/N)	Repository Storage			
Note: for privacy reasons Do Not use subject's name on this form, instead use study ID	Date	Time (24 hour clock)	Lavender Top 7 x 10mL + 1 x 4mL	Tempus Tube (3mL)			#Aliquots	Box	Position	Freezer
							PL			-80 °C WNV PL
							TMA			-80 °C WNV
Name of Phlebotomist							CE			$LiqN_2$ WNV CE
Signature			*Date*		*Condition of Specimens (If not satisfactory, please explain):*					

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Attachment 2

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Title: CoolCell PBMC Freezing Process		Page 1 of 2	
Doc. #:VRLRC 0010	**Revision:**	**Effective date:**	**10/19/09**

1	**Purpose**		The Biocision CoolCell with a -80C freezer provides the freezing rate of -1° C per minute that is idea for cryo-preservation. This eliminates the need for an alcohol based freezing process.
2	**Scope**		This procedure is used for the cryopreservation of isolated peripheral blood mononuclear cells (PBMC).
3	**Responsibility**	3.1	It is the responsibility of the Supervisor to ensure that laboratory personnel have been trained in accordance with this procedure before handling any PBMCs and to ensure that the training is documented.
		3.2	It is the responsibility of laboratory personnel to ensure he/she has read understands and follows this procedure when preparing PBMCs for LN_2 storage.
		3.3	It is the responsibility of laboratory personnel to record and notify the Supervisor of any deviations from this procedure, which are not accounted for in this procedure.
4	**Materials**	4.1	Adequate number of Biocision CoolCells for number of cryovials to be place in -80C freezer.
		4.2	Container with 1 inch of crushed dry ice.
5	**Equipment Required**	5.1	-80C mechanical freezer
		5.2	LN_2 freezer
6	**Procedure**	6.1	The entire CoolCell, and all chambers, need to be absolutely dry and at room temperature prior to use.
		6.2	The core (black ring) must be at room temperature, dry and seated properly in the bottom of the central cavity. Place sample cryovials (dry externally) one at a time into each well. No cryovial should exceed the height of the chamber.
		6.3	Make sure that the cryovials slide in and out of the wells easily.
		6.4	Place the lid onto the CoolCell body without forcing but make sure it seals the central cavity completely
		6.5	Place the CoolCell into a -80C freezer with 1 inch of free space surrounding the CoolCell.

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		6.6	The CoolCell must remain in the -80C freezer for at least 4 hours.
7	**LN_2 Storage**	7.1	Prepare a pan with 1 inch of either pulverized or crushed dry ice.
		7.2	Remove the CoolCell from the -80C freezer and gently remove the lid using a twisting and rocking motion.
		7.3	Immediately invert the CoolCell over the dry ice to recover the cryovials.
		7.4	Check the CoolCell chamber to ensure that all cryovials have been removed. If any cryovials have stuck, release the vials by tapping the inverted CoolCell on a flat surface or the palm of your hand.
8	**Reusing the CoolCell**	8.1	The CoolCell is ready when the inner core (black ring) is once again at room temperature.
		8.2	The black core ring must be dry before reinserting into the CoolCell body
		8.3	CoolCell will warm to room temperature in 10 to 15 minutes if you invert and tap out the black core ring.
		8.4	All chambers must be dry before being used again.
9	**Special Notes**	9.1	Always use dry ice to transfer the cryovials to their permanent storage in LN_2.
		9.2	Cryovial contents can warm from -75° C to over -50° C in less than a minute when exposed to room temperature air.

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Title: VRLRC Shipping of Frozen Infectious Samples			Page 1 of 3	
Doc. #: VRLRC0011	**Revision:**		**Effective date:**	**4/27/10**

1	**Purpose**		To outline the responsibilities and describe the procedure for shipping Category B infectious substances, i.e. plasma, serum and whole blood aliquots, requiring dry ice by any mode of transportation.
2	**Scope**	2.1 2.2 2.3 2.4	This procedure is used for the shipping of frozen samples on dry ice. To meet 49 CFR requirements To meet IATA PI 650 (Diagnostic Specimens) requirements To meet IATA PI 904 (Dry Ice) requirements
3	**Responsibility**	3.1 3.2 3.3	It is the responsibility of the Supervisor to ensure that VRLRC laboratory personnel have been trained in accordance with this procedure before shipping infectious samples on dry ice and to ensure that the training is documented. It is the responsibility of VRLRC laboratory personnel to ensure he/she has read, understands and follows this procedure when shipping infectious samples on dry ice. It is the responsibility of laboratory personnel to record and notify the Supervisor of any deviations from this procedure, which are not accounted for in another procedure.
4	**Materials Required**	4.1 4.2 4.3 4.4 4.5 4.6 4.7 4.8	Saf-T-Pak™ Compliance Training for Shipping Class 6 Division 6.2 – Infectious Substances -80°C frozen infectious samples Insulated shipper for temperature sensitive infectious specimens. Inner box (optional) Polystyrene cooler and lid Dry ice Hazard and handling labels: 1) Dry ice label (class 9 label) and 2) a label that reads “Biological Substance, Category B UN3373”, 3) Shipper’s name, address and phone number as well as the consignee’s name, address and phone number (on outside of outer box). Completed FedEx air bill form
5	**Equipment Required**	5.1	-80C mechanical freezer
6	**Solutions/Buffer Required**		None

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7	**Procedure**	7.1	Each tube must be placed inside a secondary container. Remove lid from polystyrene cooler.
		7.2	Place inner box into the polystyrene cooler.
		7.3	Amount of dry ice added is determined by the distance of
		7.4	the shipment.
		7.5	Place the Styrofoam lid onto the inner Styrofoam container (do not tape the styrofoam lid).
		7.6	Partially seal the cardboard box so that the dry ice vapor can vent.
		7.7	Complete the FedEx air bill: 1) BSRI shipping address, 2) amount of dry ice used, 3) Section 2 – fill in the internal billing number 4) Section 4a – Check the "FedEx Priority Overnight" box, 5) Section 5 – Check the "other" box, 6) Section 6 – Check the box that says, "Yes Shipper's Declaration not required" and check the "Dry Ice" box and write "1" in the first blank line and the "kg" amount of dry ice used on the second line, i.e. 1x9 kg, 7) Section 7 – Check sender.
		7.8	Fill in the dry ice label on the outer box with the amount of dry ice used
		7.9	Fill in the sender's and consignee's name and address on the outer box or use preprinted address stickers.
		7.9	Include with the shipment a listing of the specimen ID numbers contained in the shipment.
		7.10	Email the list of specimen ID numbers to the recipient
		7.11	**Alert the recipient a day in advance of the incoming shipment. Provide the recipient with the tracking number.**
		7.12	Shipments may only be shipped on Monday through Thursday.
8	**Special Notes**	8.1	Never place dry ice inside the certified secondary container.

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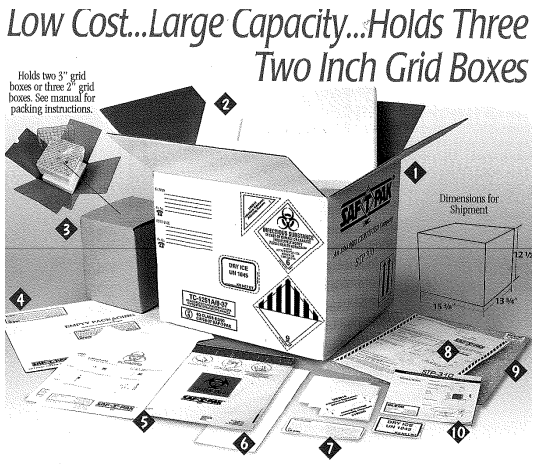

STP 310 Certified Insulated Shipper for Temperature Sensitive Specimens.

Certified insulated shipper for the shipping of temperature sensitive infectious specimens on dry ice. Note, not all items in the illustration are used in this shipping procedure.

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Title: Viral Reference Laboratory and Repository Core Quality Control and Quality Assurance Program for the Mechanical and Liquid Nitrogen Freezers contained in the basement of Blood Centers of the Pacific belonging to Blood Systems Research Institute				Page 1 of 14	
Doc. #:		**Revision:**		**Effective date:**	

1	**Purpose**		To outline the management responsibilities and describe the steps involved in the oversight and monitoring of both the mechanical and liquid nitrogen freezers by the Viral Reference Laboratory and Repository Core (VRLRC).
2	**Scope**	2.1	To provide guidelines for general practices within the freezer area.
		2.2	All freezers are designated by department.
		2.3	The temperature of all freezers within the freezer area are monitored 24/7.
		2.4	Each VRLRC departmental mechanical freezers has a contents map on the freezer door.
		2.5	Overview of daily temperature monitoring using either the built in freezer temperature display or NIST certified stand alone thermometers (-20°C mechanical freezers). (Table 6.2)
		2.6	Overview of liquid nitrogen (LN_2) monitoring (Table 6.2)
		2.7	Monitoring of ambient temperature in freezer area using a stand alone NIST certified thermometer) This is not a daily activity.
		2.8	Use of pocket folders attached to each freezer. Freezer or specimen related events are recorded on the Freezer Farm Mack Alarm Event Form (Table 6.5) within the pocket folder.
		2.9	Quarterly maintenance and unit repairs are managed by an outside contractor (Pelco Sales and Service, see Table 6.7).
3	**Responsibilities**	3.1	It is the responsibility of the Supervisor to ensure that VRLRC laboratory personnel have been trained in accordance with handling and working procedures before routinely retrieving or storing of VRLRC samples.
		3.2	It is the responsibility of personnel to ensure he/she has read, understood, and will follow these practices while working in the freezer area.
		3.3	It is the responsibility of personnel to notify the supervisor and record any event (see Table 6.5) which may account for a unit to alarm or malfunction.
4	**Materials Required**	4.1	Laboratory coat
		4.2	Insulated freezer gloves
			Face shield or goggles when working with a liquid nitrogen freezer
		4.2	Dry ice (as needed)
		4.3	Pre-labeled freezer boxes or racks
		4.4	Freezer racks
		4.5	Labtop computer
	Equipment Required	5.1	Two Oxygen Sensors from Teledyne Analytical Instruments
		5.2	MACK alarm system from MACK Information Systems with 27 MACK LabLink Data Collectors and freezer probes for -20^{0}C, -40^{0}C, -80^{0}C and

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			LN_2 freezers.
		5.3	MACK monitoring system (Blood Centers of the Pacific's Dispensing Department)
		5.4	HVAC S-6 systems
		5.5	Over forty -20^{0}C, -40^{0}C, -80^{0}C mechanical freezers (Table 6.1)
		5.6	Six LN_2 freezers (Table 6.1)
6	**Procedure - General**	6.1	Universal safety precautions are to be followed when handling sample vials in the freezer area.
		6.2	Maintain uncluttered rows and aisles. Adequate spacing is needed between freezers for the proper functioning of freezer condensers.
		6.3	Monitor mechanical freezer temperatures daily.
		6.4	Monitor liquid nitrogen level daily using both the electronic display found on each LN_2 freezer and well as physically measuring the LN_2 level.
		6.5	Record both mechanical freezer temperatures and LN_2 levels on appropriate log (Table 6.2)
		6.5	Note unusual fluctuations or drops in temperature while monitoring mechanical freezers.
		6.6	Note low liquid nitrogen levels while monitoring liquid nitrogen freezers.
		6.7	Add LN_2 to LN_2 freezers on Tuesday and Friday by pushing the Fill button on each LN_2 freezer.
		6.8	Store log record at the end of the month in the Temperature Monitoring Log for -80^{0}C Freezers and LN_2 Tanks binder
	Procedure -- Freezer Maintenance	6.9	As needed consolidate or move frozen specimens.
		6.10	Racks and boxes within a freezer should be adequately labeled
		6.11	De-ice mechanical freezers as needed. Use yellow absorbent pads to collect water in front of freezer and to the sides.
		6.12	Record all activities involving either a freezer or frozen specimens on the Freezer Farm Mack Alarm Event Form found in the pocket folder on the freezers involved in the activities (Table 6.5).
		6.13	Maintain two empty freezers as in case of emergency.
		6.14	MACK probes must be calibrated regularly by the Training-Safety-Process-Improvement Dept.
		6.15	Routinely check biohazard containers so that they are not overfilled..
		6.16	Biohazardous containers are emptied every Friday by Blood Centers of the Pacific's Facilities Department
	Procedure -- Freezer Failure	6.17	If a mechanical freezer fails during non-working hours temporarily maintain the temperature with dry ice.
		6.18	Vent door so that CO_2 gas can escape.
7	**Related Documents**	7.1	Freezer Farm freeze inventory list (Table 6.1) Freezer are identified by their MACK alarm number.

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		7.2	VRLRC Daily Temperature Monitoring Log – Per Month (Table 6.2)
			MACK Alarm action form (Used by Dispensing Personnel) (Table 6.3)
		7.3	MACK Lablink Probe Calibration form (Used by Training-Safety-Process-Improvement department personnel) (Table 6.4)
		7.4	Freezer Farm Mack Alarm Event Form (Table 6.5)
			Freezer Farm Emergency Check List (Table 6.6)
		7.5	Pelco Services Quarterly Maintenance Form (Table 6.7)
		7.6	Individual Unit Action Document (Table 6.8)
8	**Special Notes**	8.1	Freezer Farm located in a keypad secured area
		8.2	Daily Work week Freezer Farm Team in place
		8.3	24/7 Emergency Freezer Farm Team in place
		8.4	Transition from MACK alarm systems to Plexxium Remote 24/7 Alarm system is underway.

Table 6.1 Freezer farm Inventory List:

	MACK PROBE #	FREEZER TYPE	DEPT.	LOC.. (RM or AREA)	CONTACT PEOPLE	SET TEMP.	SET RANGE
	73-1	Forma Scientific	Core Immunology	Freezer Farm	Monday-Friday 9 am-7pm Freezer Farm Team	-80º C	-40º C to -90º C
	73-2	Harris uprt	Core Immunology	Freezer Farm		-80º C	-40º C to -90º C
	74-1	Harris uprt	Core Immunology	Freezer Farm		-40º C	-20º C to -60º C
	74-2	Harris uprt	Core Immunology	Freezer Farm	Monday-Friday 7pm- 9 am and Weekends Emmergency Freezer Farm Team	-40º C	-20º C to -60º C
	75-1	Harris uprt	Core Immunology	Freezer Farm		-40º C	-20º C to -60º C
	75-2	Harris uprt	Core Immunology	Freezer Farm		-40º C	-20º C to -60º C
	32-1	Forma - Lab	VRLRC	Hall Walking Refrigerator		+5º C	2.5º C to 7.5º C
	35-1	LG Refrigerator	VRLRC	mezzanine		+5º C	0.5º C to 10º C
	36-1	LG Freezer (refrig)	VRLRC	mezzanine		-15º C	-40º C to 0º C

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	36-2	Kenmore	VRLRC	mezzanine		-20° C	-40° C to -10° C
	53-2	Innova	VRLRC	Room 18		-80° C	-40° C to -90° C
	77-1	Harris chest	VRLRC	Freezer Farm		-80° C	-40° C to -90° C
	80-1	Harris uprt	VRLRC	Freezer Farm		-80° C	-40° C to -90° C
	80-2	Harris uprt	VRLRC	Freezer Farm		-80° C	-40° C to -90° C
	81-1	Harris uprt	VRLRC	Freezer Farm		-40° C	-20° C to -60° C
	83-2	Baxter SP	VRLRC	Freezer Farm		-80° C	-40° C to -90° C
	85-1	Harris chest	VRLRC	Freezer Farm		-80° C	-40° C to -90° C
	85-2	Harris chest	VRLRC	Freezer Farm		-80° C	-40° C to -90° C
	86-1	Harris chest	VRLRC responsibility	Freezer Farm		-80° C	-40° C to -90° C
	86-2	Revco	VRLRC	Freezer Farm		-80° C	-40° C to -90° C
	87-1	Sears	VRLRC	Freezer Farm		-20° C	-40° C to -10° C
	89-1	Harris uprt	VRLRC	Freezer Farm		-40° C	-20° C to -60° C
	89-2	Thermo Forma	VRLRC	Freezer Farm		-80° C	-40° C to -90° C
	90-2	Harris uprt	VRLRC	Freezer Farm		-80° C	-40° C to -100° C
	91-1	Sanyo	VRLRC	Freezer Farm		-80° C	-40° C to -90° C
	91-2	Thermo Forma	VRLRC	Freezer Farm		-80° C	-40° C to -90° C
	92-1	Sears	VRLRC	Freezer Farm		-20° C	-40° C to -10° C
	none yet	Kenmore	VRLRC	Freezer Farm		-20° C	-40° C to -10° C

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	18-1	MVE xlc 1520HE	VRLRC	Freezer Farm		LN2	-130º C to -203º C
	18-2	MVE xlc 1520HE	Immunology but VRLRC responsibility	Freezer Farm		LN2	-130º C to -203º C
	42-1	MVE 1520HE	VRLRC	Freezer Farm		LN2	-130º C to -203º C
	42-2	Taylor-Wharton 40K	VRLRC	Freezer Farm		LN2	-130º C to -203º C
	88-1	Taylor-Wharton 40K	VRLRC	Freezer Farm		LN2	-130º C to -203º C
	88-2	Taylor-Wharton 40K	VRLRC	Freezer Farm		LN2	-130º C to -203º C
	76-1	Harris chest	Molec. Transfusion Core	Freezer Farm		-80º C	-40º C to -90º C
	76-2	Harris chest	Molec. Transfusion Core	Freezer Farm		-80º C	-40º C to -90º C
	77-2	Revco Ultima Plus uprt	Molec. Transfusion Core	Freezer Farm		-80º C	-40º C to -90º C
	79-1	Harris uprt	Molec. Transfusion Core	Freezer Farm		-80º C	-40º C to -90º C
	81-2	Sanyo uprt	Molec. Transfusion Core	Freezer Farm		-80º C	-40º C to -90º C
	82-1	Harris uprt	Molec. Transfusion Core	Freezer Farm		-80º C	-40º C to -90º C
	82-2	Thermo uprt	Molec. Transfusion Core	Freezer Farm		-80º C	40º C to -90º C
	83-1	Forma Scientific	Molec. Transfusion Core	Freezer Farm		-80º C	40º C to -90º C
	84-1	VWR 1/2	Molec. Transfusion Core	Freezer Farm		-20º C	-40º C to -10º C
	84-2	Harris uprt	Molec. Transfusion Core	Freezer Farm		-80º C	-40º C to -100º C
	90-1	Forma Scientific	Molec. Transfusion Core	Freezer Farm		-80º C	-40º C to -90º C
	71-2	Forma Scientific	Epidemiology - BSRI	Freezer Farm		-80º C	-40º C to -90º C
	72-1	Forma Scientific	Epidemiology - BSRI	Freezer Farm		-80º C	-40º C to -90º C
	72-2	Forma Scientific	Epidemiology - BSRI	Freezer Farm		-80º C	-40º C to -90º C

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	71-1	**Harris upright**	**Molecular Virology**	**Freezer Farm**		**-80º C**	**-40º C to -90º C**
	78-1	**Harris chest**	**Molec. Virology**	**Freezer Farm**		**-80º C**	**-40º C to -90º C**

Table 6.2 VRLRC Daily Temperature Monitoring Log – Per Month

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Month(s)_____

Viral Reference Lab and Repository Core
Temperature Monitoring

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Date	Harris	Therma(DD)	Kenmore	Harris	Sanyo	Therma(DD)	Harris	Harris	Harris	Baxter SP	Harris	Harris	Harris	Kenmore	Harris	Revco	Innova	LG	LG	Kenmore
	89-1	89-2	92-1	90-2	91-1	91-2	80-1	80-2	81-1	83-2	77-1	85-2	85-1	87-1	86-1	86-2	53-2	35-1	36-1	36-2
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version 1-6-09

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MONTH_____

Viral ReferenceLaboratory and Repository Core
Liquid Nitrogen Temperature Monitoring

Cryospan, Inc.
tel. (650) 871-2100

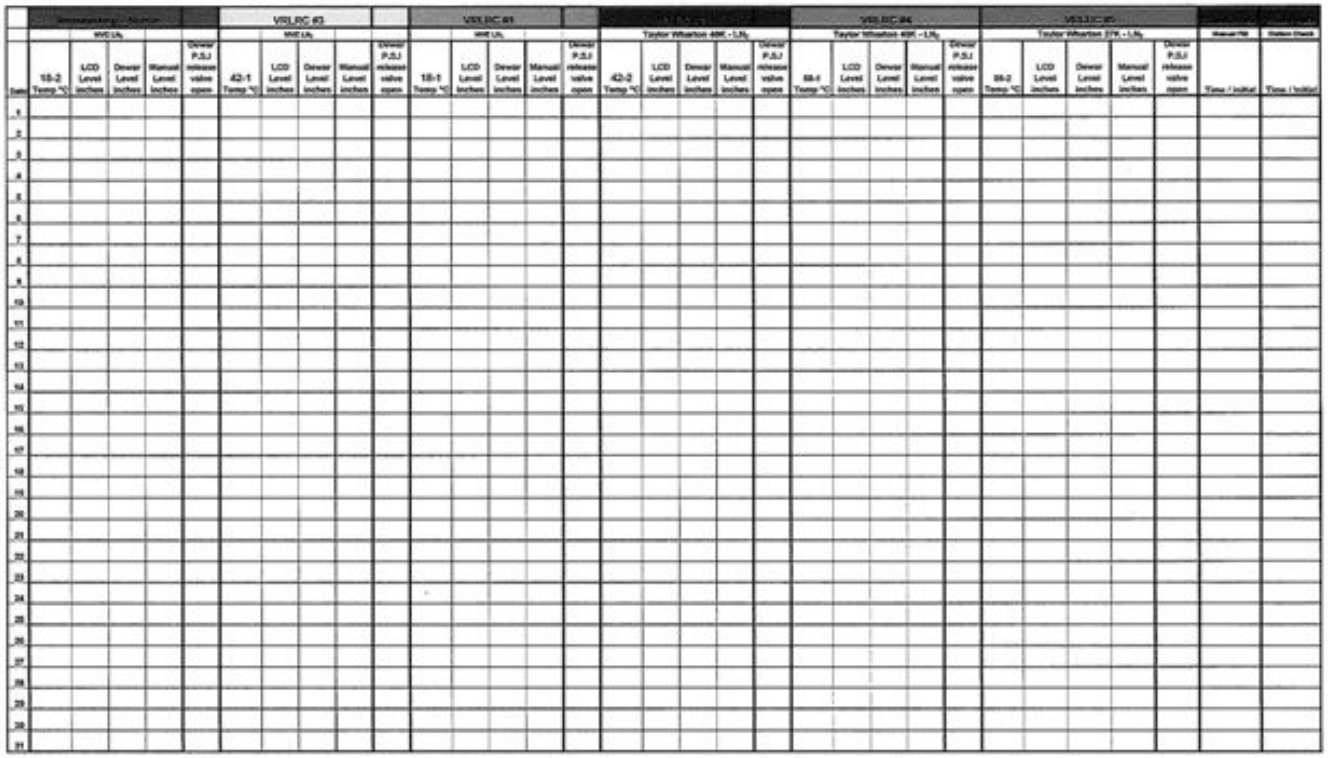

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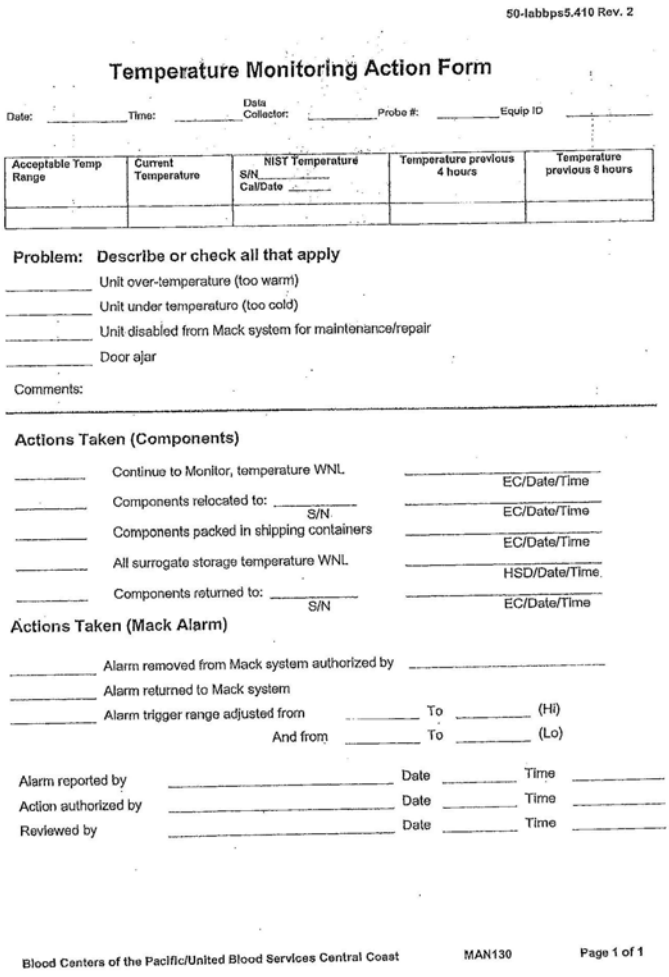
Table 6.3 Mack Alarm Action Form (Maintained by Blood Centers of Pacific's Dispensing Department



50-labbps5.410 Rev. 2

Temperature Monitoring Action Form

Date: _____ Time: _____ Data Collector: _____ Probe #: _____ Equip ID _____

Acceptable Temp Range	Current Temperature	NIST Temperature S/N_____ Cal/Date _____	Temperature previous 4 hours	Temperature previous 8 hours

Problem: Describe or check all that apply

_____ Unit over-temperature (too warm)
_____ Unit under temperature (too cold)
_____ Unit disabled from Mack system for maintenance/repair
_____ Door ajar

Comments: _____

Actions Taken (Components)

_____ Continue to Monitor, temperature WNL _____ EC/Date/Time
_____ Components relocated to: _____ S/N _____ EC/Date/Time
_____ Components packed in shipping containers _____ EC/Date/Time
_____ All surrogate storage temperature WNL _____ HSD/Date/Time
_____ Components returned to: _____ S/N _____ EC/Date/Time

Actions Taken (Mack Alarm)

_____ Alarm removed from Mack system authorized by _____
_____ Alarm returned to Mack system
_____ Alarm trigger range adjusted from _____ To _____ (Hi)
And from _____ To _____ (Lo)

Alarm reported by _____ Date _____ Time _____
Action authorized by _____ Date _____ Time _____
Reviewed by _____ Date _____ Time _____

Blood Centers of the Pacific/United Blood Services Central Coast MAN130 Page 1 of 1

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Table 6.4 MACK Lablink Probe Calibration Form

50-15.104c Rev. 1

Center:

Mack LabLink Probe Calibration

Data Collector No. _____ Data Collector Type: _____ Probe No: _____

Zero Setting Adjustment

NIST S/N: _____ Date of Calibration: _____

Probe Temp (°C)	NIST Temp (°C)	Correct NIST Temp (°C)	Adjustment (°C)	EC/Date

Temperature Probe Verification:

Probe Temp (°C)	NIST Temp (°C)	Correct NIST Temp (°C)	Difference (°C)	Pass/Fail	EC/Date

Span Setting Adjustment

NIST S/N: _____ Date of Calibration: _____

Probe Temp (°C)	NIST Temp (°C)	Correct NIST Temp (°C)	Adjustment (°C)	EC/Date

Temperature Probe Verification:

Probe Temp (°C)	NIST Temp (°C)	Correct NIST Temp (°C)	Difference (°C)	Pass/Fail	EC/Date

Reviewed By (EC/Date): _____

Blood Centers of the Pacific/Tri-Counties Blood Bank Page 1 of 1

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Table 6.5 Freezer Farm MACK alarm event form

MACK# _____

Freezer Mack Alarm Event Form

This form is to be used each time and every time any required change in status occurs with regard to our individual freezer MACK Alarms registered and connected to the B.S.R.I. Master Mack System. An additional action status note should be placed on the outside of the freezer.

Date	Time	Alarm ID (Mack#)	Action: Alarm ON or OFF	Reason: ex. Freezer temp. inaccurate, Freezer malfunction, Freezer move, Freezer loading or unloading, etc.	Individual responsible: (initial –sign off)	Any additional comments

Event Action items:

1. **Request Mack alarm off [date, time, how (in person, telephone, document), verify that the request was accomplished]**
2. **List progress status at the time a procedure is initiated [repairman, and outcome]**
3. **Request Mack alarm on [date, time, how (in person, telephone, document), verify that the request was accomplished]**
4. **Note: MACK probe alarm record from upstairs (dispensing) can be warmer than our individual freezer display.**

9/19/2005

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Table 6.6 Freezer Farm Emergency Check List

FREEZER FARM EMERGENCY CHECK LIST

Date: _____Time:_____
Initials: _____
Freezer MACK probe ID: _____

Primary Issue:

- ☐ 1.Freezer unit is too warm
- ☐ 2.Freezer unit is too cold
- ☐ 3.Freezer unit has no digital readout
- ☐ 4.Liquid Nitrogen Repository Tank

……

Secondary Actions required:

Freezer Unit is too warm

- ☐ **a**. Identify error alarm reading on freezer

  _____
- ☐ b. check all electrical connections
- ☐ c. get dry ice from Dispensing (3 slabs minimum for 1 day and place in top, middle, and bottom of freezer…vent the door

Freezer Unit is too cold

- ☐ a.Have Dispensing lower the cold range value to at least -45° C (for a -20 freezer), -50°C (for a -40 freezer), and -100°C or -105° C (for a -80 freezer).

Freezer Unit has no power

- ☐ a. check all the electrical connections
- ☐ b. if freezer is warm based on Dispensing records, get dry ice from Dispensing (3 slabs minimum for 1 day and place in top, middle, and bottom of freezer…vent the door
- ☐ c. Craig Anderson telephone (415) 749-6613

Liquid Nitrogen Repository Tank

- ☐ a. Call any member of the FREEZER FARM TEAM immediately

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Table 6.7 Pelco Services Quarterly Maintenance Form

PELCO SALES & SERVICE

Equipment Maintenance Log

Maintenance performed by:

Pelco Refrigeration
1550 Park Ave.
Emeryville, CA 94608

Phone: (510) 653-9850
Fax: (510) 653-0338

Equipment		
Mack #:	DC	Probe
Type:		
Make:		
Model:		
Serial:		
Location Dept.:		

Line/Instructions	Criteria	Measurement	Status	1Q Pelco/Initial/Date	Status	2Q Pelco/Initial/Date	Status	3Q Pelco/Initial/Date	Status	4Q Pelco/Initial/Date
1. Clean condenser coil.	Free of dust and debris									
2. Clean evaporator coil.	Free of dust and debris									
3. Clean out drain.	Unrestricted flow									
4. Clean fan blades and guards.	Free of dust and debris		/							
5. Clean water circuits; reser voir, distributor and screens.	Unrestricted flow									
6. Inspect gaskets for wear.	Provides tight seal									
7. Inspect handles, hinges and hardware.	Functioning properly									
8. Examine system wiring and electrical component integrity.	System wiring and electrical components in good condition									
9. Perform system check for refrigerant leaks.	No detectable leaks									
10. Examine piping, welds and flare connections.	No vibration or cracks									
11. Test temperature con trols.	Functioning properly									
12. Record cabinet tempera- ture.	Temperature is within its application range									
13. Lubricate all applicable motors and bearings.	Well lubricated, freely rotating									
14. Wash and/or replace fil- ters.	Free of dust and debris									
15. Examine back up system.	All components functioning properly									
16. Comments	See Reverse									
17. BCP review by/date										

Legend "P": Passed/Accomplished; "F": Failure per criteria; "N/A": Not applicable; "NP": Component not present; "IU": Unit in use, unable to perform step. Mack # DC Pr
Note Columns under status heading represent 1st, 2nd, 3rd and 4th quarters respectively.

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Table 6.8 Individual Unit Action Document

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BSRI
270 Masonic Avenue
San Francisco, CA 94118
Contact:_____
Tel._____

Freezer Farm
Freezer MACK #

Date	Action	Comments

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Title: Using Tempus Blood RNA Tubes			Page 1 of 2	
Doc. #: VRLRC0012	**Revision:**		**Effective date:**	**03/29/10**

1	**Purpose**		To outline the responsibilities and to define the steps when using Tempus tubes for the collection and isolation of RNA.
2	**Scope**	2.1	This procedure is used with protocols that require the collection and isolation of RNA. Gene expression measurements in human whole blood are becoming an increasingly important research tool.
3	**Responsibility**	3.1	It is the responsibility of the Supervisor to ensure that laboratory personnel have been trained in accordance with this procedure before using the Tempus Blood RNA tubes and to ensure that the training is documented.
		3.2	It is the responsibility of laboratory personnel to ensure he/she has read, understands and follows the procedure when using Tempus Blood RNA tubes.
		3.3	It is the responsibility of both the Supervisor and VRLRC personnel to ensure that Tempus Blood RNA tubes are stored properly post-phlebotomy.
		3.4	It is the responsibility of laboratory personnel to record and notify the Supervisor of any deviations from this procedure, which are not accounted for in this procedure.
4	**Materials Required**	4.1	Tempus™ Blood RNA Tubes with 6 mL of Applied Biosystems Stabilizing Reagent (Figure 1).
5	**Equipment Required**	5.1 5.2	-20^0C mechanical freezer -80^0C mechanical freezer
6	**Procedure**	6.1 6.2 6.3 6.5	Use Universal Safety precautions Draw 3mL of blood directly into Tempus Blood RNA tube. Shake vigorously for 10-20 seconds. Storage and shipping options are: 1. Vortex well before storage 2. Within 5 hours of receipt store at -20^0C 3. After 24 hrs move to -80^0C

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7	**Notes**	7.1	Tempus™ Blood RNA tubes can be stored at room temperature for up to 5 days.
		7.2	Therefore, Tempus™ Blood RNA tubes can be shipped at room temperature by FedEX Priority Overnight.

Figure 1. Illustration of Tempus tubes

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Title: Using Leucosep® tubes for the isolation of PBMCs			Page 1 of 8	
Doc. #: VRLRC 0013	**Revision:**		**Effective date:**	**03/29/10**

1	**Purpose**		To outline the responsibilities and to define the steps when using Leucosep® tubes for the isolation of peripheral blood mononuclear cells (PBMCs).
2	**Scope**	2.1	This procedure is used for the isolation of peripheral blood mononuclear cells (PBMCs) using Ficoll-Paque Plus™. This procedure is an alternative to the EDTA or ACD PBMC Cell Separation Overlay Procedure.
		2.2	In general there are eleven steps in the isolation of PBMCs using Leucosep® tubes: 1) 5 steps in preparing the tubes, and 2) 6 steps for the PBMC isolation procedure.
3	**Responsibility**	3.1	It is the responsibility of the Supervisor to ensure that laboratory personnel have been trained in accordance with this procedure before using the Leucosep® tubes for PBMC isolation and to ensure that the training is documented.
		3.2	It is the responsibility of laboratory personnel to ensure he/she has read, understands and follows the procedure when using Leucosep® tubes.
		3.3	It is the responsibility of laboratory personnel to record and notify the Supervisor of any deviations from this procedure, which are not accounted for in this procedure.
4	**Materials Required**	4.1 4.2 4.3 4.4 4.5 4.6 4.7 4.8 4.9 4.10 4.11	Leucosep® Tubes Ficoll-Paque Plus™ (Amersham Biosciences, density:1,077g/mL) 25mL pipets (USA Scientific) 15mL conical centrifuge tubes (VWR) 2mL cryovials for PBMC aliquots (VWR) Sterile glass Pasteur pipettes (VWR) 20µL and 1000µL pipette tips (Rainin) Freeze media (after Table 2) 9 x 9 freezer box with drains for LN_2 (Custom Biogenic Systems) Laboratory disposable gloves (E & K Scientific) Disposable laboratory coats (Market Lab)
5	**Equipment Required**	5.1 5.2 5.3 5.4 5.5	Centrifuge capable of 900x g Biohazard Safety cabinet -80^0C mechanical freezer LN2 freezer CoolCell (see CoolCell procedure)

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6	**Solutions/Buffers Required**	6.1 6.2 6.3 6.4 6.5	Mg^{++}, Ca^{++} free phosphate buffered saline (PBS) Heat inactivated Fetal bovine serum (UCSF Cell Culture Facility) Dimethyl sulfoxide, minimum 95.5% GC (Sigma/Aldrich) Ficoll-Paque Plus™ (Amersham) or Lymphoprep (AXIS-SHIELD PoC AS) Bleach (Blood Center Warehouse)
7	**Procedure**	7.1	Use Universal Safety precautions
		7.2	**Leucosep® Tube Preparation Steps:** Fill 50mL Leucosep® conical tube with 15mL of Ficoll-Paque Plus™ and cap tube.
		7.3	Centrifuge for 1 minute at 1000 x g at room temperature. After centrifugation step, the Ficoll-Pague™ will be beneath the barrier.
		7.4	Tubes may be prepared the night before and stored in the dark.
		7.5	**PBMC isolation Procedure using Leucosep® Tube:** If plasma has been removed, replace plasma volume with Ca^{++}, Mg^{++} free PBS to bring the blood volume back to its original volume.
		7.6	After replacing the plasma volume, gently mix tubes by inversion and process for PBMC's as follows.
		7.7	Pour the blood from either the ACD or EDTA tubes collectively into either a 50 mL or 225mL conical collection tube. The choice of tube size is based on the amount of blood being processed.
		7.8	Wash tubes sequentially with Ca^{++}, Mg^{++} free PBS to obtain the blood clinging to the sides of each tube. Place the PBS wash into the collection tube holding the blood.
		7.9	The ratio should be 1 volume of Ca^{++}, Mg^{++} free PBS to 1 volume of blood + PBS.
		7.10	Gently mix the PBS – Blood mixture in either a 50mL conical or a 225mL conical tube using a sterile 25mL pipette.
		7.11	The volume of PBS-Blood will determine the number of 50mL Leucosep® tubes to prepare (see Table 1).
		7.12	Pour 15 – 30mL of **PBS – Blood solution** into each of the prepared Leucosep® tubes (see Figure 1 and 2)

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		7.13	Centrifuge the tube(s), **no brake at room temperature** at 1000 x g for 10 minutes in a swinging bucket rotor using either the Sorvall RT6000B Refrigerated Centrifuge or the Sorvall Legend RT (see RPM versus g force conversion table in the VRLRC Laboratory Processes and Protocols binder).
		7.14	Once centrifugation is done, do not leave tubes in the centrifuge for an extended period of time. **The density gradient is toxic to the lymphocytes.**
		7.15	Very carefully aspirate off (with vacuum pump and glass Pasteur pipette) the upper layer (plasma+ PBS layer) up to a minimum remnant of 5 to 10 mm leaving the lymphocyte layer undisturbed at the interface (see Figure 4). This helps to prevent contamination of the PBMCs with platelets.
		7.16	Collect the PBMC interface layer from each tube and transfer into a fresh sterile conical 50 mL centrifuge tube. Note: The porous barrier effectively avoids recontamination with pelleted erythrocytes and granulocytes.
		7.17	Fill the 50 mL centrifuge tube with Ca^{++}, Mg^{++} free PBS. (1^{st} wash)
		7.18	Centrifuge with **no brake at room temperature** for 10 minutes at 200 x g in a swinging bucket rotor using either the Sorvall RT6000B Refrigerated Centrifuge or the Sorvall Legend RT (see RPM versus g force conversion table in the VRLRC Laboratory Processes and Protocols binder).
		7.19	Aspirate off Ca^{++}, Mg^{++} free PBS wash media and repeat wash step twice always filling the 50 mL centrifuge tube.
		7.20	Resuspend pellet in Ca^{++}, Mg^{++} free PBS in accordance with Table 2.
8	**Cell count using Coulter Counter**	8.1	From this cell-PBS suspension, take 10µL and add to the labeled Coulter counting vial containing 10 mL of Isoton solution.
		8.2	Follow the Coulter instrument procedure to count cells; apply raw counts, and dilution factors to get total cells in volume of PBS used to resuspend cells after 3^{rd} wash.

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		8.3	Divide the total number of cells by the number of cells (cells/mL) needed for each aliquot to get the number of cryovials that can be frozen down.
		8.4	Centrifuge as described in step 7.18.
		8.5	Gently remove Ca^{++}, Mg^{++} free PBS without disturbing the pellet.
9	**Preparing PBMCs for Liquid Nitrogen Storage**	9.1	Add Freeze Media dropwise for the first 2 to 3 mL and then gently add the remaining volume.
		9.2	Gently resuspend the cells in the freeze medium.
		9.3	Aliquot into the proper number of labeled cryovials
		9.4	Document the freezer box number and freezer box positions on the laboratory specific batch record and freeze cells in accordance with the CoolCell procedure. **The use of the CoolCell allows for optimal freezing at 1° per hour.**
		9.5	Store the laboratory specific batch record in front of the Virology and Immunology WNV- Study Shipping List for Specimens Shipping List form in the study specific binder.
10	**Special Notes**	10.1	See Figure 3 for the appearance of the Leucosep® tube after the initial centrifugation.
		10.2	15-30mL of blood-PBS solution maybe poured into a Leucosep® containing 15mL of Ficoll-Paque Plus™.
		10.3	Use and amount of FBS used through out the procedure is study specific.
		10.4	Final concentration of PBMCs/mL is study specific.
		10.5	Number of cryovials made is study specific

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Table 1.

Blood + Replacement PBS after plasma removal mixture	Mixing Tubes	Additional PBS volume (total)	Total Volume	Density Gradient per 50mL tube	Overlay volume of blood + PBS	Number of 50 mL tubes for overlay
10 mL	50 mL tube	10 mL	20 mL	15 mL	20mL	1
20 mL	50 mL tube	20 mL	40 mL	15 mL	20 mL	2
30 mL	50 mL tube	30 mL	60 mL	15 mL	30 mL	2
40 mL	225 mL tube	40 mL	80 mL	15 mL	20 mL	4
50 mL	225 mL tube	50 mL	100 mL	15 mL	25 mL	4
60 mL	225 mL tube	60 mL	120 mL	15 mL	30 mL	4
70 mL	225 mL tube	70 mL	140 mL	15 mL	28 mL	5

Table 2.

Number and size of Starting EDTA tubes	Amount of Ca^{++}, Mg^{++} free PBS used to resuspend PBMC pellet after 3rd wash
7 x 10mL EDTA tubes	5mL
1 x 10mL EDTA tube	1mL
2 x 10mL EDTA tubes	2mL
3 x 10mL EDTA tubes	3mL
4 x 10mL EDTA tubes	4mL

Media Preparation: Use .22 µ filters for media filtration.

1. **Wash media – PBS (Ca^{++}, Mg^{++} free) only, RT**
2. **Freeze Media – 45 mL FBS, 5 mL DMSO, 4°C**

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Figure 1. Filling with **PBS – Blood solution**

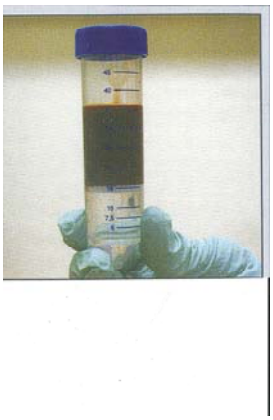

Figure 2. Appearance of Leucosep® tube before centrifugation

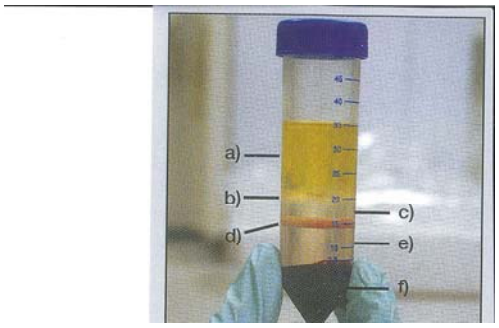

Figure 3. Appearance of the Leucosep® tube after centrifugation.

a) Plasma, b) enriched cell fraction after centrifugation, i.e. PBMCs., c) Ficoll-Paque Plus™, d) porous barrier, e) Ficoll-Paque Plus™, f) erythrocyte and granulocyte pellet.

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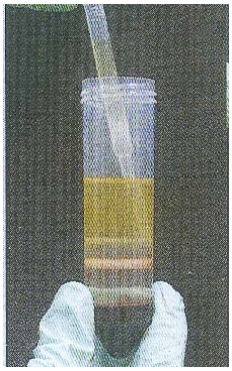

Figure 4. Carefully aspirating off the upper layer (plasma+ PBS layer) up to a minimum remnant of 5 to 10 mm above the PBMC layer.

Brief Summation of Process: PBMC Cell Separation Procedure

Preparation of Leucosep® tubes:
1. Using room temperature Ficoll-Paque™, place 15mL in to a 50mL Leucosep® tube.
2. Centrifuge closed Leucosep® tubes at 1000 x g for 1 minute at room temperature with the break off. Note: Do not over centrifuge.
3. After centrifugation the Leucosep® tubes are ready for use. Tubes can be prepared the evening before being used but then they must be stored in the dark because Ficoll-Paque™ is light sensitive.
PBMC isolation procedure using Leucosep® tubes:
1. Spin blood and remove plasma
2. Replace removed plasma with equal volume with Ca^{++}, Mg^{++} free PBS
3. Wash tubes sequentially with Ca^{++}, Mg^{++} free PBS to obtain the blood clinging to the sides of each tube. Place the PBS wash into the collection tube holding the blood.
4. The ratio should be 1 volume of Ca^{++}, Mg^{++} free PBS to 1 volume of blood + PBS.
5. Mix PBS/blood mixture very well.
6. Use Leucosep® tubes containing Ficoll-Paque™, in accordance with Table 1 (Figure 1).
7. Centrifuge the tube(s), **no brake at room temperature** at 1000 x g for 10 minutes in a swinging bucket rotor.
8. Very carefully aspirate off (with vacuum pump and glass Pasteur pipette) the upper layer (plasma+ PBS layer) up to a minimum remnant of 5 to 10 mm leaving the lymphocyte layer undisturbed at the interface (see Figure 4).

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9. Harvest the enriched PBMCs by means of a Pasteur pipette or by pouring the supernatant above the porous barrier from the Leucosep® tube into another 50 mL centrifugation tube. Note: The porous barrier effectively avoids recontamination with pelleted erythrocytes and granulocytes.
10. Fill the 50 mL centrifuge tube with Ca^{++}, Mg^{++} free PBS. (1^{st} wash)
11. Centrifuge with **no brake at room temperature** for 10 minutes at 250 x g in a swinging bucket rotor
10. Aspirate off Ca^{++}, Mg^{++} free PBS wash media and repeat wash step two more times.

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Title: Procedure for logging the Receipt and Shipping of specimens				Page 1 of 3	
Doc. #: VRLRC 0014		**Revision:**		**Effective date:**	3/29/10
1	**Purpose**		To provide a chronological record of incoming and outgoing specimen shipments within the Viral Reference Laboratory and Repository Core (VRLRC) at Blood Systems Research Institute (BSRI).		
2	**Scope**		This procedure is to be used by all VRLRC departmental personnel trained in the performance of this procedure.		
3	**Responsibility**	3.1	It is the responsibility of the Supervisor to ensure that laboratory personnel have been trained in accordance with this procedure and to ensure that the training is documented.		
		3.2	It is the responsibility of laboratory personnel to ensure he/she has read, understands and follows this procedure subsequent to training.		
		3.3	It is the responsibility of laboratory personnel to record and notify the Supervisor of any deviations from this procedure, which are not accounted for by another procedures.		
4	**Materials**	4.1	Log entry sheet		
		4.2	Log book with completed copies of the log form(s)		
		4.3	Blade cutter with safety handle use to open boxes		
		4.4	Disposable gloves and or laboratory coat		
5	**Procedure**	5.1	Use Universal Safety Precautions.		
			Receipt of Specimen Shipments:		
		5.2	Using the log sheet, the date, initials of the receiving staff member and the source/related study of each incoming shipment must be recorded.		
		5.3	On a daily basis each incoming specimen shipment will be delivered to the processing laboratory or stored in the biospecimen repository.		
		5.4	Receiving staff within the processing laboratory will initial the log sheet when they take possession of the incoming specimens, i.e. shipment.		
			Shipping Out Specimens:		
		5.5	Using the log sheet, each out going specimen shipment will be documented as follows: date of shipment, initials of the staff member responsible for the shipment, source of specimens and recipient.		

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		5.6	Once a log entry sheet is full, the sheet is placed in the Log Book in the main office of the VRLRC Department.
6	**Special Notes**	6.1	The Log Book with filled copies of the log form will be kept in the VRLRC Department.
		6.2	Specimen shipments are received Monday through Saturday
		6.3	Specimen shipments are sent Monday through Thursday.
		6.4	Specimen shipment are either received or sent by FedEx Priority Overnight or World Courier.
		6.5	The “LOG IN” process is a daily event at the time of the action. No delayed entries are acceptable.

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VRLRC Specimen Receipt and Shipment LOG

Incoming Specimen Shipments				Outgoing Specimen Shipments			
Incoming Date:	Receiver Initials	Source of Specimens	Initials of Lab Staff Receiving Specimens	Outgoing Date	Initials of Staff member packaging up the shipment	Source of Specimens	Recipient

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Title: Usage of Specimen Processing and Storage Forms			Page 1 of 5	
Doc. #: VRLTRC 0015	**Revision:**		**Effective date:**	**03/29/10**

1	**Purpose**		To outline the responsibilities and to define the steps when using study specific processing and storage form(s).
2	**Scope**	2.1	This procedure is used with studies that entail the processing and storage of aliquots made from blood drawn for a specific study.
		2.2	In general there are two steps in the use of a specimen processing and storage form: 1) staff member processing study specific blood and storing aliquots made from this blood fills out the form and 2) form is filed in study specific binder.
3	**Responsibility**	3.1	It is the responsibility of the Supervisor to ensure that laboratory personnel have been trained in accordance with this procedure before processing blood drawn for a study managed by the VRLRC Department.
		3.2	It is the responsibility of laboratory personnel to ensure he/she has read, understands and follows the procedure when processing blood drawn for a study managed by the VRLRC Department.
		3.3	It is the responsibility of laboratory personnel to record and notify the Supervisor of any deviations from this procedure, which are not accounted for in other departmental procedures.
4	**Materials Required**	4.1 4.2 4.3	UFO Aliquot Processing and Storage Form WNV Intensive Study Specimens Processing and Storage Form Microchimerism Study Specimen Processing and Storage Form
5	**Procedure**	5.1 5.2 5.3 5.4	Each staff member processing blood drawn for a specific study managed by the VRLRC Department, will fill out their own form for the aliquots they made. Place label with specimen identification number in the Study ID rectangle on the appropriate form. Record on the appropriate form the date and time that processing began as well as the staff's initials. Record on the form: 1) the number of aliquots made and 2) date and time they were placed in the -80^0C mechanical freezer once plasma, whole blood or serum processing is completed.

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		5.5	Record on the form: 1) the number of aliquots made, 2) initials of the processing staff member and 3) date and time PBMC aliquots were placed in the -80^0 C mechanical freezer once these aliquots have been made.
		5.6	Record on the form: 1) initials of the processing staff member and 2) the date and time the PBMC aliquots are transferred from -80^0 C to the LN2 freezer.
		5.7	Record on the form: 1) shelf (if rack is stored on a shelf), 2) rack, 3) box and 4) location of aliquots after the aliquots have been entered into Freezerworks.
		5.8	File form in study specific binder on top of the study shipping list for specimens.

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UFO Aliquot Processing and Storage Form

Specimen ID	Box Location	No. of Aliquots	Rack	Box	Position
	Plasma Aliquots				
	PBMC_ONE				
	PBMC_TWO				

Specimen ID	Box Location	No. of Aliquots	Rack	Box	Position
	Plasma Aliquots				
	PBMC_ONE				
	PBMC_TWO				

Specimen ID	Box Location	No. of Aliquots	Rack	Box	Position
	Plasma Aliquots				
	PBMC_ONE				
	PBMC_TWO				

Tech: ____ Sample Processing Date: _____ Time: _____

Tech: ____ Plasma freezing -80 Date: _____ Time: _____

Tech: ____ Serum freezing -80 Date: _____ Time: _____

Tech: _____PBMC freezing: -80 Date: _____ Time: _____

Tech _____ PBMC freezing: LN2 Date_____ Time: _____

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Microchimerism Study Specimen Processing and Storage Form

Study ID	Box Location	No. of Aliquots	Box	Position
	Plasma Aliquots			
	PBMC_ONE			
	PBMC_TWO			

Study ID	Box Location	No. of Aliquots	Box	Position
	Plasma Aliquots			
	PBMC_ONE			
	PBMC_TWO			

Study ID	Box Location	No. of Aliquots	Box	Position
	Plasma Aliquots			
	PBMC_ONE			
	PBMC_TWO			

Tech: _____Sample Processing Date: _____ Time: _____

Tech: _____Plasma freezing -80 Date: _____ Time: _____

Tech: _____PBMC freezing: -80 Date: _____ Time: _____

Tech _____ PBMC freezing: LN2 Date_____ Time: _____

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WNV Intensive Study Specimen Processing and Storage Form

Study ID	Number of Aliquots Made										
	TMA		Plasma		PBMC-One		PBMC-Two		Whole Blood		
	Box	Pos	Box	Pos	Box	Pos	Box	Pos	Box	Row	Pos

Study ID	Number of Aliquots Made										
	TMA		Plasma		PBMC-One		PBMC-Two		Whole Blood		
	Box	Pos	Box	Pos	Box	Pos	Box	Pos	Box	Row	Pos

Tech: ____ Sample Processing Date: _____ Time: _____

Tech: ____ Plasma/WB freezing -80 Date: _____ Time: _____

Tech: _____PBMC freezing: -80 Date: _____ Time: _____

Tech _____ PBMC freezing: LN2 Date_____ Time: _____

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Title: Using Tempus Blood RNA Tubes				Page 1 of 2	
Doc. #:	**VRLRC 0016**	**Revision:**		**Effective date:**	**4/26/10**

1	**Purpose**		The Coulter Counter Z1 provides a means of counting (quantifying) isolated peripheral blood mononuclear cells (PBMC).
2	**Scope**		This procedure is used to define the parameters of quantitative PBMC counting.
3	**Responsibility**	3.1	It is the responsibility of the Supervisor to ensure that laboratory personnel have been trained in accordance with this procedure before handling any PBMCs and to ensure that the training is documented.
		3.2	It is the responsibility of laboratory personnel to ensure he/she has read, understands and follows this procedure when preparing PBMCs for LN_2 storage.
		3.3	It is the responsibility of laboratory personnel to record and notify the Supervisor of any deviations from this procedure, which are not accounted for in this procedure.
4	**Materials**	4.1	Calibrated Coulter Counter Z1
		4.2	Blood Cell-Counter vials with snap caps (VWR)
		4.3	Isoton-II Diluent (Beckman Coulter)
		4.4	20 µL Pipet Plus (Rainin)
			20 µL Pipet Plus tips
5	**Equipment Required**	5.1	Coulter Counter Z1
6	**Procedure**	6.1	Step #1: obtain raw cell counts in duplicate using Coulter Counter Z1.
		6.2	Step #2: Calculate the average of the duplicate raw counts.
		6.3	Step #3: Multiply the average of the two raw counts times two. Note this is because the original raw counts were per 0.5 mL.
		6.4	Step #4: Multiply times the volume used for the final suspension of the PBMC pellet. Note, this volume is taken from Table 2 in the PBMC Overlay, Underlay and Leucosep procedures.
		6.5	Step #5: Multiply times a thousand. Note, 10µL of the final pellet suspension is re-suspended into 10mL of Isoton II diluent, i.e. 1:1000 dilution. This number is equal to the final PBMC cell count.
7	**Special Notes**	7.1	The basis for the PBMC final cell count calculation can be found in Figure 1.

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Coulter Count Calculation 01-22-2010

Basic Formula: Prep 1 = (C1 + C2) / 2 = Ave, times 2 = AveC / mL, times X tubes, *times 1000 (10^3) = Total # cells
Prep 2 = (C1 + C2) / 2 = Ave, times 2 = AveC / mL, times X tubes, *times 1000 (10^3) = Total # cells
Ave the two preps / # cells needed per vial

#1	Counts			
	10600			
	10631			
	Total			
	21231			
#2	Average			
	10616			
#3	x's 2	(to equal 1 mL)		
	equals			
	21231			
#4	x's 4	(multiplication factor equal to the number of tubes, but 5 or more tubes is always equal to factor of 5)		
	equals			
	84828			
#5	x's 10^3	(dilution of 1000)		
	equals			
	84,928,000	or	84.92 x 10^6	TOTAL
*	NOTE:			
	10 µL equals 0.01 mL			
	10 mL isoton / 0.01 mL equals 1000			

version 1-22-2010

Figure 1

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Title: VRLRC procedure for receiving specimens			Page 1 of 3	
Doc. #: VRLRC 0017	**Revision:**		**Effective date:**	4/26/10

1	**Purpose**		To outline the responsibilities and define the steps to be followed when receiving specimens to ensure consistency.
2	**Scope**		The procedure is used for receiving specimens within the Viral Reference Laboratory and Repository Core (VRLRC) at Blood Systems Research Institute in San Francisco.
3	**Responsibility**	3.1	It is the responsibility of the Supervisor to ensure that laboratory personnel have been trained in accordance with this procedure before receiving specimens within the VRLRC department and to ensure that the training is documented.
		3.2	It is the responsibility of laboratory personnel to ensure he/she has read, understands and follows this procedure when receiving specimens within the VRLRC department.
		3.3	It is the responsibility of laboratory personnel to record and notify the Supervisor of any deviations from this procedure, which are not accounted for by another procedure.
	Materials	4.1	Copy of the Shipping List.
		4.2	FedEx delivered packages containing either 1) Virology and Immunology WNV- 2009 Study Shipping List for Specimens or 2) another type of invoice.
		4.3	In-house study specific log sheet
5	**Procedure**	5.1	Use Universal Safety Precautions
		5.2	Receive and deliver FedEx package(s) to the appropriate laboratory.
		5.3	Unpack the contents of each package carefully
		5.4	Retain original box for reuse and return to the shipping area within the VRLRC.
		5.5	Verify sample identification numbers with the invoice that arrives with the package.
		5.6	Copy specimen identification numbers along with any comments onto a VRLRC study specific log sheet.
		5.7	Date VRLRC study specific log sheet. Steps 5.3 and 5.4 are study specific.
		5.8	Place invoice(s) and study specific log sheet(s) into the study specific log book.
		5.9	Make “in house” working labels using appropriate labeling program. Note, the labeling of tubes is study specific.

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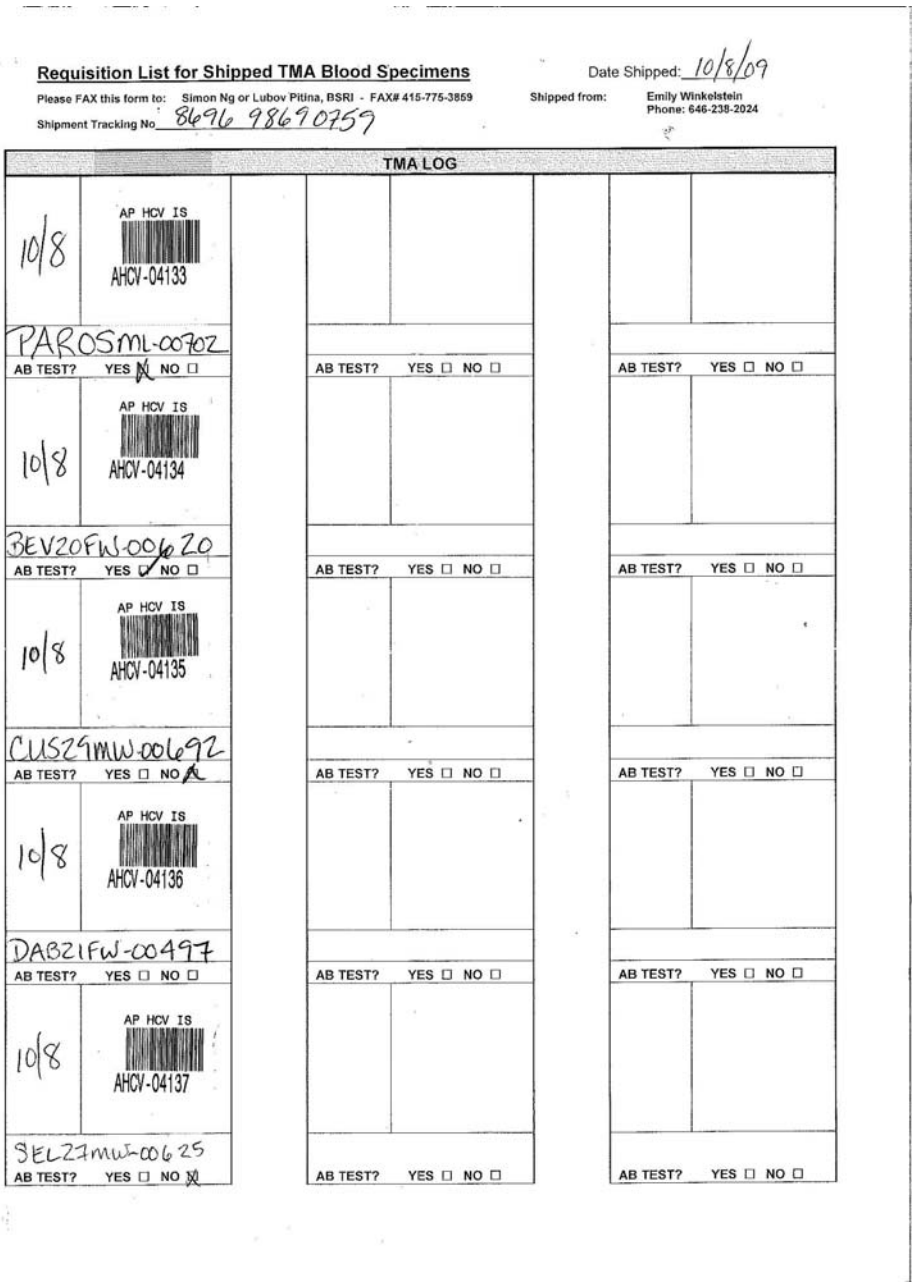
Attachments below are examples of: 1) a study specific invoice and 2) a study specific log sheet.



Requisition List for Shipped TMA Blood Specimens

Date Shipped: 10/8/09

Please FAX this form to: Simon Ng or Lubov Pitina, BSRI - FAX# 415-775-3859

Shipped from: Emily Winkelstein Phone: 646-238-2024

Shipment Tracking No 8696 9869 0759

TMA LOG

10/8 AP HCV IS AHCV-04133			
PAROSML-00702 AB TEST? YES ☒ NO ☐	AB TEST? YES ☐ NO ☐	AB TEST? YES ☐ NO ☐	
10/8 AP HCV IS AHCV-04134			
BEV20FWJ-00620 AB TEST? YES ☑ NO ☐	AB TEST? YES ☐ NO ☐	AB TEST? YES ☐ NO ☐	
10/8 AP HCV IS AHCV-04135			
CUS29MW-00692 AB TEST? YES ☐ NO ☒	AB TEST? YES ☐ NO ☐	AB TEST? YES ☐ NO ☐	
10/8 AP HCV IS AHCV-04136			
DAB21FW-00497 AB TEST? YES ☐ NO ☐	AB TEST? YES ☐ NO ☐	AB TEST? YES ☐ NO ☐	
10/8 AP HCV IS AHCV-04137			
SEL27MW-00625 AB TEST? YES ☐ NO ☒	AB TEST? YES ☐ NO ☐	AB TEST? YES ☐ NO ☐	

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SWAN (Brian Edlin) samples

Processing date: 10/09/09

#	Specimens ID	TMA	HCV EIA	Plasma	Rack	Box	Positions
1	AHCV- 04133	1	✓	1	4	45	3
2	AHCV- 04134	1	✓	2	↓	↓	4-5
3	AHCV- 04135	1		1	↓	↓	6
4	AHCV- 04136	1		1	↓	↓	7
5	AHCV- 04137	1		2	↓	↓	8-9
6	AHCV-						
7	AHCV-						
8	AHCV-						
9	AHCV-						
10	AHCV-						
11	AHCV-						
12	AHCV-						
13	AHCV-						
14	AHCV-						
15	AHCV-						
16	AHCV-						
17	AHCV-						
18	AHCV-						
19	AHCV-						
20	AHCV-						
21	AHCV-						
22	AHCV-						

Comments_____

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Title: Procedure for the requisition of study specific specimens stored at -40^{0}C and -80^{0}C			Page 1 of 2	
Doc. #: VRLRC 0018	**Revision:**		**Effective date:**	**4/26/10**

1	**Purpose**		To outline the responsibilities and to define the steps to be followed for the requisitioning study specific specimens from the Biorepository.
2	**Scope**	2.1	This procedure is applicable to all personnel within the VRLRC department who are engaged in the methodical and efficient retrieval of study specific specimens while preserving sample integrity.
3	**Responsibility**	3.1	It is the responsibility of the Supervisor to ensure that laboratory personnel have been trained in accordance with this procedure before retrieving any specimens from the Biorepository and to ensure that the training is documented.
		3.2	It is the responsibility of laboratory personnel to ensure he/she has read, understands and follows this procedure when retrieving any specimens from the Biorepository.
		3.3	It is the responsibility of both the Supervisor and VRLRC personnel to ensure that the integrity of all specimens retrieved from the Biorepository is maintained.
4	**Materials Required**	4.1	Hardcopy of a specimen pull list
		4.2	Dry Ice
		4.3	Laboratory disposable gloves (E&K Scientific and MRI Company)
		4.4	Disposable laboratory coats (Market Lab)
		4.5	Freezer box (Market lab)
		4.6	22 x 17 ½ inches Tray (Quantum Storage Systems)
		4.7	11 x 8 inches Tray (Quantum Storage Systems)
5	**Equipment Required**	5.1	-40°C Mechanical Freezer -80^{0}C Mechanical Freezer
6	**Procedure**	6.1	Using the proper Personal Protective Equipment (PPE), place a single layer of dry ice on a 22” x17 ½” or 11” x 8” tray, sufficient to cover the length of the freezer box being used.

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		6.2 6.3 6.4 6.7	Pull out specimens listed on the specimen sample pull list and insert into a new freezer box for storage and shipment. Verify sample identification numbers on the aliquot tubes with the information listed on the specimen pull list. Label the new freezer box with the appropriate information and place in an -80°C mechanical freezer for storage until specimens can be released for shipment. Place the original freezer box back in the location and freezer from which it was taken. If all samples in the original box have been pulled, discard the freezer box.
7	**Special Note**	7.1	Use Universal Safety Precautions

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Title: Procedure for the requisition of study specific specimens stored at -40^{0}C and -80^{0}C			Page 1 of 2	
Doc. #: VRLRC 0018	**Revision:**		**Effective date:**	**4/26/10**

1	**Purpose**		To outline the responsibilities and to define the steps to be followed for the requisitioning study specific specimens from the Biorepository.
2	**Scope**	2.1	This procedure is applicable to all personnel within the VRLRC department who are engaged in the methodical and efficient retrieval of study specific specimens while preserving sample integrity.
3	**Responsibility**	3.1	It is the responsibility of the Supervisor to ensure that laboratory personnel have been trained in accordance with this procedure before retrieving any specimens from the Biorepository and to ensure that the training is documented.
		3.2	It is the responsibility of laboratory personnel to ensure he/she has read, understands and follows this procedure when retrieving any specimens from the Biorepository.
		3.3	It is the responsibility of both the Supervisor and VRLRC personnel to ensure that the integrity of all specimens retrieved from the Biorepository is maintained.
4	**Materials Required**	4.1	Hardcopy of a specimen pull list
		4.2	Dry Ice
		4.3	Laboratory disposable gloves (E&K Scientific and MRI Company)
		4.4	Disposable laboratory coats (Market Lab)
		4.5	Freezer box (Market lab)
		4.6	22 x 17 ½ inches Tray (Quantum Storage Systems)
		4.7	11 x 8 inches Tray (Quantum Storage Systems)
5	**Equipment Required**	5.1	-40°C Mechanical Freezer -80^{0}C Mechanical Freezer
6	**Procedure**	6.1	Using the proper Personal Protective Equipment (PPE), place a single layer of dry ice on a 22” x17 ½” or 11” x 8” tray, sufficient to cover the length of the freezer box being used.

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		6.2 6.3 6.4 6.7	Pull out specimens listed on the specimen sample pull list and insert into a new freezer box for storage and shipment. Verify sample identification numbers on the aliquot tubes with the information listed on the specimen pull list. Label the new freezer box with the appropriate information and place in an -80°C mechanical freezer for storage until specimens can be released for shipment. Place the original freezer box back in the location and freezer from which it was taken. If all samples in the original box have been pulled, discard the freezer box.
7	**Special Note**	7.1	Use Universal Safety Precautions

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Title: Procedure for the Requisition of Study Specific Specimens Stored in Liquid Nitrogen		Page 1 of 2	
Doc. #: VRLRC 0019	**Revision:**	**Effective date:**	**5/17/10**

1	**Purpose**		To outline the responsibilities and to define the steps to be followed for the requisition of study specific specimens stored in liquid nitrogen.
2	**Scope**	2.1	This procedure is used to pull study specific specimens in a methodical and efficient fashion while preserving the integrity of the sample.
		2.2	This procedure is applicable to all personnel within the VRLRC department.
3	**Responsibility**	3.1	It is the responsibility of the Supervisor to ensure that laboratory personnel have been trained in accordance with this procedure before pulling study specific samples.
		3.2	It is the responsibility of the Viral Reference Laboratory and Repository Core (VRLRC) personnel to ensure he/she has read, understands and follows this procedure.
		3.3	It is the responsibility of VRLRC personnel to record and notify the Supervisor of any deviation from this procedure, which is not accounted for in another procedure.
4	**Materials Required**	4.1	Hard copy of a specimen pull list
		4.2	Dry Ice
		4.3	Laboratory disposable gloves (E&K Scientific)
		4.4	Waterproof Cryo-gloves (Lab Safety Supply)
		4.5	Full Face Shield
		4.6	Disposable laboratory coats (Market Lab)
		4.7	Freezer box (Market Lab)
		4.8	22 x 17 ½ inches Tray with dividers (Quantum Storage Systems) or 11 x 8 inches Tray with dividers (Quantum Storage Systems)
5	**Equipment Required**	5.1	Liquid Nitrogen Freezers (Taylor Wharton or MVE)
6	**Procedure**	6.1	Use Universal Safety Precautions throughout this procedure
		6.2	Obtain specimen sample pull list.
		6.3	Using the proper Personal Protective Equipment (PPE), place a single layer of dry ice on a 22 x17 ½ or on 11 x 8 inches tray sufficient to cover the length of the Freezer Box being used.

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		6.4	Label the “transfer to freezer box” prior to opening the LN2 freezer.
		6.5	Quickly pull out the specimens listed on the specimen sample pull list and insert them into a new freezer box for storage.
		6.6	Transfer the cryovials from the LN2 freezer to the solid CO_2 as quickly as possible, as the cryovial will warm up at ~10-20^0 C/min. and must not rise above -50^{0}C.
		6.7	Verify sample identification numbers on the aliquot cryovials with the information listed on the specimen sample pull list.
		6.8	Quickly replace the original box in the designated LN2 freezer. If all samples in the original box have been pulled, discard the freezer box.
		6.9	Place freezer box in an -80°C mechanical freezer for storage until specimens can be released.

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Title: Daily Monitoring of Mechanical and Liquid Nitrogen Freezers as well as Teledyne Oxygen Monitors Procedure			**Page 1 of 7**	
Doc. #: VRLRC 0020	**Revision:**		**Effective date:**	**4/26/10**

1	**Purpose**		To outline the responsibilities and define the steps to be following during the daily monitoring (Monday – Friday) procedures during the daily monitoring of the mechanical refrigeration and freezer units, the liquid nitrogen freezer and the Teledyne Oxygen monitors.
2	**Scope**	2.1	This procedure is used for the daily: 1) temperature monitoring of the mechanical freezers using the digital displays on the mechanical freezers (-40°C and -80°C), as well as the NIST certified stand alone thermometers in mechanical -4^{0}C, -20^{0}C freezers and 5^{0}C refrigerator, 2) temperatures, LCD LN2 levels, dewar LN2 level and manually read LN2 levels in the LN2 freezers, and 3) oxygen levels read off the Teledyne Oxygen monitors.
		2.2	In general there are five steps in this procedure: 1) daily freezer and refrigerator temperature monitoring, 2) dewar pressure monitoring (measured in inches of water--capsuhelic gauge), 3) monitoring LCD LN2 levels as well as manually read LN2 levels in each LN2 freezers, 4) monitor oxygen levels read off the two Teledyne Oxygen monitors in the Freezer Farm and, 5) monitor the ambient Freezer Farm temperature off the stand alone NIST certified thermometer in the Freezer Farm.
3	**Responsibilities**	3.1	It is the responsibility of the Supervisor to ensure that laboratory personnel have been trained in accordance with this procedure before assuming this responsibility and to ensure that the training is documented.
		3.2	It is the responsibility of laboratory personnel to ensure he/she has read, understood, and will follow this procedure while performing daily monitoring tasks.
		3.3	It is the responsibility of personnel to notify the Supervisor and record (on the unit action sheet) any event, which may account for a unit alarm or unit malfunction.
4	**Materials Required**	4.1 4.2 4.3 4.4 4.5 4.6	Viral Reference Lab and Repository Core Temperature Monitoring log (monthly)—Attachment 1 Viral Reference Laboratory and Repository Core Liquid Nitrogen Temperature Monitoring log (monthly)---Attachment 2 Ambient Freezer Farm Temperature Monitoring Log (monthly)—Attachment 3 Daily Oxygen Monitor Log—Unit #1 (monthly)---Attachment 4 Daily Oxygen Monitor Log—Unit #2 (monthly)---Attachment 5 1st floor Dispensing MACK station

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5	**Procedures**		**Daily Procedures:**
		5.1	Daily temperature monitoring (Monday – Friday) using the Viral Reference Lab and Repository Core Temperature Monitoring form for the mechanical freezers.
		5.2	Note any fluctuations and/or temperature trends.
		5.3	Daily temperature monitoring using the Viral Reference Laboratory and Repository Core Liquid Nitrogen Temperature Monitoring form for the LN2 freezers.
		5.4	Record ambient Freezer Farm temperature using the Ambient Freezer Farm Temperature Monitoring Log form. Note, this thermometer is in the back left hand corner of the Freezer Farm.
		5.5	At the end of each month file completed log forms in appropriate binder.
		5.6	Record the daily oxygen level from the two (#1 and #2) Teledyne Oxygen Monitors in the Freezer Farm on the Daily Oxygen Monitor Log – Units #1 and 2 forms. Monitor the span variations if any from the set oxygen level of 20.8.
			Bi-weekly Procedure
		5.6	Manually fill each LN2 freezers on Tuesday and Friday using the LCD soft pad “fill” function. Note, this ensures that we don’t have a lapse in liquid nitrogen levels.
			Weekly Procedure
		5.8	Check 1st floor Dispensing MACK station to ensure that all monitored units are enabled on the MACK Alarm system.
6	**Special Notes**	6.1	All mechanical refrigerators and freezers as well as LN2 freezers are on the MACK alarm system
		7.1	Document all trouble incidents on individual unit logs and alert rest of Freezer Farm team.

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Month(s)_____

Viral Reference Lab and Repository Core
Temperature Monitoring

Date	Harris uprt -40°C	Therma(DD) uprt -80°C	Kenmore uprt -20°C	Harris uprt -80°C	Sanyo uprt -80°C	Therma(DD) uprt -80°C	Harris uprt -80°C	Harris uprt -80°C	Harris uprt -40°C	Baxter SP uprt -80°C	Harris chst -80°C	Innova chst -80°C	Harris chst -80°C	Harris chst -80°C	Kenmore uprt -20°C	Harris chst -80°C	Revco chst -80°C	Innova uprt -80°C	LG refrig 5°C	LG frzr -4°C	Kenmore frzr -20°C
	89-1	89-2	92-1	90-2	91-1	91-2	80-1	80-2	81-1	83-2	77-1	79-2	85-2	85-1	87-1	86-1	86-2	53-2	35-1	36-1	36-2
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Attachment 1

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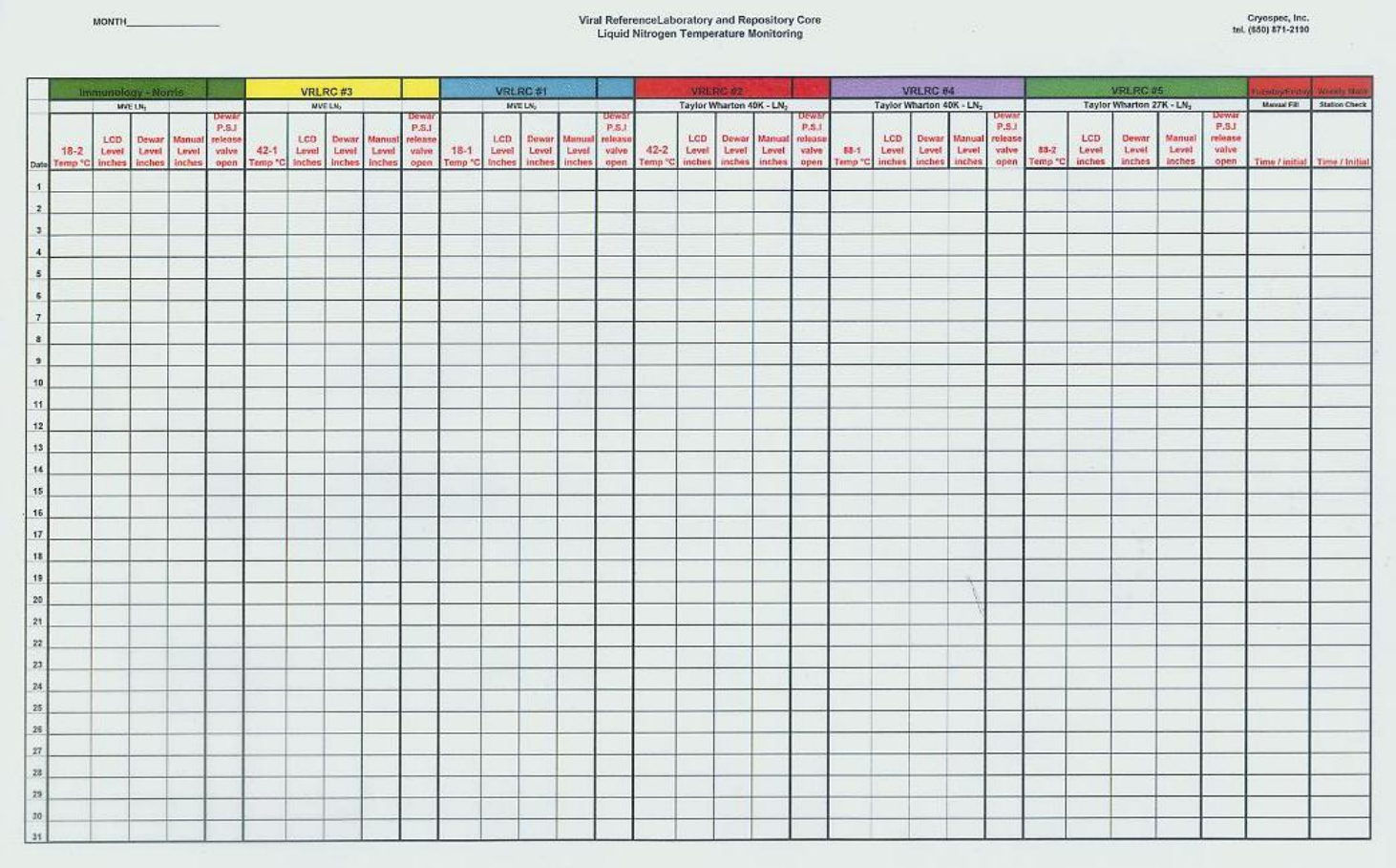

MONTH_____

Viral ReferenceLaboratory and Repository Core
Liquid Nitrogen Temperature Monitoring

Cryospec, Inc.
tel. (650) 871-2150

	Immunology - Norris					VRLRC #3					VRLRC #1					VRLRC #2					VRLRC #4					VRLRC #5					Tuesday/Friday	Weekly Mode
	MVE LN_2					MVE LN_2					MVE LN_2					Taylor Wharton 40K - LN_2					Taylor Wharton 40K - LN_2					Taylor Wharton 27K - LN_2					Manual Fill	Station Check
Date	18-2 Temp °C	LCD Level inches	Dewar Level inches	Manual Level inches	Dewar P.S.I release valve open	42-1 Temp °C	LCD Level inches	Dewar Level inches	Manual Level inches	Dewar P.S.I release valve open	18-1 Temp °C	LCD Level inches	Dewar Level inches	Manual Level inches	Dewar P.S.I release valve open	42-2 Temp °C	LCD Level inches	Dewar Level inches	Manual Level inches	Dewar P.S.I release valve open	88-1 Temp °C	LCD Level inches	Dewar Level inches	Manual Level inches	Dewar P.S.I release valve open	88-2 Temp °C	LCD Level inches	Dewar Level inches	Manual Level inches	Dewar P.S.I release valve open	Time / Initial	Time / Initial
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Attachment 2

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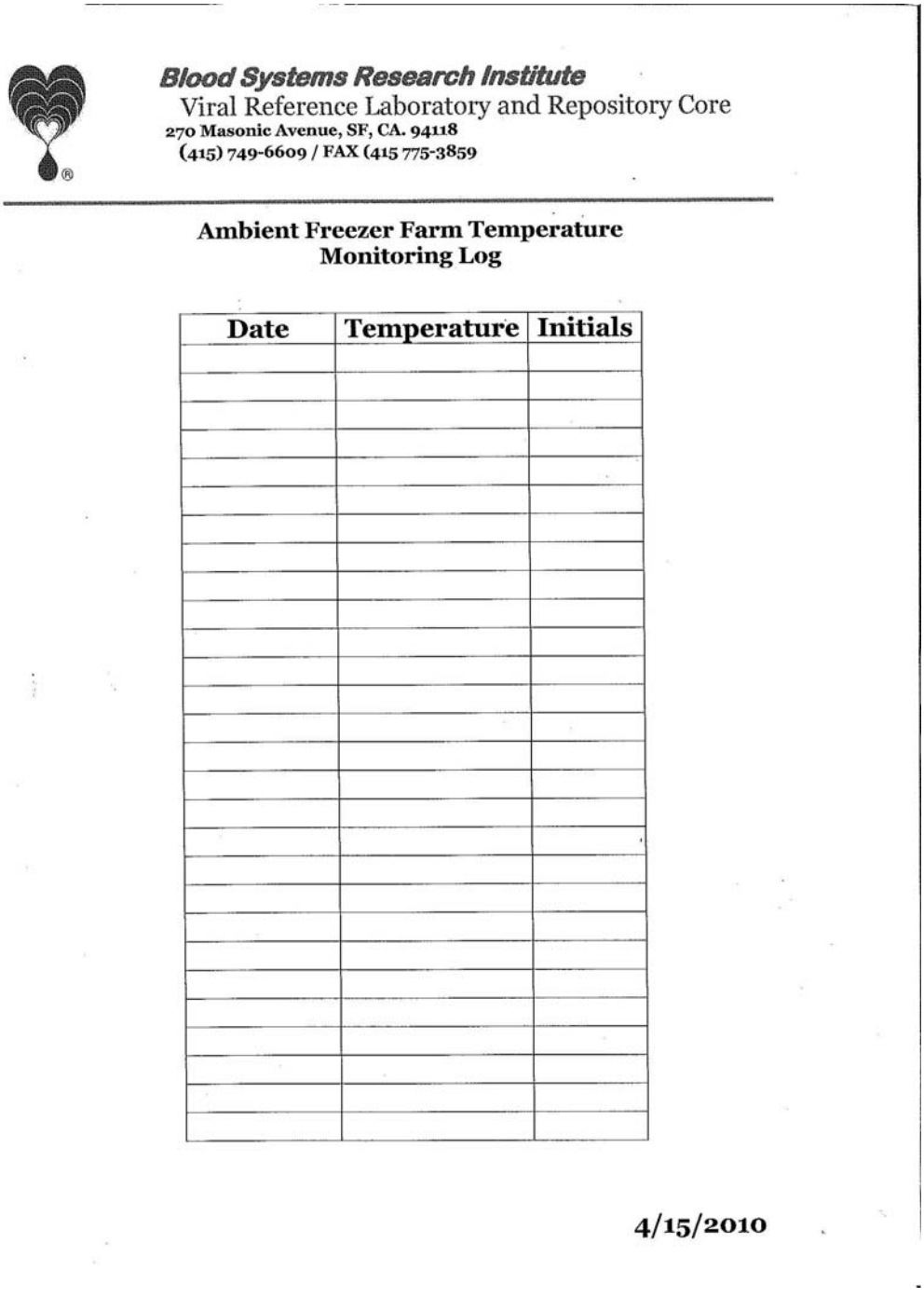

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Ambient Freezer Farm Temperature Monitoring Log

Date	Temperature	Initials

4/15/2010

Attachment 3

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DAILY OXYGEN MONITOR LOG – UNIT #1

Date:	Time:	Tech:	Oxygen Monitor Reading	Alarm 1	Explanation	Alarm 2	Explanation	Notes

Attachment 4

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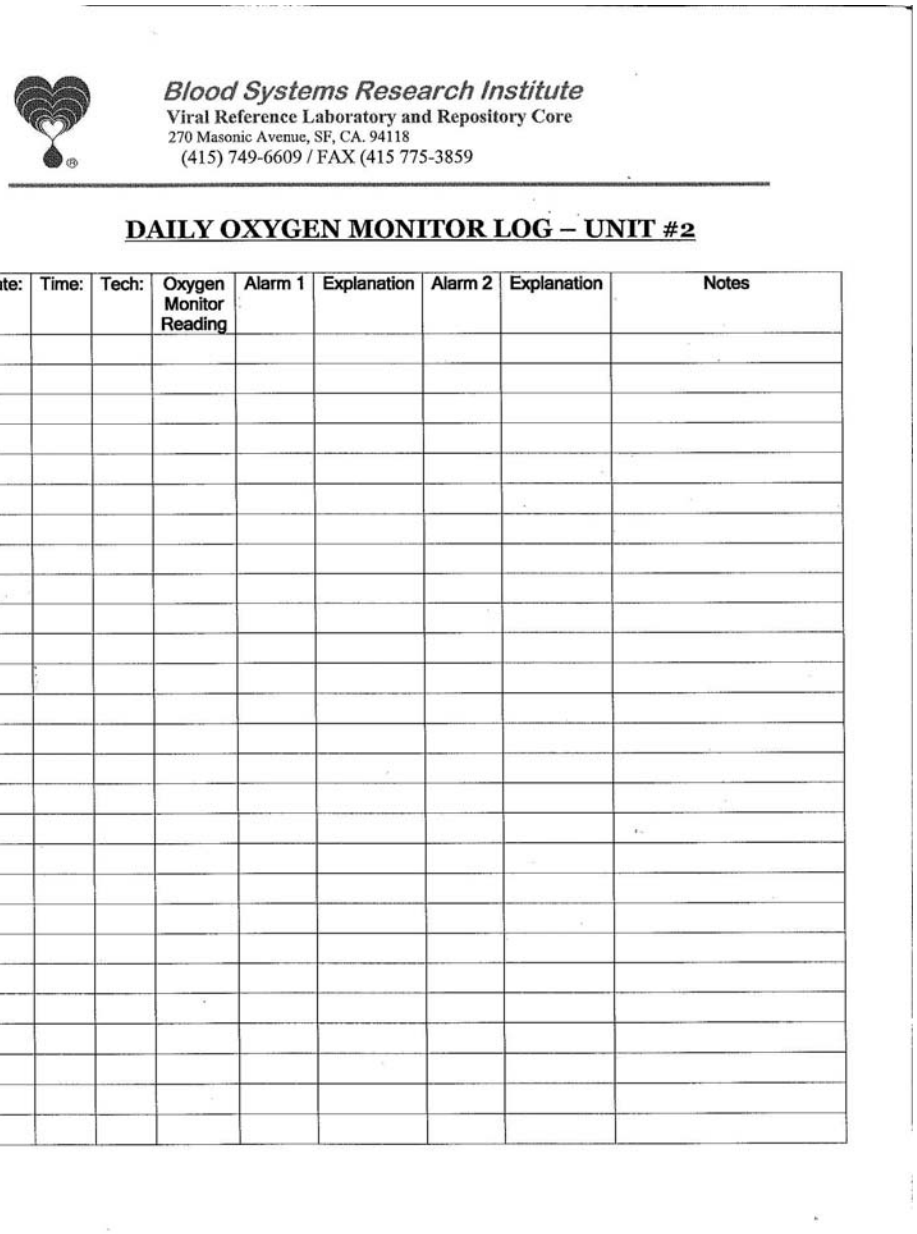

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DAILY OXYGEN MONITOR LOG – UNIT #2

Date:	Time:	Tech:	Oxygen Monitor Reading	Alarm 1	Explanation	Alarm 2	Explanation	Notes

Attachment 5

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Title: Oxygen Monitor Documentation and Alarm Response Procedure			Page 1 of 2
Doc. #: VRLRC 0021	**Revision:**		**Effective date: 5/12/10**

1	**Purpose**		To outline the responsibilities and to define the steps when responding to a caution level alarm 1 (oxygen 20.0%), a danger level alarm 2 (oxygen 19.5%) or a sensor failure alarm produced by the Teledyne 3350 oxygen monitors in the Biorepository located in the basement.
2	**Scope**	2.1	Applies to all VRLRC personnel responding to any alarm produced by the Teledyne 3350 oxygen monitoring systems in BSRI's Biorepository.
3	**Responsibility**	3.1	It is the responsibility of the Supervisor to ensure that laboratory personnel have been trained in accordance with this procedure before responding to a Teledyne 3350 alarm in the BSRI Biorepository.
		3.2	It is the responsibility of laboratory personnel to ensure he/she has read, understands and follows the procedure when responding to a Teledyne 3350 alarm in the BSRI Biorepository.
		3.3	It is the responsibility of both the Supervisor and VRLRC personnel to ensure that the Teledyne 3350 alarms in the BSRI Biorepository are maintained properly.
		3.4	It is the responsibility of laboratory personnel to record and notify the Supervisor of any deviations from this procedure, which are not accounted for in this procedure.
4	**Materials Required**	4.1	OSHA required action sheet located at each of the Teledyne 3350 oxygen monitors in the BSRI Biorepository located in the basement.
		4.2	Readout Monitoring Log
5	**Equipment Required**	5.1	Teledyne 3350 oxygen monitors
6	**Procedure**		**Teledyne 3350 oxygen monitor ----Alarm level 1 (CAUTION) is activated at 20% oxygen.**
		6.1	Reset the monitor. If the monitor will not reset, contact Teledyne technical assistance at (626) 934-1500.
			Teledyne 3350 oxygen monitor goes into "Sensor Fail" alarm, turn off the monitor.
		6.2	Either replace the oxygen sensor or turn off the monitor.
		6.3	If necessary immediately order a new oxygen sensor
		6.4	Replace the old oxygen sensor as soon as the new oxygen

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			sensor arrives.
		6.5	Date and initial the sensor when new sensor is installed.
		6.6	Turn monitor immediately back on.
			Teledyne 3350 oxygen monitor ----Alarm level 2 (DANGER) is activated at 19.5% oxygen.
		6.7	In all cases assume the alarm is valid and do not enter the room while the alarm is sounding.
		6.8	Immediately evacuate the basement
		6.9	Immediately notify Facilities staff, the Safety Specialist and Call 911
		6.10	If the situation is considered an emergency or life threatening, the fire alarm should be activated to alert the rest of the building to evacuate.
		6.11	Do not return to the area unless instructed to do so by Facilities staff, the Safety Specialist or the fire department.
7	**Special Notes**	7.1	Nitrogen vapor is a colorless, odorless, and tasteless gas constituting 78% by volume of the Earth's atmosphere.
		7.2	The Teledyne Electronic Technologies Analytical Instrument Model 3350 is a microprocessor-based oxygen alarm monitor for real-time measurement of the oxygen content of the atmosphere surrounding its sensor.
		7.3	Asphyxiation can occur when greater than normal quantities of nitrogen gas are present in the air.
		7.4	Alarm level 1 (CAUTION) is activated at an oxygen level of 20.0%. Note, the monitor will give off a continuous loud, high- pitched sound accompanied by a blinking LED readout on the monitor. that alternates between the oxygen level and the letters "CAUt" for Caution.
		7.5	Alarm level 2 (DANGER) is activated at 19.5% oxygen. Note, the monitor will give off a loud continuous high-pitched alarm accompanied by a continuously lit LED readout on the monitor. The readout will alternate between the oxygen level and the letters "dAng" for DANGER.
		7.7	Supervisory staff must account for all staff in the basement.
		7.7	The "Sensor Fail" alarm actuates when the output of the MicroFuel Cell sensor falls below the acceptable level (0.05% oxygen).

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Title: VRLRC Laminar Flow Biological Safety Cabinet maintenance			Page 1 of 3	
Doc. #: VRLRC0022	**Revision:**		**Effective date:**	**5/12/10**

1	**Purpose**		To outline the responsibilities and to define the steps to be followed when working in a laminar flow biological safety cabinet.
2	**Scope**	2.1	This procedure applies to all laboratory staff working in a laminar flow biological safety cabinet.
		2.2	A laminar flow biological safety cabinet is designed to provide three basic types of protection, 1) personnel protection from harmful agents inside the cabinet, 2) product protection to avoid contamination of the work, experiment or process, and 3) environmental protection from contaminants contained within the cabinet.
		2.3	In general there are four steps that need to be followed when working with a laminar flow biological safety cabinet: 1) daily procedures, 2) monthly, 3) yearly, and 4) as needed.
3	**Responsibility**	3.1	It is the responsibility of the Supervisor to ensure that laboratory personnel have been trained in accordance with this procedure and to ensure that the training is documented before using a laminar flow biological safety cabinet.
		3.2	It is the responsibility of laboratory personnel to ensure he/she has read, understands and follows this procedure when using a laminar flow biological safety cabinet.
		3.3	It is the responsibility of both the Supervisor and VRLRC personnel to ensure that all laminar flow biological safety cabinets are maintained in accordance with this procedure.
		3.4	It is the responsibility of laboratory personnel to record and notify the Supervisor of any deviations from this procedure, which are not accounted for in this procedure or another procedure.
4	**Materials Required**	4.1	Biohazardous Waste Containers (BCP Warehouse)
		4.2	Stainless steel discard bucket
		4.3	70% Ethyl Alcohol with hazard class 3 label (Flammable Liquids)
		4.4	PDI Sani-Cloth® Plus (Germicidal Disposable cloth) (BCP Warehouse)
		4.5	Clinisorb Non-Woven Sponges (Dukal Corporation) (BCP Warehouse)

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5	**Equipment**		Laminar flow biological safety cabinet
6	**Procedure**		**Daily Maintenance**
		6.1	Disinfect work surfaces with 70 % alcohol or an equivalent disinfectant before use.
		6.2	Disinfect work surfaces with 70 % alcohol or an equivalent disinfectant after use.
		6.3	After cleaning working surface, turn on the UV light or place a UV light in the safety cabinet.
			Monthly Maintenance
		6.4	Remove bottom panels and disinfect entire cabinet working chamber with zorbicide or other suitable disinfectant including walls, top and as much glass as possible.
			Yearly Maintenance
		6.5	Certify laminar flow biological safety cabinet using an outside vendor. Certification means that the laminar flow biological safety cabinet complies with the National Sanitation Foundation's Standard 49 (NSF-49). The NSF-49 certification method ensures that air balance is correct and filters are leak free (Attachment 1). Furthermore, certification ensures that the model tested will provide personnel, product, and cross contamination protection.

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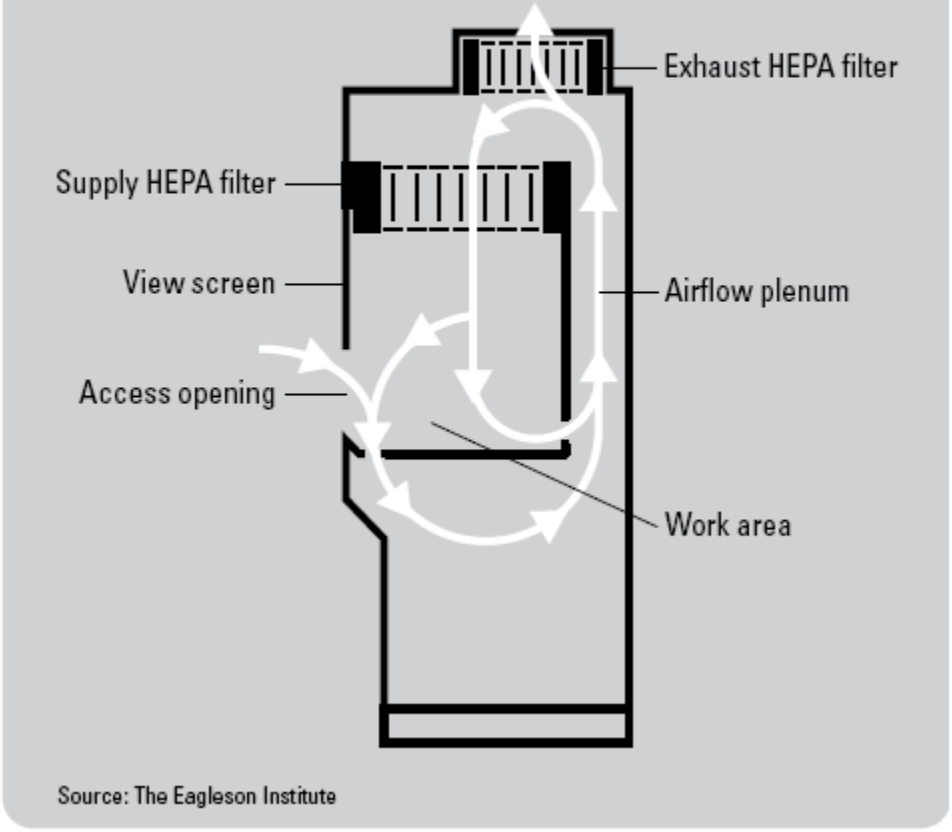
Figure 1: Class II BSC airflow schematic



Attachment 1

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Title: Procedure for Freezerworks Unlimited Database Sample Check-out Process		Page 1 of 2	
Doc. #:VRLRC0023	Revision:	Effective date:	5/17/10

1	**Purpose**		To outline the responsibilities and to define the steps to be followed for checking-out samples from the Freezerworks Unlimited Database.
2	**Scope**	2.1 2.2	This procedure is used after study specific specimens have been pulled from their repository location and need to be removed from the Freezerworks Unlimited Database. This procedure is applicable to all personnel within the VRLRC department.
3	**Responsibility**	3.1 3.2 3.3	It is the responsibility of the Supervisor to ensure that laboratory personnel have been trained in accordance with this procedure before removing from Freezerworks pulled study specific samples. It is the responsibility of the Viral Reference Laboratory and Repository Core (VRLRC) personnel to ensure he/she has read, understands and follows this procedure. It is the responsibility of VRLRC personnel to record and notify the Supervisor of any deviation from this procedure, which is not accounted for in another procedure.
4	**Materials Required**	4.1	Freezerworks Unlimited (version 4.0.26)
5	**Equipment Required**	5.1 5.2	A PC-compatible Pentium III with a 1280x1040 monitor resolution and 1 GB of available RAM. Operation system: Microsoft Windows XP Professional (version 5.1.2600)
6	**Procedure**	6.1	Open the Freezerworks Unlimited database.
		6.2	Enter username and password.
		6.3	Select “Shipping” on the menu bar at the top of the screen
		6.4	Next, select “Check Out Aliquots.”
		6.5	In the drop-down menu, select a previously created shipping list. A pop-up window will appear.
		6.6	Select option “Delete Position Information and Set Amount to Zero.” Click on the “OK” button.
		6.7	A window will appear asking you to confirm the deletion of aliquots. Click on “Continue”.
		6.8	When asked about the ASCII Export File Format, select “Skip.”
		6.9	When asked to “Select Report to Print”, click on “Skip.”

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		6.10	The next window, Freezerworks will ask for a second confirmation about the deletion of the aliquots. Again, click "Continue".
		6.11	The next window will state that the "Shipping Check Out Process" has been completed. Click on "OK".

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Title: GO grant WNV Sample ID assignment			Page 1 of 5
Doc. #: VRLRC0024		Revision:	Effective date: 5/17/10
1	**Purpose**		To outline the responsibilities and to define the steps to be followed when assigning West Nile Virus sample ID numbers during the GO grant.
2	**Scope**	2.1	This procedure is used to assign blinding sample numbers to aliquots made during the WNV arm of the GO grant.
		2.2	This procedure is applicable to all personnel within the VRLRC department performing data entry into Freezerworks Unlimited.
3	**Responsibility**	3.1	It is the responsibility of the Supervisor to ensure that laboratory personnel have been trained in accordance with this procedure before assigning Sample ID numbers.
		3.2	It is the responsibility of the Viral Reference Laboratory and Repository Core (VRLRC) personnel to ensure he/she has read, understands and follows this procedure before assigning Sample ID numbers.
		3.3	It is the responsibility of VRLRC personnel to record and notify the Supervisor of any deviation from this procedure, which is not accounted for in a study specific procedures.
4	**Materials Required**	4.1	10 mL EDTA vacutainer tubes filled with blood. Note the donor's identification number will be on the tubes..
		4.2	List of Sequential BSI ID numbers: GW 000001 – GW 001000 (Attachment #1)
		4.3	Virology and Immunology WNV- Study Shipping List for Specimens (Attachment #2).
		4.4	WNV Intensive Study Specimen Processing and Storage Form (Attachment #3)
5	**Equipment Required**	5.1	N/A
6	**Procedure**	6.1	Receive priority overnight shipped WNV follow-up specimens in "ready-to-go-shipper" from shipping area.
		6.2	Unpack EDTA vacutainer tubes from "ready-to-go-shipper".
		6.3	Compare Donor ID number on EDTA tubes to Donor ID number on Virology and Immunology WNV- Study Shipping List for Specimens

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		6.4	Choose next unused BSI ID number from the list of BSI ID numbers in room 18 next to the computer.
		6.5	Write the Donor ID number from the EDTA vacutainer tubes in the Donor ID number column next to the chosen BSI ID number.
		6.6	Cross out from List of Sequential BSI ID numbers, the BSI ID number you have just chosen for your set of WNV follow-up specimens
		6.7	Make a set of labels with BSI ID number plus sequence numbers.
		6.8	Place BSI ID numbers on: 1) Virology and Immunology WNV- Study Shipping List for Specimens, 2) the WNV Intensive Study Specimen Processing and Storage form, 3) on the 10mL EDTA vacutainer tubes from the set of follow-up specimens, 4) on the plastic tubes that will be consumed during PBMC isolation, and 5) on the repository cryovials for plasma, whole blood aliquots and PBMCs.
7	**Special Note**	7.1	This procedure is specifically for the WNV arm of the GO grant.

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BSI Sequential ID# for WNV Arm of GO Grant

BSI Sequential Number	Study ID# (Donor ID# + Time Point in Sequence of Follow-up Visits)
GW000001	
GW000002	
GW000003	
GW000004	
GW000005	
GW000006	
GW000007	
GW000008	
GW000009	
GW000010	
GW000011	
GW000012	
GW000013	
GW000014	
GW000015	
GW000016	
GW000017	
GW000018	
GW000019	
GW000020	
GW000021	
GW000022	
GW000023	

Attachment 1

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Please fax this form to: Simon Ng, BSRI
(No cover sheet required) Fax #: (415) 775-3859

Page: _____ of _____

Virology and Immunology WNV - Study Shipping List for Specimens

Blood Center Location: _____ **FedEx Tracking #:** _____

1. Collection center please fill in the left columns. **The study ID# for study subject can be located directly on the blood tube.**
2. Fax this form to Simon Ng (BSRI-(415)-775 3859) the same day the specimens are sent to BSRI, this alerts our lab staff of sample's arrival.
3. Include this form with shipment, fold and place inside box.
4. Shaded regions to be completed at BSRI lab upon arrival.

For Phlebotomy use only **For Lab use only**

Study ID# (Donor ID #)	Phlebotomy		Vacutainer tubes shipped to BSRI		Date/ Time tubes received	All tubes received? (Y/N)	Repository Storage			
Note: for privacy reasons Do Not use subject's name on this form, instead use study ID	Date	Time (24 hour clock)	Lavender Top 7 x 10mL + 1 x 4mL	Tempus Tube (3mL)			#Aliquots	Box	Position	Freezer
							PL			-80 °C WNV PL
							TMA			-80 °C WNV
Name of Phlebotomist							CE			$LiqN_2$ WNV CE
Signature			*Date*		*Condition of Specimens (if not satisfactory, please explain):*					

Version 03.30.10

Attachment 2

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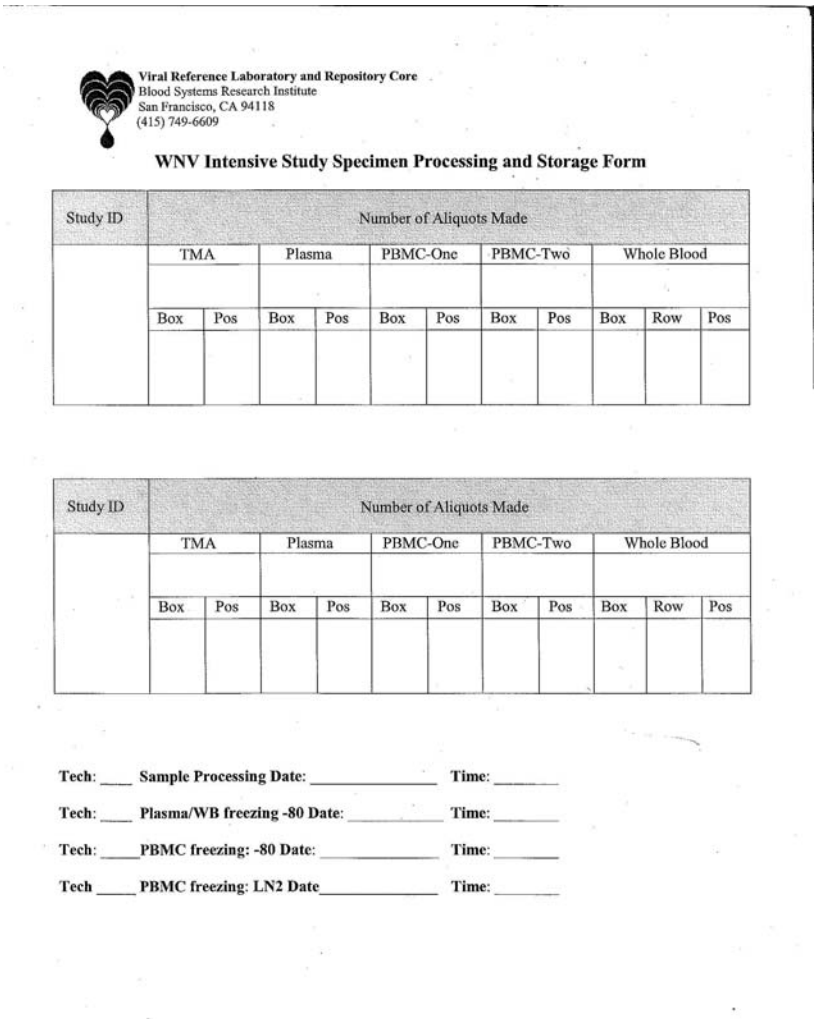

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WNV Intensive Study Specimen Processing and Storage Form

Study ID	Number of Aliquots Made										
	TMA		Plasma		PBMC-One		PBMC-Two		Whole Blood		
	Box	Pos	Box	Pos	Box	Pos	Box	Pos	Box	Row	Pos

Study ID	Number of Aliquots Made										
	TMA		Plasma		PBMC-One		PBMC-Two		Whole Blood		
	Box	Pos	Box	Pos	Box	Pos	Box	Pos	Box	Row	Pos

Tech: ____ Sample Processing Date: _____ Time: _____

Tech: ____ Plasma/WB freezing -80 Date: _____ Time: _____

Tech: ____ PBMC freezing: -80 Date: _____ Time: _____

Tech ____ PBMC freezing: LN2 Date_____ Time: _____

Attachment 3

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Title: Freezerwork's Data Entry for the GO Grant		**Page:** 1 of 5
Doc. #: VRLRC 0025	**Revision:**	**Effective date: 5/27/10**

1	**Purpose**		To outline the responsibilities and to define the steps to be followed when entering WNV data for the GO grant into the Freezerworks Unlimited database.
2	**Scope**	2.1 2.2	This procedure is used to track aliquot creation and storage using the Freezerworks Unlimited Database software. This procedure is applicable to all personnel within the VRLRC department performing WNV GO grant data entry into the Freezerworks Unlimited database.
3	**Responsibility**	3.1 3.2 3.3	It is the responsibility of the Supervisor to ensure that laboratory personnel have been trained in accordance with this procedure before entering WNV data from the GO grant into our Freezerworks Unlimited database. It is the responsibility of the Viral Reference Laboratory and Repository Core (VRLRC) personnel to ensure he/she has read, understands and follows this procedure while entering WNV data from the GO grant into our Freezerworks Unlimited database. It is the responsibility of VRLRC personnel to record and notify the Supervisor of any deviation from this procedure, which is not accounted for in study specific procedures.
4	**Materials Required**	4.1	Freezerworks Unlimited (version 4.0.26)
5	**Equipment Required**	5.1 5.2	A PC-compatible Pentium III with a 1280x1040 monitor resolution and 1 GB of available RAM. Operation system: Microsoft Windows XP Professional (version 5.1.2600)
6	**Procedure**	6.1 6.2 6.3 6.4	Open Freezerworks Unlimited Enter your username and password. Click on "Sample Mgmt" in the menu bar, From the drop down menu click on "Select Entry Format" and then select "West Nile 2010"from the drop down menu.
		6.5	Having selected the entry format, select "Sample Mgmt" again from the menu bar and click on "Add New Samples".
		6.6	Fill out the form four separate times, i.e. once for each sample type (plasma, whole blood, Tempus tube and PBMCs.
		6.7	**Fill in the following fields for Plasma Aliquots:** *Sample ID, Donor ID, Date Received, Received time, Study Name, Draw Date, Draw Time, Sample Type, Date Processed, Processed By, Plasma/WB Freeze date, and Plasma/WB Freeze time (Figure 1).*

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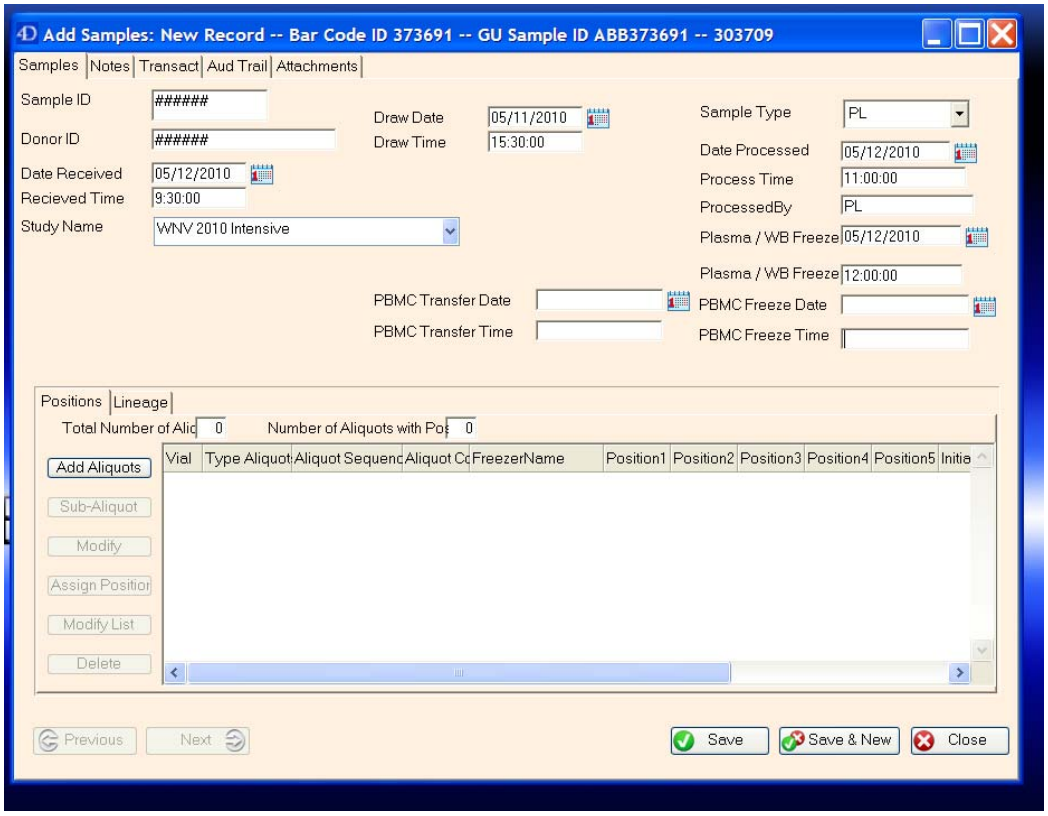

Figure 1. “Add Samples” Screen for plasma aliquots

		6.8 6.9 6.10 6.11 6.12	Select the “Positions” tab half way down the form. Fill in the “Total Number of Aliquots” box and then click on “Add Aliquots”. In the Modify Aliquot Data Window, select “WNV Int. Plasma” from the “Select Freezer” drop-down list. Click on the “Page 2” tab. Fill in the “Initial amount”, “Current amount” and set the units to “mL” for plasma. When adding data for PBMCs add “viability”. (*see Attachment 1 and 2 for typical aliquot information*)
		6.13	Click on the Page 1 tab.
		6.14	Select Plasma in the “Type Aliquot” drop down list.
		6.15	Choose “yes” on the pop up dialogue box asking you if you want to change the Aliquot Code.
		6.17	Click on “Assign” and then “Save”.
		6.18	Finally, Click on “Save & New” at the bottom of the “Add New Samples” screen. *(See Attachment 1 for typical WB, Tempus and PBMC aliquot information).*
		6.19	Repeat steps 6.5 to 6.18 for the whole blood aliquots. *(Fill in the same dates and times as you did for the plasma aliquots).*

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		6.20	Repeat steps 6.5 to 6.18 for Tempus tubes. *(Leave Plasma/WB freeze date and time boxes blank as well as dates and times PBMC Freeze -80 and PBMC transfer).*
		6.21	Repeat steps 6.5 to 6.18 for PBMCs. **NOTE:** When filling in the "Add New Samples" form for the PBMCs aliquot leave the boxes for the dates and times for Plasma/WB aliquots blank (Figure 2).

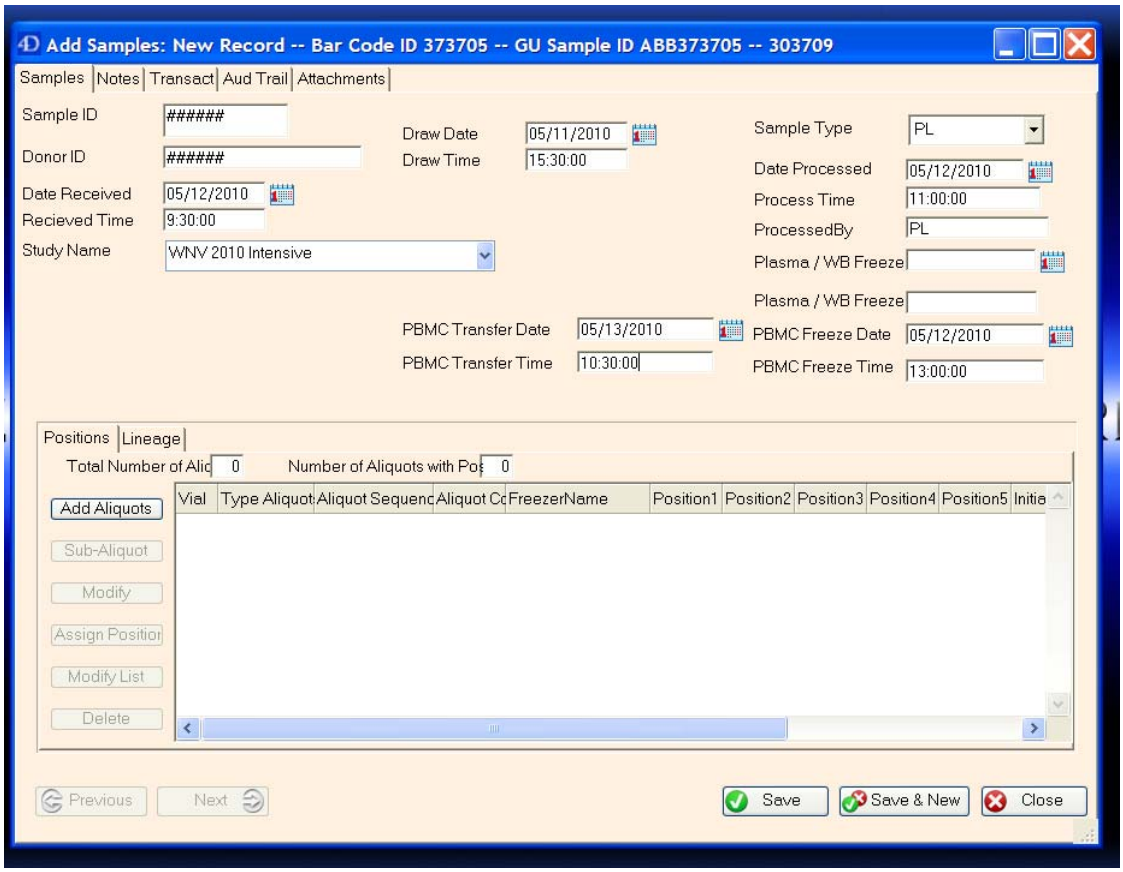

Figure 2. "Add Samples" Screen for PBMCs

		6.23	After entering the final PBMC aliquot, click on Save and then Close. Click on Close in the next window. Click on file, then Quit.

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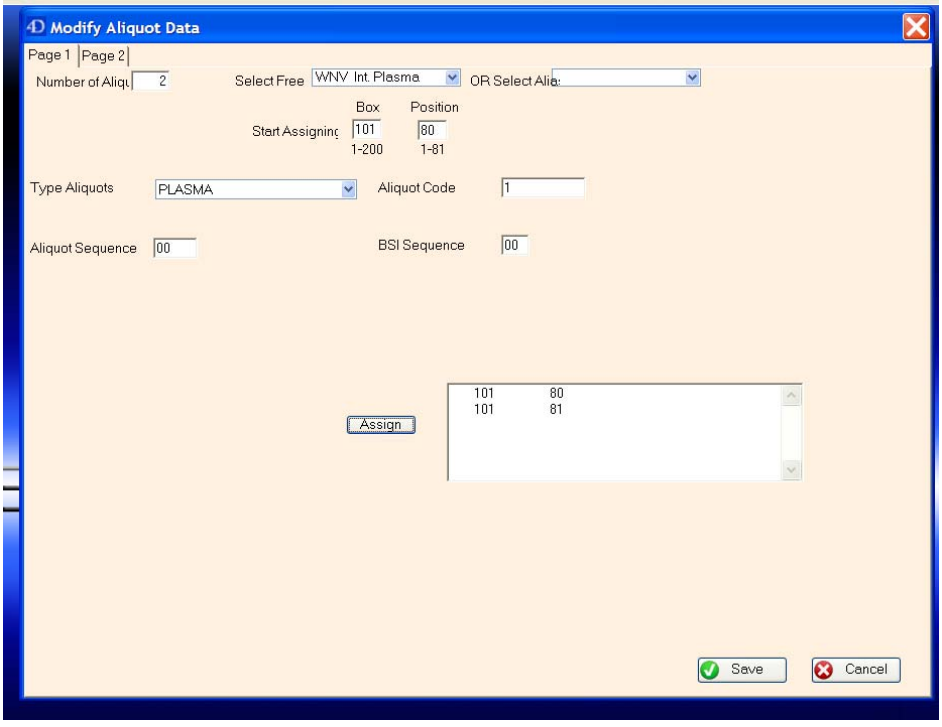
7	**Special Notes**	7.1	This data entry procedure is for the West Nile Virus portion of the GO grant only.
		7.2	PBMC aliquots are evenly split between liquid nitrogen freezers PBMC_ONE and PBMC_TWO.
		7.3	The Sample ID corresponds to the BSI ID.
		7.4	The Donor ID corresponds to the donor identification number plus the time point in the phlebotomy schedule for the donor, i.e. 1867838-01,1867838-02, and 1867838-03 for the phlebotomy during weeks one, two and three.
		7.5	Aliquot information varies depending on aliquots type, i.e. plasma, whole blood, PBMC and Tempus tube.

	Plasma	**Whole Blood**	**PBMC**	**Tempus Tube**
Freezer	WNV Int. Plasma	WNV Study WB	WNV Int. PBMC_ONE & WNV Int. PBMC_TWO	WNV 2010 Tempus Tube
Type Aliquot / Sample Type	Plasma	WB	PBMC	Tempus
Initial Amount	1.8	1	10	9
Current Amount	1.8	1	10	9
Units	mL	mL	million	mL
Viability			Value Varies	

Attachment 1. Typical GO grant aliquot information in Freezerworks.



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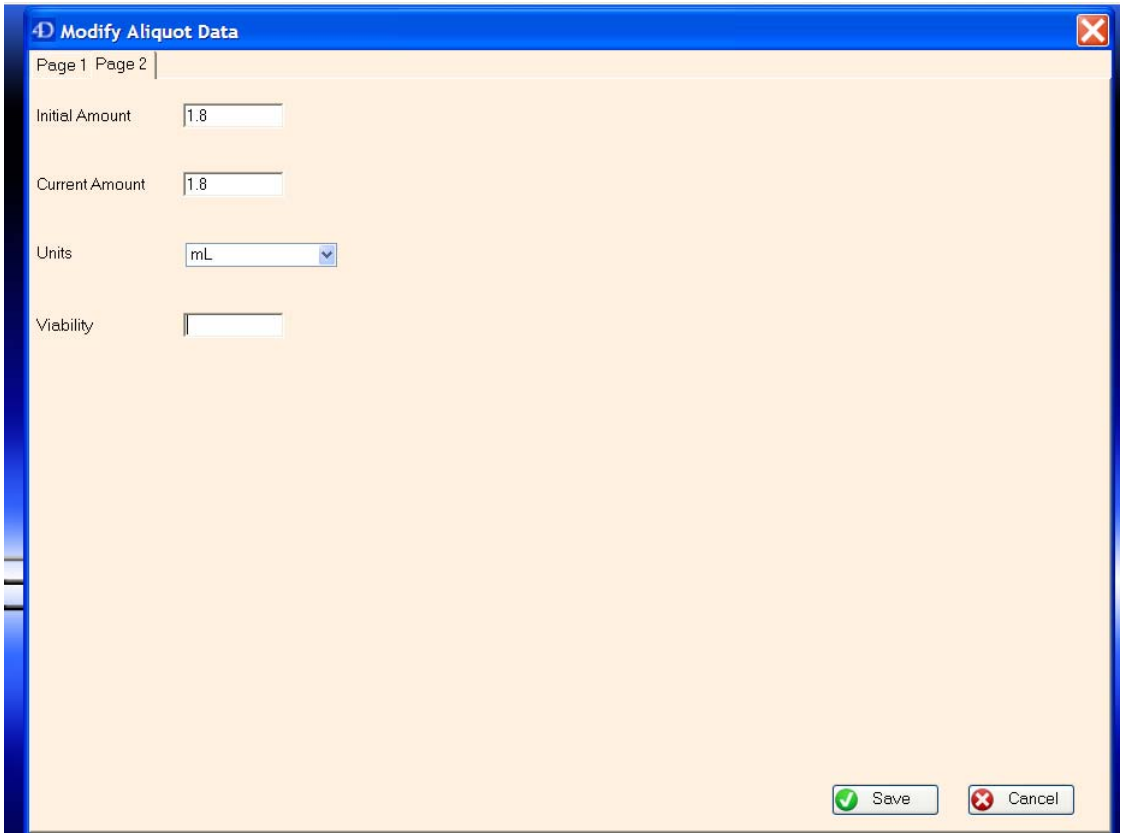

Attachment 2. “Modify Aliquot Data” screen for plasma.

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Title: LabScan Luminex Reader Maintenance			Page 1 of 3		
Doc#	Imm003	**Revision:**		**Effective Date:**	12/10/09

1	Purpose		To provide instructions on how to maintain the Luminex reader
2	Scope	2.1	
3	Responsibilities	3.1	It is the responsibility of the supervisor to ensure that the Core Immunology Laboratory personnel have been trained in accordance with using the Labscan Luminex reader.
		3.2	It is the responsibility of the supervisor to ensure that the personnel have been trained in properly handling human specimens and wearing PPE.
4	Materials Required	4.1	LabScan Luminex 100 IS Reader
		4.2	Computer
		4.3	Bio-Rad MCV Plate
		4.4	Bio-Plex Manager Software
		4.5	Deionizer Water
		4.6	70% Isopropanol
		4.7	10% Bleach
		4.8	20 mL syringe
		4.9	3/32 inch hex wrench
		4.10	Sonicator
5	Procedure	**5.1**	**Daily Start-up**
		5.1.1	Make sure that the shield fluid cube contains sufficient volume for assays
		5.1.2	Turn power on the Luminex 100 analyzer, Luminex XYP platform and the Luminex Sheath Fluid Delivery System
		5.1.3	Start the BioPlex Manager software
		5.1.4	Software will connect with the reader and automatically start a 30 minute timer to warm up the laser, during which the prime and start-up program may be run
		5.1.5	Select *Prime* from the drop down menu
		5.1.6	Reader will perform a 2 minute prime of the machine, no plate is needed
		5.1.7	Add deionized water and 70% Isopropanol to the appropriate wells in the MCV plate

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		5.1.8	Click on the *Start-up* icon on the software toolbar
		5.1.9	Click on open tray and place the MCV plate on the tray platform
		5.1.10	Close tray and click on start
		5.1.11	Remove MCV plate after program is finished
		5.2	**Daily Shut Down**
		5.2.1	Add 10% bleach to the appropriate well in the MCV plate
		5.2.2	Click on the *Shut Down* icon the software toolbar
		5.2.3	Click on open tray and place the MCV plate on the tray platform
		5.2.4	Close tray and click on start
		5.2.5	Shut down program takes 10 minutes
		5.2.6	Remove MCV plate after program is finished
		5.2.7	Turn off the power on the Luminex 100 analyzer, Luminex XYP platform and the Luminex Sheath Fluid Delivery System
		5.2.8	Close the BioPlex Manager Software program
		5.3	**Clean the sample probe**
		5.3.1	Remove the clear plastic housing that covers the sample probe area
		5.3.2	Unsnap the light housing located above the probe
		5.3.3	Unscrew the fitting on top of the probe completely
		5.3.4	Gently move the probe up out of the housing
		5.3.5	Clean the probe by placing the narrow tip into the sonicator for 2 to 5 minutes
		5.3.6	Using a 20 ml syringe, back flush the probe with distilled water from the narrow end out through the larger end
		5.3.7	Replace the probe
		5.3.8	Run 3 Backflushes, 2 Alcohol Flushes, and 3 Washes with deionized water
		5.4	**Adjust the sample probe height**
		5.4.1	Remove the clear plastic housing that covers the sample probe area
		5.4.2	Using an old Millipore plate, add three small round metal alignment discs into position H12

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		5.4.3	Select the maintenance tab, then click Eject/Retract to eject the plate platform
		5.4.4	Place the Millipore plate onto the plate platform
		5.4.5	Click Eject/Retract to retract the plate
		5.4.6	Use the 3/32 inch hex wrench to loosen the height adjustment locking screw
		5.4.7	Click sample probe down
		5.4.8	Using the thumb wheel, lower the probe until it just touches the top of the alignment discs
		5.4.9	Use the 3/32 hex wrench to tighten the height adjustment locking screw
		5.4.10	Click Sample Probe Up to raise the sample probe
		5.4.11	Check the alignment by clicking Sample Probe Down and watching the sample probe as it touches the top of the alignment discs
		5.4.12	There should be a very slight downward movement of the Millipore plate as the sample probe touches the alignment discs
		5.4.13	Readjust if necessary
		5.4.14	Replace the plastic shield that covers the probe area

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Title: Milliplex High Sensitivity Human Cytokine / Chemokine Kit				Page 1 of 4	
Doc#	Imm002	**Revision:**		**Effective Date:**	4/22/10

1	Purpose		To provide instruction on performing the Milliplex Cytokine Kit assay
2	Scope	2.1	To measure high sensitivity cytokines in plasma or serum.
3	Responsibilities	3.1	The Antibody-Immobilized Beads are light sensitive and must be protected from light.
		3.2	Cover the assay plate containing beads with opaque plate lid or aluminum foil duringall incubation steps.
		3.3	Reagents must be at room temperature before use.
		3.4	The bottom of the Microtiter Filter Plate must not be in direct contact with any surface during assay setup or incubation times. Use the plate stand at all times.
		3.5	Blot the bottom of the Microtiter Filter Plate after each wash with a paper towel.
		3.6	Keep vacuum settings at lowest possible level.
		3.7	After hydration, all Standards and Controls must be transferred to polypropylene tubes.
		3.8	Standards prepared by serial dilution must be used within one hour.
		3.9	Plate shaker should be set between 500 to 800 RPM.
		3.10	Frozen plasma or serum must be completely thawed, vortexed and centrifuged before addition to plate.
		3.11	Vortex all reagents well before adding to plate.
4	Materials Required	4.1	LabScan Luminex 100 IS Reader
		4.2	Computer
		4.3	Bio-Plex Manager Software
		4.4	Plate Shaker
		4.5	4° C Refrigerator
		4.6	Pipetman P1000 and P200 with tips
		4.7	Rainin Multichannel Pipette 20 ul – 200 ul

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		4.8	Polypropylene Microfuge Tubes
		4.9	Laboratory Vortex Mixer
		4.10	Vacuum Filtration Unit
		4.11	Vacuum Pump
		4.12	Reagent Reservoirs
		4.13	Plate Stand
		4.14	Plate Map Form
5	Procedure	5.1	Preparation of Reagents
		5.1.1	Preparation of Antibody-Immobilized Beads
		5.1.2	If premixed beads are used, sonicate bottle for 30 seconds then vortex for 1 minute before use.
		5.1.3	For individual beads, sonicate each vial for 30 seconds then vortex for 1 minute.
		5.1.4	Add 60 µl from each bead vial into the mixing bottle and then bring the final volume up to 3.0 mL with Bead Diluent. Vortex the mixed beads well.
		5.1.5	Quality Controls
		5.1.6	Reconstitute QC 1and QC 2 vials with 250 µL deionized water.
		5.1.7	Invert vial several times to mix and vortex.
		5.1.8	Let stand 5 – 10 minutes and transfer to polypropylene tubes.
		5.1.9	Wash buffer
		5.1.10	Dilute 30 mL of 10X Wash Buffer with 270 mL deionized water.
		5.1.11	Store at 2-8°C for up to one month.
		5.2	Preparation of Serum Matrix
		5.2.1	Add 1.0 mL deionized water to the lyophilized Serum Matrix. Mix well.
		5.2.2	Allow at least 10 minutes for complete reconstitution.
		5.3	Preparation of Human Cytokine Standards
		5.3.1	Reconstitute Human Cytokine Standard with 250 µL deionized water.
		5.3.2	Invert vial several times to mix and vortex.
		5.3.3	Let stand 5 – 10 minutes and transfer to polypropylene tube.
		5.3.4	This will be used as the 2,000 pg/mL standard.
		5.3.5	Label five polypropylene microfuge tubes 400, 80,

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			16, 3.2, 0.64 and 0.13 pg/mL.
		5.3.6	Add 200 µL Assay Buffer to each tube.

Prepare serial dilution:

Standard Concentration	Volume of Assay Buffer	Volume of Standard to add
2000	200 µL	50 µL of 10,000 pg/mL
400	200 µL	50 µL of 2000 pg/mL
80	200 µL	50 µL of 400 pg/mL
16	200 µL	50 µL of 80 pg/mL
3.2	200 µL	50 µL of 16 pg/mL

		5.4	Immunoassay Procedure
		5.4.1	Fill in the Milliplex High Sensitivity Human Cytokine Plate Map with the tech ID, lot number, date performed, and expiration date of the kit.
		5.4.2	Indicate the location of all the samples to be run on the Plate Map.
		5.4.3	Prewet the plate with 200 µL of Wash Buffer into each well.
		5.4.4	Seal and mix on the plate shaker for 10 minutes.
		5.4.5	Remove Wash Buffer by vacuum. Blot bottom of plate with a paper towel.
		5.4.6	Vortex the Bead Bottle and add 25 µL to each well.
		5.4.7	Remove liquid from plate by vacuum. Blot bottom of plate with a paper towel.
		5.4.8	Add 50 µL of each Standard and Control into the appropriate wells.
		5.4.9	Add 50 µL of Assay Buffer to the sample wells.
		5.4.10	Add 50 µL of serum matrix to the background, standards, and control wells.
		5.4.11	Add 50 µL of sample into the appropriate wells.
		5.4.12	Seal the plate with a plate sealer and cover it with a lid or aluminum foil.
		5.4.13	Place on the Shaker at 4° C for 16 to 18 hours.
		5.4.14	Remove fluid by vacuum
		5.4.15	Wash plate 2 times with 200 µL/well Wash Buffer,

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			removing Wash Buffer by vacuum filtration between each wash.
		5.4.16	Blot plate with paper towel.
		5.4.17	Add 50 µL of Detection Antibodies into each well.
		5.4.18	Seal, cover plate with lid, and incubate one hour at room temperature on the shaker.
		5.4.19	Do Not Vacuum After Incubation
		5.4.20	Add 50 µL Streptavidin-Phycoerythin to each well.
		5.4.21	Seal, cover plate with lid, and incubate 30 minutes at room temperature on the shaker.
		5.4.22	Remove fluid by vacuum.
		5.4.23	Wash plate 2 times with 200 µL/well Wash Buffer, removing Wash Buffer by vacuum filtration between each wash.
		5.4.24	Blot plate with paper towel.
		5.4.25	Add 100 µL of Sheath Fluid to all wells.
		5.4.26	Resuspend the beads on the plate shaker for 5 minutes.
		5.4.27	Run plate on the Luminex 100. See settings below for reader setup
		5.4.28	Save file to the G drive after completion.

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Title: Milliplex Human Cytokine / Chemokine Kit			Page 1 of 4		
Doc#	Imm001	**Revision:**		**Effective Date:**	4/22/10

1	Purpose		To provide instruction on performing the Milliplex Cytokine Kit assay
2	Scope	2.1	To measure cytokines in plasma or serum.
3	Responsibilities	3.1	The Antibody-Immobilized Beads are light sensitive and must be protected from light.
		3.2	Cover the assay plate containing beads with opaque plate lid or aluminum foil duringall incubation steps.
		3.3	Reagents must be at room temperature before use.
		3.4	The bottom of the Microtiter Filter Plate must not be in direct contact with any surface during assay setup or incubation times. Use the plate stand at all times.
		3.5	Blot the bottom of the Microtiter Filter Plate after each wash with a paper towel.
		3.6	Keep vacuum settings at lowest possible level.
		3.7	After hydration, all Standards and Controls must be transferred to polypropylene tubes.
		3.8	Standards prepared by serial dilution must be used within one hour.
		3.9	Plate shaker should be set between 500 to 800 RPM.
		3.10	Frozen plasma or serum must be completely thawed, vortexed and centrifuged before addition to plate.
		3.11	Vortex all reagents well before adding to plate.
4	Materials Required	4.1	LabScan Luminex 100 IS Reader
		4.2	Computer
		4.3	Bio-Plex Manager Software
		4.4	Plate Shaker
		4.5	4° C Refrigerator
		4.6	Pipetman P1000 and P200 with tips
		4.7	Rainin Multichannel Pipette 20 ul – 200 ul
		4.8	Polypropylene Microfuge Tubes

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		4.9	Laboratory Vortex Mixer
		4.10	Vacuum Filtration Unit
		4.11	Vacuum Pump
		4.12	Reagent Reservoirs
		4.13	Plate Stand
		4.14	Plate Map Form
5	Procedure	5.1	Preparation of Reagents
		5.1.1	Preparation of Antibody-Immobilized Beads
		5.1.2	If premixed beads are used, sonicate bottle for 30 seconds then vortex for 1 minute before use.
		5.1.3	For individual beads, sonicate each vial for 30 seconds then vortex for 1 minute.
		5.1.4	Add 60 µl from each bead vial into the mixing bottle and then bring the final volume up to 3.0 mL with Bead Diluent. Vortex the mixed beads well.
		5.1.5	Quality Controls
		5.1.6	Reconstitute QC 1and QC 2 vials with 250 µL deionized water.
		5.1.7	Invert vial several times to mix and vortex.
		5.1.8	Let stand 5 – 10 minutes and transfer to polypropylene tubes.
		5.1.9	Wash buffer
		5.1.10	Dilute 30 mL of 10X Wash Buffer with 270 mL deionized water.
		5.1.11	Store at 2-8°C for up to one month.
		5.2	Preparation of Serum Matrix
		5.2.1	Add 1.0 mL deionized water to the lyophilized Serum Matrix. Mix well.
		5.2.2	Allow at least 10 minutes for complete reconstitution.
		5.3	Preparation of Human Cytokine Standards
		5.3.1	Reconstitute Human Cytokine Standard with 250 µL deionized water.
		5.3.2	Invert vial several times to mix and vortex
		5.3.3	Let stand 5 – 10 minutes and transfer to polypropylene tubes.
		5.3.4	This will be used as the 10,000 pg/mL standard.
		5.3.5	Label five polypropylene microfuge tubes 2000, 400, 80, 16, and 3.2.

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		5.3.6	Add 200 µL Assay Buffer to each tube.

Standard Concentration	Volume of Assay Buffer	Volume of Standard to add
2000	200 µL	50 µL of 10,000 pg/mL
400	200 µL	50 µL of 2000 pg/mL
80	200 µL	50 µL of 400 pg/mL
16	200 µL	50 µL of 80 pg/mL
3.2	200 µL	50 µL of 16 pg/mL

		5.4	Immunoassay Procedure
		5.4.1	Fill in the Milliplex Human Cytokine/Chemokine Plate Map with the tech ID, lot number, date performed, and expiration date of the kit.
		5.4.2	Indicate the location of all the samples to be run on the Plate Map.
		5.4.3	Prewet the plate with 200 µL of Assay Buffer into each well.
		5.4.4	Seal and mix on the plate shaker for 10 minutes.
		5.4.5	Remove Assay Buffer by vacuum. Blot bottom of plate with a paper towel.
		5.4.6	Add 25 µL of each Standard and Control into the appropriate wells.
		5.4.7	Add 25 µL of Assay Buffer to the sample wells.
		5.4.8	Add 25 µL of serum matrix to the background, standards, and control wells.
		5.4.9	Add 25 µL of sample into the appropriate wells.
		5.4.10	Vortex the Bead Bottle and add 25 µL to each well.
		5.4.11	Seal the plate with a plate sealer and cover it with a lid or aluminum foil.
		5.4.12	Place on the Shaker at 4° C for 16 to 18 hours.
		5.4.13	Remove fluid by vacuum
		5.4.14	Wash plate 2 times with 200 µL/well Wash Buffer, removing Wash Buffer by vacuum filtration

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			between each wash.
		5.4.15	Blot plate with paper towel.
		5.4.16	Add 25 µL of Detection Antibodies into each well
		5.4.17	Seal, cover plate with lid, and incubate one hour at room temperature on the shaker.
		5.4.18	Do Not Vacuum After Incubation
		5.4.19	Add 25 µL Streptavidin-Phycoerythin to each well.
		5.4.20	Seal, cover plate with lid, and incubate 30 minutes at room temperature on the shaker. Remove fluid by vacuum.
		5.4.21	Wash plate 2 times with 200 µL/well Wash Buffer, removing Wash Buffer by vacuum filtration between each wash.
		5.4.22	Blot plate with paper towel.
		5.4.23	Add 150 µL of Sheath Fluid to all wells.
		5.4.24	Resuspend the beads on the plate shaker for 5 minutes.
		5.4.25	Run plate on the Luminex 100. See settings below for reader setup
		5.4.26	Save file to the G drive after completion

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Title: Luminex Quality Control			Page 1 of 3		
Doc#	Imm004	**Revision:**		**Effective Date:**	12.11.09

1	Purpose		To provide instructions on how to perform calibration and validation on the Lab Scan Luminex Reader
2	Scope	2.1	
3	Responsibilities	3.1	It is the responsibility of the supervisor to ensure that the Core Immunology Laboratory personnel have been trained in accordance with using the Labscan Luminex reader.
		3.2	It is the responsibility of the supervisor to ensure that the personnel have been trained in properly handling human specimens and wearing PPE.
4	Materials and Equipment	4.1	LabScan Luminex Reader
		4.2	Bio-Rad MCV Plate
		4.3	Vortexer
		4.4	Deionizer Water
		4.5	70% Isopropanol
		4.6	Bio-Plex Calibration Kit #
		4.7	Bio-Plex Validation Kit #
		4.8	Bio-Plex Manager Software
		5.1	Turn reader on and allow one-half hour warm up
5	Procedure	5.2	Turn on computer and start the Bio-Plex Manager Software
		5.3	Rinse MCV plate in tap water and blot
		5.4	Add di-water to the well label H2O
		5.6	Add 70% Isopropanol to well labeled 70% Isopropanol
		5.7	Perform start-up procedure
		5.8	Remove Bio-Plex Calibration kit from refrigerator
		5.9	Vortex Cal 1 bead bottle for 30 seconds
		5.10	Add 5 drops to well labeled Cal 1
		5.11	Vortex Cal 2 bead bottle 30 seconds
		5.12	Add 5 drops to well labeled Cal 2
		5.13	Open reader draw and place MCV plate onto platform
		5.14	Close reader draw
		5.15	Click on calibration Button
		5.16	Check that the calibration bottle information is correct

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6	Calibration	6.1	Rinse the MCV plate with tap water and blot
		6.2	Add deionized water and 70% Isopropanol to the appropriate wells in the MCV plate
		6.3	Remove the Calibration kit from the refrigerator
		6.4	Vortex the Cal 1 and Cal 2 bottles for 30 seconds each
		6.5	Place five drops from the Cal 1 bottle into the Cal 1 well
		6.6	Place five drops from the Cal 2 bottle into the Cal 2 well
		6.7	Click on Calibration icon in the toolbar
		6.8	Calibrate menu will display
		6.9	Cal 1 & Cal 2 button should be marked in the Select Calibration type
		6.10	Check that Cal 1 and Cal 2 Control Numbers correspond to the numbers on the bottles
		6.11	Check that the DD Target, CL1 Target, and the CL2 Target correspond to the values on the Cal 1 bottle
		6.12	Check that the RP1 value correspond to the value on the Cal 2 bottle
		6.13	Make sure the calibration bottles are within the expiration date
		6.14	Make sure the correct high or low value is used by referring to the kit insert for the assay to be run
		6.15	Click the Eject/Retract button and place the MCV plate on the platform
		6.16	Retract the drawer and click OK
		6.17	Reader will perform the calibration automatically and display a pass or fail message when finished
		6.18	Click on View, then the Calibration Log to view and print results
		6.19	Place in the LabScan 100 QC Reports binder

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7	Validation	7.1	A successful Calibration must be run before Validation can be performed
		7.2	Isopropanol to the appropriate wells in the MCV plate
		7.3	Rinse the MCV plate with tap water and blot
		7.4	Add deionized water and 70%
		7.5	Remove the Validation kit from the refrigerator
		7.6	Each of the 16 bottles must be vortexed for 30 seconds each and the five drops placed into the appropriate wells on the MCV plate
		7.7	Click on the Validation icon in the toolbar
		7.8	Validation menu will display
		7.9	Check that the Validation control number corresponds to the number on the Validation Kit box and is within the expiration date; all should be marked in the Validation Type
		7.10	Click the Eject/Retract button and place the MCV plate on the platform
		7.11	Retract the drawer and click OK
		7.12	Reader will perform the validation automatically and display a report when finished
		7.13	Save the report in C:\Luminex Files\Reports Print the report and place in the LabScan 100 QC Reports binder

Milliplex Cytokine Assay

Study:	Cytokine kit run:	Plate Number:

	1	2	3	4	5	6	7	8	9	10	11	12
A	0 pg/mL	80 pg/mL	QC1									
B	0 pg/mL	80 pg/mL	QC1									
C	0.64 pg/mL	400 pg/mL	QC2									
D	0.64 pg/mL	400 pg/mL	QC2									
E	3.2 pg/mL	2000 pg/mL										
F	3.2 pg/mL	2000 pg/mL										
G	16 pg/mL	10,000 pg/mL										
H	16 pg/mL	10,000 pg/mL										

Tech	Date	Lot #	Expiration Date	4C -16-18hr	Antibody -1hr	PE 30 minutes	READ
				Start			
				Finish			

Title: Pre-Amplification Protocol			Page 1 of 3		
Doc#	MTC-0001	**Revision:**		**Effective Date:**	08/31/1995

1	Purpose	1.1	To provide guidelines and procedures to be followed in pre-amplification work areas. This protocol is designed to prevent contamination of equipment, supplies, reagents and samples.
2	Scope	2.1	This protocol applies to all Molecular Transfusion Core personnel.
		2.2	This protocol applies to pre-amplification work areas, which includes the reagent preparation laboratory and the sample preparation laboratory.
3	Responsibilities	3.1	It is the responsibility of all Molecular Transfusion Core personnel to adhere to the universal precautions.
		3.2	It is the responsibility of each Molecular Transfusion Core personnel working in the specified areas to adhere to these guidelines and procedures.
4	Materials and Equipment	4.1	Disposable gloves
		4.2	Disposable lab coats
		4.3	Bleach, 10%
		4.4	Alcohol, 70%
		4.5	Plastic wash bottles
		4.6	Gauze sponges, 4 x 4 inches
		4.7	Soak containers
5	Basic Principles	5.1	Physical separation of reagent, sample and amplification laboratories prevents contamination of “clean” areas from “hot” areas.
		5.2	Supplies, instruments and personal protective equipments are not interchangeable between laboratories.
		5.2	Use of dedicated equipment assigned to each workstation allows containment of possible contamination.
		5.3	Unidirectional flow of worksheets, from “clean” to “hot” areas, prevents contamination of “clean” areas.
		5.4	Unidirectional flow of racks, from “clean” to “hot” areas, prevents contamination of “clean” areas, until decontamination is performed.
		5.5	Decontamination procedures during and at the end of the day provides prevents spread and accumulation of

			contaminating substances.
6	Supplies and Reagents	6.1	Supplies will originate from a “clean” area.
		6.2	Instruments, calculators, pens, pipettes will not be shared between laboratories.
		6.3	Instruments, calculators, pens, pipettes are dedicated for specific tasks.
		6.4	Each workstation is designated by color according to the level of use.
7	Gloves and Lab coats	7.1	Gloves will be worn before touching any item in the pre-amplification areas.
		7.2	Lab coats will be worn whenever working in the pre-amplification areas
		7.3	Gloves and lab coats will not be worn from one laboratory to another.
		7.4	Gloves and lab coats will be discarded when soiled.
8	Housekeeping, Clean-up, Decontamination	8.1	The workstation garbage bins will be lined by a Ziploc bag before use.
		8.2	Discard the used Ziploc bag daily.
		8.2	When soiled and after use, the workstation, centrifuges, pipettes, pens will be decontaminated with 10% bleach and 70% alcohol.
		8.3	Racks will be soaked in 10% bleach and rinsed immediately after use.
9	Unidirectional Workflow of Paperwork	9.1	Paperwork, like experimental designs and worksheets, must follow a one way-flow, from the Reagent Prep Lab to Sample Prep Lab to PCR Lab.
		9.2	All paperwork entering the Reagent Prep Lab must be faxed. Paperwork faxed to the Reagent Prep Lab may be moved to the Sample Prep Lab but once in the Sample Prep Lab, may not go back to the Reagent Prep Lab.
		9.3	All paperwork entering the Sample Prep Lab must be faxed, unless the paper originated from the Reagent Prep Lab. Paperwork faxed to the Sample Prep Lab may not be moved to the Reagent Prep Lab.
		9.4	All paperwork which entered the PCR Lab may not be moved back to either Reagent or Sample Prep Labs.
		9.5	Speed dials to both fax numbers are set-up in the basement fax machine. a. Reagent Prep Lab Fax: 749-6689 b. Sample Prep Lab Fax: 749-6666

10	Racks Reverse Flow- Bleach Twice	10.1	Racks that were moved to the PCR lab must be immersed in 10% bleach, for 5 minutes, and rinsed with water before they can be moved to the Sample Prep Lab. Once in the Sample Prep Lab, the racks should be immediately immersed in 10% bleach, for 5 minutes, and rinsed again before use.
		10.2	Racks that were moved to the Sample Prep Lab must be immersed in 10% bleach, for 5 minutes, and rinsed with water before they can be moved to the Reagent Prep Lab. Once in the Reagent Prep Lab, the racks should be immediately immersed in 10% bleach, for 5 minutes, and rinsed again before use.

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Title: Quality Control Procedures for the Quantitative Reverse Transcription Real-Time PCR Assays Using SyBr Green			Page 1 of 2		
Doc#	MTC-0002	**Revision:**		**Effective Date:**	04/01/10

1	Purpose	1.1	To provide the reverse transcription assays a method for evaluating the efficiency of the RNA extraction, reverse transcription and PCR amplification.
2	Scope	2.1	This protocol applies to all Molecular Transfusion Core personnel.
		2.2	This protocol serves as a QC procedure for the quantitative reverse transcription real-time PCR assays using SyBr Green.
		2.3	This protocol applies to assays using Qiagen columns as RNA extraction procedure.
		2.4	This protocol applies to assays which quantifies RNA viruses.
3	Responsibilities	3.1	It is the responsibility of the supervisor to ensure that the technical staff performing the assay is trained to include and analyze the QC samples in every run.
		3.2	It is the responsibility of the supervisor to ensure that variances or deviance are documented and addressed.
		3.3	It is the responsibility of the staff performing the assay to include the QC samples in each run.
		3.4	It is the responsibility of the staff performing the assay to document deviance to the protocol.
		3.5	It is the responsibility of all Molecular Transfusion Core personnel to adhere to the universal precautions and MTC-0001.
4	Materials and Equipment	4.1	Quantitative Positive QC Plasma Standards: Plasma spiked with RNA virus, (1000 copies/100µL, 100 copies/100µL, 10 copies/100µL, 1 copy/100µL)
		4.2	Negative QC Plasma samples: Unspiked plasma negative for either Dengue Virus or West Nile Virus
		4.3	Negative QC sample: No template control (Solution A and B)
		4.4	Real-time Thermal Cycler
5	RNA Extraction	5.1	One each of positive QC plasma standard will be added during RNA extraction of experimental samples.
		5.2	One negative QC plasma samples unspiked with virus will

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			be added during RNA extraction of experimental samples.
6	Reverse Transcription	6.1	The positive standards and the negative control will be processed along with the experimental samples.
7	PCR Amplification	7.1	PCR amplification will include two wells containing Solution A and B, a no template control reagent.
		7.2	The positive standards and the negative control will be processed along with the experimental samples.
8	Evaluation of RNA Extraction and Reverse Transcription Efficiency	8.1	The quantitative standards will be evaluated for linearity and efficiency.
		8.2	The standards will be compared to values in the control chart. The control chart will have values of at least 20 standards ran before the assay is used.
		8.3	Experimental unknowns will be quantified by interpolation using the quantitative standards.
		8.4	Negative Control Plasma sample will be used to evaluate non-specific amplification.
		8.5	No template controls will be used to evaluate generation of primer dimers.
9	Evaluation of PCR Specificity	9.1	The melting temperatures of the experimental unknowns will be compared to melting temperatures of the positive controls. Experimental unknowns with the same melting temperature will be counted as positive.
		9.2	The melting temperatures of the experimental unknowns will be compared to melting temperatures of the negative control. Experimental unknowns with the same melting temperature will be counted as negative.
		9.3	The melting temperatures of the experimental unknowns will be compared to melting temperatures of the no template control. Experimental unknowns with the same melting temperature will be counted as negative.
10	Deviance	10.1	A run where the quantitative standards are outside the acceptable values of the control chart will be invalidated.
		10.2	A run where the melting temperatures of the quantitative standards are inconsistent with expected melting temperatures will be invalidated.
		10.3	A run where the linearity of standards is below the acceptable value will be invalidated.

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Title: Preventative Maintenance, Calibration and Validation of Equipment Performed By External Service Contractors			Page 1 of 2		
Doc#	MTC-0003	**Revision:**		**Effective Date:**	04/29/10

1	Purpose	1.1	To provide information on the schedule of preventative maintenance, calibration and validation of equipments. To provide instructions on how to handle the documentations provided by external service contractors.
2	Scope	2.1	This protocol applies to equipments used by the Molecular Transfusion Core laboratory.
		2.2	This protocol applies to equipments which are serviced by companies certified to perform validation and calibration. Specifically, the equipments pertain to: Applied BioSystems 7500, Roche LightCycler 480, centrifuges, michrocentrifuges and pipettes.
3	Responsibilities	3.1	It is the responsibility of the supervisor to ensure that the procedure is performed on a regular schedule.
		3.2	It is the responsibility of the supervisor to ensure that documentations are filed in the designated binder or folder.
4	Materials and Equipment	4.1	Service Reports or Calibration Certificates
		4.2	Equipment specific folders
5	Thermal Cyclers: ABI 7500 and Roche LC 480	5.1	The ABI 7500 and Roche LC 480's are scheduled for annual maintenance by a service engineer from Applied BioSystems, Inc. and Roche Diagnostics Corp, respectively.
		5.2	The service engineer will perform the preventative maintenance per instrument requirement.
		5.2	A service report will be provided by the service engineer to indicate that the instrument passed all required parameters.
		5.3	The service report will be filed in their respective folders.
6	Centrifuges and microcentrifuges	6.1	Centrifuges and microcentrifuges will be maintained annually by a qualified laboratory service repair company.
		6.2	Service stickers are placed on the centrifuges to indicate that the instrument passed the maintenance quality controls.
		6.3	A service report will be provided by the service engineer to indicate that the instrument passed all required

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			parameters.
		6.4	The service report will be filed in its respective folders.
7	Pipettes	7.1	Pipettes are scheduled for service annually.
		7.2	Pipettes are sent out to qualified pipette service laboratories.
		7.3	Each pipette will be provided a sticker on the pipette indicating that the pipette had been validated. Each pipette will also be provided a calibration certificate.
		7.4	The calibration certificate will be filed in its respective folder.

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Title: Creation of a Control Chart			Page 1 of 2		
Doc#	MTC-0004	**Revision:**		**Effective Date:**	04/01/10

1	Purpose	1.1	To create a control chart to be used to generate an acceptable range and standard deviations for positive controls.
2	Scope	2.1	This protocol applies to all Molecular Transfusion Core personnel.
		2.2	This protocol applies to control charts to be used for evaluating acceptability of standard curves and qualitative positive controls.
3	Responsibilities	3.1	It is the responsibility of the supervisor to ensure that the technical staff performing the assay is trained to perform the assay.
		3.2	It is the responsibility of the supervisor to ensure that variances or deviance are documented and addressed.
		3.3	It is the responsibility of the staff performing the assay to document deviance to the protocol.
		3.4	It is the responsibility of the technical staff performing the assays to abide by the universal precaution and Protocol number MTC-0001.
4	Materials and Equipment	4.1	20 Positive control samples
		4.2	Negative sample controls
		4.3	No template controls (Solution A and B)
		4.4	Real-time Thermal Cycler
		4.5	All materials and equipment to run the assay. See specific assay procedure.
5	Running the assay	5.1	The 20 positive controls should be placed on 5 different runs, four controls per run.
		5.2	Negative sample controls should be included in each run.
		5.3	No template controls should be included in each run.
6	Evaluation of PCR Specificity	6.1	The melting temperatures of the amplicons of the positive controls will be compared to each other for homogeneity.
		6.2	The melting temperatures of the positive controls will be compared to melting temperatures of the negative control. Positive controls with the same melting temperature as the negative control will be invalidated.
		6.3	The melting temperatures of the positive controls will be

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			compared to melting temperatures of the no template controls. Positive controls with the same melting temperature as the no template controls will be invalidated.
7	Control Chart	7.1	At least twenty data points will be collected before generating the control chart.
		7.1	The cycle threshold for each data will be charted.
		7.2	Acceptability will be set at 2x standard deviation.

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Title: West Nile Virus RT-PCR Assay			Page 1 of 4		
Doc#	MTC-0006	**Revision:**		**Effective Date:**	04/01/10

1	Purpose	1.1	To provide instructions on how to perform the West Nile Virus RT-PCR assay.
2	Scope	2.1	This protocol applies to testing samples to be interrogated for the presence of West Nile Virus.
3	Responsibilities	3.1	This procedure is to be performed by personnel from the Molecular Transfusion Core.
		3.2	It is the responsibility of the Molecular Transfusion Core supervisor to ensure that the laboratory personnel have been trained in properly handling human specimens and wearing PPE.
		3.3	It is the responsibility of all Molecular Transfusion Core personnel to adhere to the universal precautions and MTC-0001.
4	Materials and Equipment	4.1	Roche 480
		4.2	PCR workstation
		4.3	Pipettes
		4.4	Heat block
		4.5	Centrifuge
		4.6	Microcentrifuge
		4.7	QIAamp Viral RNA Mini Kit
		4.8	Pipette tips
		4.9	Ethanol (200 proof)
		4.10	RNase-free 1.5 mL tubes
		4.11	96 well PCR plate
		4.12	Buffer
		4.13	dNTPs
		4.14	Primers
		4.15	Probe
		4.15	Probe
		4.16	FastStart Taq
		4.17	10x Solution A+B
		4.18	RNase inhibitor
		4.19	MuLV reverse transcriptase
		4.20	Lab coat
		4.21	Gloves

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5	RNA Extraction via QIAamp Viral RNA Spin Protocol	5.1	Pipet 800 uL of prepared Buffer AVL containing Carrier RNA into a 1.5 mL microcentrifuge tube.
		5.2	Add 200 uL of plasma to the Buffer AVL/Carrier RNA in the microcentrifuge tube. Mix by pulse-vortexing for 15 sec.
		5.3	Incubate at room temperature (15-25^{o}C) for 10 min.
		5.4	Briefly centrifuge the 1.5 mL microcentrifuge tube to remove drops from the inside of the lid.
		5.5	Add 800 uL of ethanol (96-100%) to the sample, and mix by pulse-vortexing for 15 sec. After mixing, briefly centrifuge the 1.5 mL microcentrifuge tube to remove drops from inside the lid.
		5.6	Carefully apply 600 uL of the solution from step 5 to the QIAamp spin column without wetting the rim. Close the cap, and centrifuge at 8,000 rpm for 1 min. Place the QIAamp spin column into a clean 2 mL collection tube, and discard the tube containing the filtrate.
		5.7	Carefully open the QIAamp spin column, and repeat step 6.
		5.8	Carefully open the QIAamp spin column and add 500 uL of Buffer AW1. Close the cap and centrifuge at 8,000 rpm for 1 min. Place the QIAamp spin column in a clean 2 mL collection tube and discard the tube containing the filtrate.
		5.9	Carefully open the QIAamp spin column and add 500 uL of Buffer AW2. Close the cap and centrifuge at full speed (14,000 rpm) for 3 min.
		5.10	Place the QIAamp spin column in a new 2 mL collection tube and discard the old collection tube with the filtrate. Centrifuge at full speed (14,000 rpm) for 1 min.
		5.11	Place the QIAamp spin column in a clean 1.5 mL microcentrifuge tube. Discard the old collection tube containing the filtrate.
		5.12	Add 120 uL of H_2O equilibrated to room temperature. Close the cap, and incubate at room temperature for 1 min. Centrifuge at 10,000 rpm for 2 min.
6	Reverse Transcription	6.1	Thaw dGTP, dATP, dTTP, dCTP, and downstream primer to room temperature and vortex.
		6.2	Add the following to a labeled 1.5 mL screwcap tube: 12.0 uL 10X Solution A+B 1.2 uL total 100 mM dNTPs (without dUTP)

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			3.0 uL RNase inhibitor (40U/uL) 1.5 uL MuLV reverse transcriptase (100 U/uL) 0.45 uL of downstream primer
		6.3	Add sample to bring final volume up to 120 uL. (To dilute the 10X Sol A+B to 1X Sol A+B)
		6.4	Vortex mixture and centrifuge.
		6.5	Reverse transcribe at 42^{o}C for 40 min in heat block.
		6.6	Vortex mixture and centrifuge.
		6.7	Incubate at 100^{o}C for 10 min.
		6.8	Vortex and centrifuge.
7	PCR Reaction Mix Preparation	7.1	Add 30 uL of mineral oil to each well of a 96-well amplification plate.
		7.2	Thaw primers and probe to room temperature and vortex.
		7.3	Add the following to a labeled 1.5 mL screwcap tube: 50 uL/rxn Buffer 52 0.5 uL/rxn Forward primer (VWNVA1) 0.5 uL/rxn Reverse primer (VWNVA2) 1.0 uL/rxn Probe (WNV PROBE) 1.0 uL/rxn FastStart Taq
		7.4	Add 50 uL of reaction mix to each well of amplification plate.
		7.5	Add 25 uL of sample to each well.
		7.6	Centrifuge plate at 1600 rpm for 1 min
8	Set up of Roche 480	8.1	Push button on instrument to open plate holder drawer. Both lights have to be steady green. Place plate in the instrument and close the plate holder drawer.
		8.2	Go to Overview screen. Click on “New Experiment” button.
		8.3	From drop-down menu choose “Dual Color Hydrolysis Probe.”
		8.4	Choose “Apply Template” then select template “PCR with Probes” in the Run Template folder. Cycle conditions are: 1 cycle of 95^{o}C for 1 min followed by 45 cycles of 95^{o}C for 30 sec and 56^{o}C for 1 min.
		8.5	Save in WNV folder and hit “Start Run” button.
9	Analysis on Roche 480	9.1	Click on “Analyze” button.

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		9.2	Select “Absolute Quant/Fit Point.”
		9.3	Adjust the threshold bar, moving it above the background signal.
		9.4	Choose dye for signal such as “FAM” for WNV probe.
		9.5	Click on “Calculate.”
		9.6	Click on “Save” (floppy disk icon on right side).
		9.7	Click on “Report” and choose parameters to be reported.
		9.8	Click on “Generate.”
		9.9	Print report by clicking printer icon on top left of the report generated.

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Title: Quality Control Procedures for the Quantitative Reverse Transcription Real-Time PCR Assays Using a Fluorescent Probes			Page 1 of 2		
Doc#	MTC-0007	**Revision:**		**Effective Date:**	04/01/10

1	Purpose	1.1	To provide the reverse transcription assays a method for evaluating the efficiency of the RNA extraction, reverse transcription and PCR amplification.
2	Scope	2.1	This protocol applies to all Molecular Transfusion Core personnel.
		2.2	This protocol serves as a QC procedure for the quantitative reverse transcription real-time PCR assays using fluorescent probes.
		2.3	This protocol applies to assays using Qiagen columns as RNA extraction procedure.
		2.4	This protocol applies to assays which quantifies RNA viruses.
3	Responsibilities	3.1	It is the responsibility of the supervisor to ensure that the technical staff performing the assay is trained to include and analyze the QC samples in every run.
		3.2	It is the responsibility of the supervisor to ensure that variances or deviance are documented and addressed.
		3.3	It is the responsibility of the staff performing the assay to include the QC samples in each run.
		3.4	It is the responsibility of the staff performing the assay to document deviance to the protocol.
		3.5	It is the responsibility of all Molecular Transfusion Core personnel to adhere to the universal precautions and MTC-0001.
4	Materials and Equipment	4.1	Quantitative Positive QC Plasma Standards: Plasma spiked with RNA virus, (1000 copies/100µL, 100 copies/100µL, 10 copies/100µL, 1 copy/100µL)
		4.2	Negative QC Plasma samples: Unspiked plasma negative for either Dengue Virus or West Nile Virus
		4.3	Negative QC sample: No template control (Solution A and B)
		4.4	Real-time Thermal Cycler
5	RNA Extraction	5.1	One each of positive QC plasma standard will be added during RNA extraction of experimental samples.

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		5.2	One negative QC plasma samples unspiked with virus will be added during RNA extraction of experimental samples.
6	Reverse Transcription	6.1	The positive standards and the negative control will be processed along with the experimental samples.
7	PCR Amplification	7.1	PCR amplification will include two wells containing Solution A and B, a no template control reagent.
		7.2	The positive standards and the negative control will be processed along with the experimental samples.
8	Evaluation of RNA Extraction and Reverse Transcription Efficiency	8.1	The quantitative standards will be evaluated for linearity and efficiency.
		8.2	The standards will be compared to values in the control chart. The control chart will have values of at least 20 standards ran before the assay is used.
		8.3	Experimental unknowns will be quantified by interpolation using the quantitative standards.
		8.4	Negative Control Plasma sample will be used to evaluate non-specific amplification.
		8.5	No template controls will be used to evaluate generation of primer dimers.
9	Deviance	9.1	A run where the quantitative standards are outside the acceptable values of the control chart will be invalidated.
		9.2	A run where the linearity of standards is below the acceptable value will be invalidated.

Title: West Nile Virus RT-PCR Assay for Whole Blood Samples			Page 1 of 4		
Doc#	MTC-0008	**Revision:**		**Effective Date:**	04/01/10

1	Purpose	1.1	To provide instructions on how to perform the West Nile Virus RT-PCR assay on whole blood samples.
2	Scope	2.1	This protocol applies to testing samples to be interrogated for the presence of West Nile Virus.
3	Responsibilities	3.1	This procedure is to be performed by personnel from the Molecular Transfusion Core.
		3.2	It is the responsibility of the Molecular Transfusion Core supervisor to ensure that the laboratory personnel have been trained in properly handling human specimens and wearing PPE.
		3.3	It is the responsibility of all Molecular Transfusion Core personnel to adhere to the universal precautions and MTC-0001.
4	Materials and Equipment	4.1	Roche 480
		4.2	PCR workstation
		4.3	Pipettes
		4.4	Heat block
		4.5	Centrifuge
		4.6	Microcentrifuge
		4.7	QIAamp Viral RNA Mini Kit
		4.8	Pipette tips
		4.9	Ethanol (200 proof)
		4.10	RNase-free 1.5 mL tubes
		4.11	96 well PCR plate
		4.12	Buffer
		4.13	dNTPs
		4.14	Primers
		4.15	Probe
		4.15	Probe
		4.16	FastStart Taq
		4.17	10x Solution A+B
		4.18	RNase inhibitor
		4.19	MuLV reverse transcriptase
		4.20	Lab coat

		4.21	Gloves
5	RNA Extraction via QIAamp Viral RNA Spin Protocol	5.1	Pipet 400 uL of prepared Buffer AVL containing Carrier RNA into a 1.5 mL microcentrifuge tube.
		5.2	Add 100 uL of plasma to the Buffer AVL/Carrier RNA in the microcentrifuge tube. Mix by pulse-vortexing for 15 sec.
		5.3	Incubate at room temperature (15-25^{o}C) for 10 min.
		5.4	Briefly centrifuge the 1.5 mL microcentrifuge tube to remove drops from the inside of the lid.
		5.5	Add 400 uL of ethanol (96-100%) to the sample, and mix by pulse-vortexing for 15 sec. After mixing, briefly centrifuge the 1.5 mL microcentrifuge tube to remove drops from inside the lid.
		5.6	Carefully apply 450 uL of the solution from step 5 to the QIAamp spin column without wetting the rim. Close the cap, and centrifuge at 8,000 rpm for 1 min. Place the QIAamp spin column into a clean 2 mL collection tube, and discard the tube containing the filtrate.
		5.7	Carefully open the QIAamp spin column, and repeat step 6.
		5.8	Carefully open the QIAamp spin column and add 500 uL of Buffer AW1. Close the cap and centrifuge at 8,000 rpm for 1 min. Place the QIAamp spin column in a clean 2 mL collection tube and discard the tube containing the filtrate.
		5.9	Repeat step 8.
		5.10	Carefully open the QIAamp spin column and add 500 uL of Buffer AW2. Close the cap and centrifuge at full speed (14,000 rpm) for 3 min.
		5.11	Repeat step 10.
		5.12	Place the QIAamp spin column in a new 2 mL collection tube and discard the old collection tube with the filtrate. Centrifuge at full speed (14,000 rpm) for 1 min.
		5.13	Place the QIAamp spin column in a clean 1.5 mL microcentrifuge tube. Discard the old collection tube containing the filtrate.

		5.14	Add 60 uL of H_2O equilibrated to room temperature. Close the cap, and incubate at room temperature for 1 min. Centrifuge at 8,000 rpm for 1 min.
6	Reverse Transcription	6.1	Thaw dGTP, dATP, dTTP, dCTP, and downstream primer to room temperature and vortex.
		6.2	Add the following to a labeled 1.5 mL screwcap tube: 12.0 uL 10X Solution A+B 1.2 uL total 100 mM dNTPs (without dUTP) 3.0 uL RNase inhibitor (40U/uL) 1.5 uL MuLV reverse transcriptase (100 U/uL) 0.45 uL of downstream primer
		6.3	Add sample to bring final volume up to 120 uL. (To dilute the 10X Sol A+B to 1X Sol A+B)
		6.4	Vortex mixture and centrifuge.
		6.5	Reverse transcribe at 42^{o}C for 40 min in heat block.
		6.6	Vortex mixture and centrifuge.
		6.7	Incubate at 100^{o}C for 10 min.
		6.8	Vortex and centrifuge.
7	PCR Reaction Mix Preparation	7.1	Add 30 uL of mineral oil to each well of a 96-well amplification plate.
		7.2	Thaw primers and probe to room temperature and vortex.
		7.3	Add the following to a labeled 1.5 mL screwcap tube: 50 uL/rxn Buffer 52 0.5 uL/rxn Forward primer (VWNVA1) 0.5 uL/rxn Reverse primer (VWNVA2) 1.0 uL/rxn Probe (WNV PROBE) 1.0 uL/rxn FastStart Taq
		7.4	Add 50 uL of reaction mix to each well of amplification plate.
		7.5	Add 25 uL of sample to each well.
		7.6	Centrifuge plate at 1600 rpm for 1 min
8	Set up of Roche 480	8.1	Push button on instrument to open plate holder drawer. Both lights have to be steady green. Place plate in the instrument and close the plate holder drawer.
		8.2	Go to Overview screen. Click on “New Experiment” button.

		8.3	From drop-down menu choose “Dual Color Hydrolysis Probe.”
		8.4	Choose “Apply Template” then select template “PCR with Probes” in the Run Template folder. Cycle conditions are: 1 cycle of 95^{o}C for 1 min followed by 45 cycles of 95^{o}C for 30 sec and 56^{o}C for 1 min.
		8.5	Save in WNV folder and hit “Start Run” button.
9	Analysis on Roche 480	9.1	Click on “Analyze” button.
		9.2	Select “Absolute Quant/Fit Point.”
		9.3	Adjust the threshold bar, moving it above the background signal.
		9.4	Choose dye for signal such as “FAM” for WNV probe.
		9.5	Click on “Calculate.”
		9.6	Click on “Save” (floppy disk icon on right side).
		9.7	Click on “Report” and choose parameters to be reported.
		9.8	Click on “Generate.”
		9.9	Print report by clicking printer icon on top left of the report generated.

KINETIC PCR AMPLIFICATION LAYOUT AND CONDITIONS

Project Name_____ Specificity_____

Experiment Title_____ Date of Amplification_____ By_____

Notes/Comments:

A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12
B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12
C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12
D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12
F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12
H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12

Kinetic Software

File Saved As_____

Temp	Time
95 C	10 min
95 C	30 sec
___C	30 sec
72 C	45 sec

Threshold value:_____

Normalization Range:_____ to _____

Reaction Mix

Buffer Number_____ Date/Initial of Buffer Prep_____

# of Samples_____

Total Buffer Volume Req._____

	Conc.		Lot#	Vol.
dNTPs	50 uL/mL		_____	_____
Primer A___	_____	_____	_____	_____
Primer B____	_____	_____	_____	_____
Syber Green	0.15uL/rxn		_____	_____
Dilution 1:400 FastStart	0.14uL/rxn		_____	_____

Protocol for infection of Vero cells with frozen samples from BSRI

Samples:
For V1 passage:
Refer to sample list sent by BSRI.
Negative control (media)
Positive control (plasma from sample NY03-11)

For M1V1 passage:
Day 7 supernatants from macrophage passage 1 from all samples, including positive and negative controls
Negative control (media)

Media for WNV infection:
MEM, with Pen-Strep and L-glutamine 1:100 (cMEM)

Other materials:
FBS
dPBS w/o Ca++/Mg++
5 ml serological pipets
P1000 and tips
Qiagen buffer AVL with carrier RNA (from kit 52926)
Labeled 15 ml centrifuge tubes

Prepare T-25 flasks to be 70% confluent at the time of infection.
Thaw plasma and whole blood samples to be used for culture at 37º. Warm PBS and culture media to 37º.
Remove culture media and wash once with 3 ml sterile prewarmed PBS. Remove PBS immediately prior to adding CCB or plasma.
For each flask, add 250 ul of thawed CCB or plasma. Rock flask to ensure that cell surface is fully covered by the 250 ul.
Incubate cells for 1 hour at 37º, 5% CO_2, rocking every 15 minutes.
After 1 hour, add 5 ml of prewarmed cMEM + 2% FBS to each flask without removing the inoculum. Return cells to 37º, 5% CO_2.
Remaining plasma or CCB (if any) will be stored at -70 in BSL3.

For infection of Veros with macrophage passage 1 supernatants, the above protocol will be followed, except that a sample volume of 500 ul of supernatant will be used.

On day 7, CPE will be assessed and supernatants will be harvested.
2 x 140 ul aliquots of each supernatant will be mixed with 560 ul Qiagen buffer AVL and stored at -20º until ready to extract. The remainder of each supernatant will be stored at -70º in BSL3. Supernatant samples will be extracted using the Qiagen ViralAmp RNA mini kit (52926) in a QiaCube instrument and eluted in a volume of 50 ul.

Viral loads will be quantified by qRT-PCR using the Applied Biosystems One-Step RNA to Ct kit (cat # 4392938) in an AB Taqman 7300 instrument. The qRT-PCR assays will use primers and probes specific for the WNV 3' noncoding region: WN3ncF, 5′-CAGCCACGCTACGGCG-3′; WN3ncR, 5′-CAGTCCTCCTGGGGCACTA-3′; and WN3ncP, 5′-TCTGCGGAGAGTGCAGTCTGCGAT-3′ and amplification conditions were previously described in (Rios M, et al *Transfusion 2006;46:659-67)*. Samples will be run in a volume of 10 ul, in duplicate in two independent assays for a total of four replicates for each sample. Data will be analyzed using SDS software v. 1.4 (Applied Biosystems).

Protocol for infection of monocyte-derived macrophages (MDM) with frozen samples from BSRI

Samples:
Refer to sample list sent by BSRI.
Negative control (media)
Positive control (plasma from sample NY03-11)

Media for WNV infection:
MEM, with Pen-Strep and L-glutamine 1:100 (cMEM)

Other materials for culture:
FBS
M-CSF (Sigma M6518), 10 ug/500 ml media
dPBS w/o Ca++/Mg++
5 ml serological pipets
P1000 and tips
Qiagen buffer AVL with carrier RNA (from kit 52926)
Labeled 15 ml centrifuge tubes

Plate elutriated monocytes from NIH Division of Transfusion Medicine at 1x10^6 cells/flask in 5 ml cMEM +10% FBS + M-CSF. Cells will be used for infection on days 7 and 8.
Thaw plasma and whole blood samples to be used for culture at 37º. Warm PBS and culture media to 37.
Remove culture media and wash once with 3 ml sterile prewarmed PBS. Remove PBS immediately prior to adding CCB or plasma.
For each flask, add 250 ul of thawed CCB or plasma. Rock flask to ensure that cell surface is fully covered by the 250 ul.
Incubate cells for 1 hour at 37º, 5% CO_2, rocking every 15 minutes.
After 1 hour, add 5 ml of prewarmed cMEM + 5% FBS to each flask without removing the inoculum. Return cells to 37º, 5% CO_2. Most supernatants will gel due to combining plasma/CCB with complete media.
Remaining plasma or CCB (if any) will be stored at -70 in BSL3.

On day 7, cultures will be observed on a phase contrast microscope, supernatants will be harvested (tapping corner of flask gently to remove any supernatants that are still gelled), and cultures fed with 5 ml fresh prewarmed cMEM + 5% FBS. On day 13, cultures will be observed on a phase contrast microscope, and supernatants will be harvested.
2 x 140 ul aliquots of each supernatant will be mixed with 560 ul Qiagen buffer AVL and stored at -20º until ready to extract. The remainder of each supernatant will be stored at -70º in BSL3. Supernatant samples will be extracted using the Qiagen ViralAmp RNA mini kit (52926) in a QiaCube instrument and eluted in a volume of 50 ul.

Viral loads will be quantified by qRT-PCR using the Applied Biosystems One-Step RNA to Ct kit (cat # 4392938) in an AB Taqman 7300 instrument. The qRT-PCR assays will use primers and probes specific for the WNV 3' noncoding region: WN3ncF, 5′-CAGCCACGCTACGGCG-3′; WN3ncR, 5′-CAGTCCTCCTGGGGCACTA-3′; and WN3ncP, 5′-TCTGCGGAGAGTGCAGTCTGCGAT-3′ and amplification conditions were previously described in (Rios M, et al *Transfusion 2006;46:659-67)*. Samples will be run in a volume of 10 ul, in duplicate in two independent assays for a total of four replicates for each sample. Data will be analyzed using SDS software v. 1.4 (Applied Biosystems).

UCSF
COMMITTEE ON HUMAN RESEARCH
FULL COMMITTEE REVIEW APPLICATION

Please date form: 5/4/09

General Instructions | View Complete Set of Linked Instructions

PART 1: ADMINISTRATIVE REQUIREMENTS

- Eligibility requirements for Principal Investigator, Co-Principal Investigator and Contact Person
- Training requirements

A. Principal Investigator:		
Name and degree Michael P. Busch, M.D., Ph.D.	University Title Adjunct Professor	Department Laboratory Medicine
Campus Mailing Address (Box No.) 270 Masonic Avenue	Phone Number (415) 749-6615	E-mail Address mbusch@bloodsystems.org
Co-Principal Investigator:		
Name and degree Philip J. Norris, M.D.	University Title	Department Blood Systems Research Institute
Campus Mailing Address (Box No.) 270 Masonic Avenue	Phone Number (415) 923-5769	E-mail Address pnorris@bloodsystems.org
Additional Contact Person (if any):		
Name Michelle Quintos	University Title Research Services Mgr.	Department Blood Systems Research Institute
Campus Mailing Address (Box No.) 270 Masonic Avenue	Phone Number (415) 749-6606 x782	E-mail Address mquintos@bloodsystems.org
Send correspondence to (check *one*):	[ ]PI only [ ]PI and Co-PI [x]PI and Additional Contact Person	
Study Title: Natural history & pathogenesis of WNV in viremic donors	Application Type: [ ]New Full Committee Application [ ]Response to "Contingent" or "Return" letter [X]Modification [X]Renewal Current CHR #: H5866-25624-05 Expiration date: 06/19/09	
Sites (Check all that apply): [ ]UCSF [ ]SFGH [ ]VAMC [ ]Fresno [ ]Cancer Center [ ]UC Berkeley [ ]GCRC (Moffitt/Mt. Zion) [ ]GCRC (SFGH) [ ]PCRC [ ]Foreign Country [x]Other(s): Blood Systems Research Institute		

B. Funding: If this study is eligible for "Just in Time" NIH review, do not submit your application to the CHR until you have received notification from the federal granting agency that your study appears to be in a fundable range. Check all that apply:

Type of funding	Source of funding	Funds will be awarded to/through:
[ ]Contract/Grant [ ]Subcontract [ ]Gift [ ]Drug/device donation [ ]Student project **[x]**Other: internal BSRI funding Have funds been awarded? **[x]**Yes **[ ]**Pending [ ]No **[Blood Systems Foundation]** Award No.:	[ ]Federal Government [ ]Other Gov. (e.g., State, local) [ ]Industry* [x]Other Private [ ]Campus/UC-Wide program [ ]Departmental Funds [ ]Other: Sponsor Name: Blood Systems Foundation	Dept./ORU: Blood Systems Research Institute 00006454 *Institution* — *Federal Wide Assurance (FWA) No.* [ ]UCSF ..... 00000068 [ ]Blood Centers of the Pacific ..... 00002111 [ ]Gallo Institute..... 00000304 [ ]Gladstone Institute ..... 00000087 [ ]Goldman Institute on Aging..... 00002525 [ ]NCIRE ..... 00000256 [ ]S.F. Dept. of Public Health ..... 00000162 [ ]VA Research Office 00000280
*UCSF (or affiliate) financial contact person for recharge:	Jerry Michaelson, 415-923-4765	
Grant Title and PI (if different from above):		
Secondary sponsors: If there are multiple sources of funding for this study, please describe the additional funding:		

C. Key Personnel: All key personnel must be listed below along with a brief statement of their qualifications. *If the SF VAMC is a study site*, please identify the principal VAMC investigator, unless already listed as PI or CoPI above. For questions regarding the VAMC application process, please contact the VA Clinical Research Office at 221-4810 ext.4655.

Investigator (and institution):	Qualifications:
Michael Busch, M.D., Ph.D. (BSRI)	Adjunct Professor of laboratory medicine with extensive experience in the study of viral infections through the acute phase, with an emphasis on the understanding of viral-immune interactions.
Philip Norris, M.D. (BSRI)	Experienced T cell immunologist with a background in CD4+ T cell immunopathogenesis and HIV infection.
Marion Lanteri, Ph.D. (BSRI)	Staff Scientist experienced in virology with skills related to T cell and immunology assays.

D. Drugs, Devices and Biologics:

Investigational drugs, biologics and IND Numbers:		
Investigational devices and IDE Numbers:		[ ] NSR determination requested
Who holds the IND/IDE?	[ ]Sponsor [ ]Investigator	
Approved Drugs and/or Devices:		
Are investigational drugs, devices, or biologics prepared or manufactured in UCSF research labs?	[ ]Yes [ ]No If "Yes," identify the lab:	

E. Other Approvals/Regulated Materials: Does this study require approval or authorization from any of the following regulatory committees, or involve the use of the regulated materials listed below? Follow the hyperlinks for more information. If "Yes," complete the applicable section(s) below.		[ ]Yes [x]No
[ ]	Biological Safety Committee OSHA compliant on-site safety policy	BUA #:
[ ]	Institutional Animal Care and Use Committee	IACUC #:
[ ]	Controlled Substances	
[ ]	Human Stem Cells	Submit stem cell supplement
[ ]	Radiation Safety Committee	RUA #:

F. Scientific Merit Review: This study has received or will receive scientific merit review from (check all that apply):

[ ]NIH [ ]Cancer Center* [ ]GCRC or PCRC [ ]SFVAMC [ ]Dept. Review [x]Other: Blood Systems Blood Systems Foundation Scientific Advisory Board

*Required prior to final CHR approval for oncology studies.

G. Statement of Financial Interest: Do you or the other investigators have a financial interest in the outcome of this study? If "Yes," please describe below and describe briefly in Purpose and Background section of the consent form.	[ ]Yes [x]No

H. Principal Investigator's Certification:

- I certify that the information provided in this application is complete and correct.
- I accept ultimate responsibility for the conduct of this study, the ethical performance of the project, and the protection of the rights and welfare of the human subjects who are directly or indirectly involved in this project.
- I will comply with all policies and guidelines of UCSF and affiliated institutions where this study will be conducted, as well as with all applicable federal, state and local laws regarding the protection of human subjects in research.
- I will ensure that personnel performing this study are qualified, appropriately trained and will adhere to the provisions of the CHR-approved protocol.

- I will not modify this CHR-certified protocol or any attached materials without first obtaining CHR approval for an amendment to the previously approved protocol.
- I assure that the protected health information requested, if any, is the minimum necessary to meet the research objectives.
- I assure that the protected health information I obtain, if any, as part of this research will not be reused or disclosed to any parties other than those described in the CHR-approved protocol, except as required by law.

Principal Investigator's Signature Date

PART 2: STUDY DESIGN

Complete items A-E using clear, concise, non-technical, lay language (i.e., the type of language used in a newspaper article for the general public) wherever possible. Define all acronyms. Use caution when cutting and pasting from another application or protocol to ensure that information is complete, supplemented where necessary, is pasted in a logical order, and is relevant to the specific section.

Space limits are recommendations and should be adjusted as needed, but the total length for sections A-E should not exceed 5 pages.

For modifications and renewals, please highlight in *italics* all changes from previously approved version.

A. Synopsis (Briefly summarize the study.) Space limit: quarter page

The proposed study would focus on a small subset of WNV RNA positive donors to perform in-depth virologic and immunologic studies. Additional cases of severe WNV will be drawn from hospitalized patients identified by clinicians at UCSF and UCLA medical centers and the California Department of Health Services.
Initial studies focused on the prevalence of WNV infection in blood donors. In examining a large cohort of blood donors a number of WNV infected individuals with detectable viremia were identified (about five percent of eligible blood donors in affected areas of the country). Initial follow-up in these individuals has identified the spectrum of symptomatic disease in infected blood donors. The degree to which immune responses correlate with control of infection and contribute to symptomatic disease is not known. The focus of the studies to be conducted under this protocol and consent form is on the interrelationship between virus load, cellular proliferative responses, cytotoxic T lymphocyte responses, and the interplay between master regulators of the inflammation regulatory T cells and Th17 cells. To achieve these research goals, subjects who are WNV RNA+ at the time of blood donation will be identified and enrolled. Study subjects will be followed with blood draws of 75 mL at enrollment, at week one, two, three, six post-enrollment, then at month two, three, six, nine, and twelve post-enrollment.

B. Purpose (Specify the hypotheses, aims and/or objectives.) Space limit: half page

WNV infection results in variable penetrance of disease manifestations, ranging from asymptomatic infection to severe meningo-encephalitis and death. The immune correlates of protection from disease have not been described, particularly in humans. Previously, human T cell responses were identified for a subset of 8 peptides from the membrane, envelope, nonstructural 3 and 4b proteins of WNV. Further phenotypic studies characterized the WNV-specific T cells as cytotoxic CD8 T cells secreting granzyme A and perforin. The set of peptides identified might be used for T cell stimulation studies and might also be of interest for vaccination studies, relevant to WNV infection as well as Flaviviruses in general. Additionally, the role that the immune system might play in the pathogenesis of WNV infection is not understood but a strong correlation between lower levels of regulatory T cells (T_{reg}) and symptomatic outcome was found comparing PBMCs from asymptomatic versus symptomatic WNV+ donors.
Our specific aims are as follows:
1. To determine if T_{reg} cells expanded in acute WNV infection are WNV-specific and whether they more efficiently suppress WNV-specific immune responses in asymptomatic than in symptomatic individuals.
2. To observe the post-infection dynamics of Th17 cells and to study how the balance between pro-inflammatory Th17 cells and anti-inflammatory T_{reg} cells relates to T cell activation and disease outcomes.

C. Background (Summarize previous studies. Explain rationale for the proposed investigation.) Space limit: one page

WNV was introduced to the Northeastern United States in late summer of 1999. The virus was determined to be almost identical genetically to strains prevalent in Israel[1].[1]. Since its introduction, WNV has spread relentlessly westward, with large outbreaks in the Midwest and Colorado in 2004 and in Arizona and Southern California in 2005. The transmission period mirrors that of mosquito activity, peaking from May through August. It is expected that WNV will continue its westward expansion in the coming transmission season this spring and summer. During an outbreak of WNV in non-immune populations, approximately 5% of blood donations are positive for WNV IgM. Blood transfusion of WNV contaminated units has resulted in transfusion-associated transmission of the virus, with severe disease and death sometimes resulting. Pooled blood donations are now routinely screened for WNV RNA, though the sensitivity of the screening process is not likely adequate to prevent 100% protection from transfusion associated WNV transmission. Given that WNV will represent an ongoing health problem and threat to the blood supply, greater understanding of the pathogenesis of the virus is required.

Both humoral and cellular immune responses have been implicated in the control of WNV infection. The bulk of the pathogenesis data relating to WNV comes from murine models [2]. Mice deficient in secreted IgM have been shown to be more susceptible to lethal challenge with WNV, and passive transfer of polyclonal IgM can protect against lethal infection with WNV[3].[2]. Additionally, low WNV IgM titer in infected wild type mice is correlated with higher mortality. T cells also likely play a role in control of the virus. CD8+ T cell deficient mice infected with low-dose WNV show increased mortality compared to wild-type controls[4].[3]. Unpublished data suggest that T cell deficient mice initially control WNV replication, but ultimately fail to eradicate the virus, leading to recrudescence of viremia. The role of T cell responses appears not only to be protective, as CD8+ T cells have been isolated in the inflammatory regions of meningoencephalitits in mice and humans [5] [4]. We will assess the role of T cells in neurological manifestations of disease by studying the properties of CSF lymphocytes in hospitalized subjects who undergo diagnostic lumbar puncture.

Our laboratory has traditionally focused on the role of HIV-specific T cell responses in the control of virus replication [5, 6].[6, 7]. In HIV and flaviviruses analogous to WNV, it has been demonstrated that some regions of the viral genome are more susceptible to recognition by T cells than others [8-10]. In collaboration with the Biodefense and Emerging Infections Research Resources Repository we acquired overlapping peptide sets spanning the WNV genome synthesized and coupled with our unique access to patient samples through a large blood donation network, we were able to do comprehensive analysis of WNV-specific T cell responses [11]. We showed several epitopes inducing CD8 T cell responses in humans. We were able to demonstrate that control of acute viremia in WNV-infected blood donors is associated with interferon and interferon-induced chemokine expression [12].

We will be able to monitor the effect T cell responses and especially regulatory T cells [13-18] and Th17 cells [19-21] have on the dynamics of viral infection, the correlation with disease manifestations, and the durability of T cell immune responses to the virus.

D. Design (Check all that apply):

[ ]Phase I [ ]Phase II [ ]Phase III [ ]Phase IV [ ]Randomized [x]Blinded

[x]Multicenter: If so, is UCSF the coordinating center? [ ]Yes [x]No

[ ]Open Label Extension: If so, specify CHR Approval Number for original study: __

[ ]Behavioral

Additional description of *general* study design. Attach flow diagram if appropriate. Space limit: half page

The study is designed as an observational study. This is a longitudinal study of cellular and humoral immune function, which will be related to viral load and disease manifestations. A portion of the study will be dedicated to determining the persistence of WNV-specific immune responses over time, so there will be no specified endpoint for the study.

Subjects will be identified at the time of blood donation by the presence of a WNV RNA positive specimen. Age-matched WNV RNA negative and WNV seronegative control subjects will be derived from the donor population who initially test false-positive for WNV RNA at the time of donation. Samples will be obtained from study subjects at enrollment, then at week one, two, three, and six post-enrollment, and at month two, three, six, nine, and twelve post-enrollment. After that period a subset of donors will be sampled every three months for two years for monitoring of persistence of immune responses. Severe cases of WNV infection with neurological manifestations will be identified by clinicians at UCSF and UCLA medical centers and the California Department of Public Health. In addition to blood samples, hospitalized patients with neurological symptoms will have CSF (5 ml) sampled at the time of diagnostic lumbar puncture.

E. Data Analysis (How and by whom will data be analyzed?) Space limit: half page

Data analysis will be performed by the PI in collaboration with statisticians and epidemiologists on site at BSRI. This is a longitudinal study of viral RNA and immune markers following initial detection of a donor as viremic by nucleic acid testing (NAT). To estimate how rapidly, on average, viral load increased during the ramp-up phase, we first need to select for each panel the RNA+/IgM- bleeds collected when viral load was increasing exponentially. Assuming that the first RNA quantifiable bleed ("time 0" bleed) probably occurred in ramp-up and that viral load increased at a constant rate (i.e., in a linear fashion) on a log scale during ramp-up, the rate of increase in log viral load can be calculated. Information on log viral load and collection days for the selected ramp-up bleeds will be entered in a repeated measures regression model where each panel subject had their own intercept and slope (PROC MIXED, SAS Institute Inc.). The average viral load doubling time during ramp-up will then be calculated as log(2)/average slope. Longitudinal regression analysis will be used to estimate time periods from selected viral RNA thresholds to IgM and IgG seroconversion, as well as the duration of time following IgM or IgG seroconversion that low-level viremia is detectable by replicate TMA. This analysis will use PROC LIFEREG (SAS Institute Inc.), and assume a normal distribution for these window periods.

For the correlation of immune responses and disease outcomes, we expect that for some of the immune measurements (e.g. T cell responses), a sizeable number of participants will have values either below the limit of detection or clustered just above the limit of detection with a rightwards skewed distribution. For these measurements, we will use non-parametric statistical tests (e.g., Wilcoxon rank-sum test) to compare distributions between groups. For measurements for which all participants have observable values and for which distributions are approximately normal, we will use parametric tests (e.g., two-sample t-test).

PART 3: PROCEDURES

Check all that apply.

[x] Biological Specimen Banking (attach supplement) [x] Genetic Testing [ ] HIV Testing

Please list, in sequence, all study procedures, tests, and treatments required for the study. Indicate which would be done even if a subject does not enroll in the study. Include a detailed explanation of any experimental procedures. Attach table if available.

The only procedure to be performed on the outpatient study subjects is phlebotomy and research lab testing. Hospitalized patients will also have CSF sampling performed at the time of diagnostic lumbar puncture (an additional 5 ml will be obtained for research purposes). None of the results will be used for clinical decision making and they will not be relayed to the patient or the patient's physician. The WNV infected subjects would be advised of potential disease manifestations and advised to follow up with their regular physicians in case of symptoms.

The maximum amount of blood obtained from subjects is **375** ml over an 8 week period, and **750** ml over the total one-year period. If a person is hospitalized, we will follow the guidelines for unhealthy individuals and the amount of blood drawn may not exceed the less of 50 ml or 3 ml per kg in an 8 week period. Each phlebotomy will collect 75 ml blood, except in hospitalized patients where 25 ml blood will be collected per phlebotomy. In addition to the phlebotomy schedule described for WNV+ subjects, blood donors' initial donation blood products will be retrieved for study if operationally feasible. This will allow study of the earliest viremic time point.

In addition to phlebotomy, two questionnaires will be administered by telephone. Questionnaire A will be administered within a week of infection being identified. The second will be administered approximately three weeks after infection was identified. Both questionnaires focus on symptoms associated with WNV infection (see questionnaires in Appendix).

Category	Specific Activities
Visit schedule non-hospitalized	1) Index donation (day 0) 2) Enrollment visit (days 2-4) 3) Phlebotomies at weeks one, two, three, six post-enrollment (days 9-46) 4) Phlebotomy at two, three, six, nine, and twelve months post-enrollment.
Visit schedule hospitalized	1) Index donation (25 ml, day 0) 2) Index CSF sample (5ml, day 0 or when first performed) 3) Phlebotomy at week one, two, three, six if still hospitalized (25 ml) 4) Phlebotomy at two and three months post- index donation after hospital discharge (50 ml) 5) Phlebotomy every three months for up to one year after enrollment
Visit procedures	1) Donor consent at enrollment visit 2) Donor risk/symptom questionnaire at enrollment and follow up questionnaire at one month 3) Donor phlebotomy at each visit (draw 7~~6~~x10~~7~~mL EDTA plus 1x2.54mL EDTA plus one 2.5 mL PAXgene tube) 4) Anticoagulated whole blood will be shipped using Federal Express to Blood Systems Research Institute.
Tests that may be performed	1) WNV TMA (5x) 2) WNV IgM and IgG (Focus) 3) PRNT (CDC protocol) 4) Quantitative WNV PCR (viral load) on index unit and TMA-reactive specimens 5) WNV Viral culture 6) WNV genome sequencing 7) Cytotoxic T cell response 8) CD4 proliferative responses 9) Regulatory T cell frequencies and WNV-specific T cell responses 10) Th17 cells frequencies and WNV-specific responses 11) Cytokine quantification 12) HLA typing 13) Generation of immortalized B and T cell lines

To provide appropriate experimental controls, the ability of study subjects' cells to combat other viruses such as Epstein Barr virus, hepatitis C virus, HIV, influenza virus, cytomegalovirus, and human herpes virus 8 (HHV-8) may also be tested.

List the clinics and/or other specific locations where study procedures will be performed. Indicate how much time will be required of the subjects, per visit and in total for the study.

Subjects will be recruited from within the network of blood banks in Blood Systems, Inc., concentrated in the West and Midwest of the United States. Candidate sites will be located in regions of the country experiencing outbreaks of West Nile virus, as documented through routine screening of blood donors. Infectious disease physicians at UCSF and UCLA medical centers will also refer patients for contact about enrollment in the study only if the patients are interested. The patients will be identified and initially contacted by their treating physician; the treating physician will document patient consent to be contacted for the study in the patient chart. In order to boost enrollment of symptomatic subject with WNV infection, patients will also be recruited by Dr. Carol Glaser at the California Department of Public Health and those subjects will undergo consent through the

state IRB. Blood will be drawn at each study visit. It is not anticipated that more than half an hour per visit will be required, for a total of 4.5 hours over a one year period.

Will any interviews, questionnaires, surveys or focus groups be conducted for the study? If "Yes," please name any standard instruments used for this study and attach any non-standard instruments.	[x]Yes [ ]No

See attached questionnaires.

Will any procedures or tests be done off-site by non-UCSF personnel? If "Yes," please explain.	[x]Yes [ ]No

Consent will be obtained by a study coordinator employed by Blood Systems Inc., and research will be performed at the Blood Systems Research Institute. Initial donor identification will be performed at Blood Systems Laboratories in Scottsdale, AZ. Additional testing will be performed at GenProbe in San Diego, CA and Chiron Corporation in Emeryville, CA (see Letters of Support).

Will subjects or their health care provider be given the results of any experimental tests that are performed for the study? If "Yes," please describe the tests, provide a rationale for providing subjects with the experimental test results and explain what, how and by whom subjects and their health care provider will be told about the meaning, reliability, and applicability of the test results for health care decisions.	[ ]Yes [x]No

PART 4: ALTERNATIVES

Describe the alternatives to study participation that are available to prospective subjects.

Participation in the study is completely voluntary. The alternative to participation is not to participate. As the study is observational and does not provide therapy, there would be no need to take any alternative action if a subject opts not to participate in the study.

Is study drug or treatment available off-study? If "Yes," discuss this in the consent form.	[ ]Yes [ ]No [x]N/A

PART 5: RISKS AND BENEFITS

A. Risks and Discomforts: Describe the risks and discomforts of any investigational or approved drugs, devices and procedures being used or assigned for study purposes. Describe the expected frequency of particular side effects. If subjects are restricted from receiving standard therapies during the study, please also describe the risks of those restrictions.

Sampling blood may cause a bruise and/or bleeding at the needle site. Occasionally, a person feels faint when their blood is drawn. Rarely, an infection may develop at the needle site. As subjects will have had a full unit (500 mL) phlebotomy immediately prior to enrollment and up to seven 75 ml phlebotomies during the initial three months of the study, they may be deferred from donating blood while participating in the study.

Lumbar puncture carries the rare risk of introduction of infection to the central nervous system (CNS), post-lumbar puncture headache, spinal trauma, and in very rare cases, brainstem herniation and death.

Describe the steps you have taken to minimize the risks/discomforts to subjects (e.g., stopping rules, special monitoring):

If a person demonstrates any sign of illness, such as fever, malaise, or recent weight loss, we will follow the guidelines for unhealthy individuals and the amount of blood drawn may not exceed the lesser of 50 ml or 3 ml per kg in an 8 week period.

To minimize the risks to hospitalized study subjects, CSF will be obtained at the time of a clinically warranted lumbar puncture, eliminating the need for additional procedures to obtain study CSF samples and making the incremental risk of obtaining the study sample minimal.

B. Data and Safety Monitoring Plan:

Lab tests: Cellular immune function assays will be monitored on a quarterly basis in the laboratory using standardized reagents to monitor for quality control.

Independent monitoring: These are basic immunological studies, which give both qualitative and quantitative readouts. No need for independent monitoring of the data is anticipated.

C. Confidentiality and Privacy: Describe the consequences to subjects of a loss of privacy (e.g., risks to reputation, insurability, other social risks):

West Nile virus is a self-limited disease in most individuals without social stigma attached, so loss of privacy would not be anticipated to have a major impact on the study subjects.

Identifiers: Please indicate all identifiers that may be included in the research records for the study. Check all that apply.

[ ] Names	[ ] Social Security Numbers	[ ] Device identifiers/Serial numbers
[ ] Dates	[ ] Medical record numbers	[ ] Web URLs
[ ] Postal address	[ ] Health plan numbers	[ ] IP address numbers
[ ] Phone numbers	[ ] Account numbers	[ ] Biometric identifiers
[ ] Fax numbers	[ ] License/Certificate numbers	[ ] Photos and comparable images
[ ] Email address	[ ] Vehicle id numbers	[x] Any other unique identifier
[ ] None of the 18 identifiers listed above		Blood Unit Identifier

Determining Whether HIPAA Regulations Apply to This Study: Please answer the questions below for the items identified in the above section. Check all that apply:

Is any of the study data: [ ] Derived from a medical record? ***Please identify source***: [ ] Added to the hospital or clinical medical record? [ ] Created or collected as part of health care? [ ] Used to make health care decisions?	**HIPAA regulations apply.** The information identified in section B above is PHI
[x] Obtained from the subject, including interviews, questionnaires? [ ] Obtained from a foreign country or countries only? [ ] Obtained from records open to the public? [x] Obtained from existing research records? Blood donor records [ ] None of the above.	**HIPAA regulations do not apply.** The information identified in section B above is not PHI.

If HIPAA regulations apply, you are required to obtain individual subject authorization or a CHR-approved waiver of authorization, or both, to be allowed access to medical records. For the VA, use the SFVAMC authorization. (The one exception to these requirements is the use of a Limited Data Set along with a Data Use Agreement.)

Use and Disclosure of Personal Health Information: Please indicate to whom or where you may disclose any of the identifiers listed above as part of the study process. Check all that apply:

[x] We do not plan to share any of the personally identifying information listed above outside the research team.
[ ] The subject's medical record
[ ] The study sponsor: ***please indicate***:
[ ] The US Food & Drug Administration (FDA)
[ ] Others: ***please indicate***:
[ ] A Foreign Country or Countries

Data Security: Please indicate how study data is kept secure. Check all that apply:

[ ] Data is coded; data key is destroyed at end of study or ***provide date***:
[x] Data is coded; data key is kept separately and securely

[ ] Data is kept in locked file cabinet	[ ] Electronic data are protected with a password
[ ] Data is kept in locked office or suite	[ ] Data is stored on a secure network

Describe any additional steps taken to assure that identities of subjects and any of their health information which is protected under the law is kept confidential. If video or audio tapes will be made as part of the study, disposition of these tapes should be addressed.

Reportable Information: Is it reasonably foreseeable that the study will collect information that State or Federal law requires to be reported to other officials (e.g., child or elder abuse) or ethically requires action (e.g., suicidal ideation)? If "Yes," please explain below and include a discussion of the reporting requirements in the consent form.	[ ]Yes [x]No

D. Benefits: Are there potential direct benefits to study subjects? If "Yes," please describe below.	[ ]Yes [x]No

What are the potential benefits to society?
The proposed studies will further our knowledge of how the immune system interacts with WNV. These studies will not only lend an understanding of WNV pathogenesis, but also hold the potential to assist in vaccine development and testing.

E. Risk/Benefit Analysis: How do the benefits of the study outweigh the risks to subjects?
The risks of the study to the subjects are minimal. While there is no direct benefit to the study subjects, the potentially large benefit to society balances the very small risks posed to the study subjects.

PART 6: SUBJECT INFORMATION

A. Number of Subjects: How many subjects will be enrolled at UCSF and affiliated institutions?	0
How many subjects will be enrolled at all sites (i.e., if multicenter study)?	300
How many people do you estimate you will need to consent and screen here (but not necessarily enroll) to get the needed subjects?	0

B. Types of Subjects: Check all that apply. Click on links for additional instructions.	
[ ]	Minors: Complete and attach "Inclusion of Minors" Supplement
[x]	Subjects unable to provide informed consent
[ ]	Subjects unable to read or speak English
[ ]	Pregnant Women
[ ]	Fetuses
[ ]	Neonates
[ ]	Prisoners: Complete and attach "Inclusion of Prisoners" Supplement
[x]	Inpatients
[x]	Outpatients
[x]	Normal Volunteers
[ ]	Staff of UCSF/affiliated institution

C. Eligibility Criteria: General description of subject population(s):
Study subjects will be drawn from the population of volunteer blood donors within the United States population.

Inclusion Criteria:
Subjects will be identified from the pool of WNV+ blood donors. WNV RNA and WNV seronegative individuals will also be included as control subjects. Subjects will range in age from 18 to 100 years.
Exclusion Criteria:

Pregnancy, age less than 18, prisoners

How (chart review, additional tests/exams for study purposes), when and by whom will eligibility be determined?

Subjects will be identified through routine screening of blood donation specimens for WNV RNA. Control subjects will be drawn from age and location matched donors in WNV affected areas of the country.

Are there any inclusion or exclusion criteria based on *gender*, *race* or *ethnicity*? If "Yes," please explain the nature and rationale for the restrictions below.	[ ]Yes [x]No

PART 7: RECRUITMENT

Please review CHR Recruitment Guidelines for more information about acceptable recruitment methods. Note that all advertisements, whether posted or broadcast, and all correspondence used for purposes of recruitment require CHR review and approval before they are used. Check all that apply:

[x]	Study investigators recruit their own patients directly and/or nurses or staff working with researchers approach patients. ***Provide detail in the space below*** *(i.e., how, when and where potential subjects are approached).*

Study subjects will be contacted if their blood donation tests positive for WNV RNA. This group will include WNV infected donors and those who test false-positive for WNV RNA (seronegative control subjects). Initial false positive donors will be identified through negative repeat RNA testing and failure to seroconvert WNV-reactive antibody responses. Both groups of subjects will be invited to enroll in the study upon presentation to their local blood donation center for follow up. In addition, infectious disease physicians at UCSF and UCLA medical centers and Dr. Carol Glaser from the California Department of Health Services will also refer patients with neurological symptoms whom they identify as having possible WNV, for contact about enrollment in the study only if the patients are interested. The patients will be identified and initially contacted by their treating physician; the treating physician will document patient consent to be contacted for the study in the patient chart.

[X]	Study investigators send a CHR-approved letter to colleagues asking for referrals of eligible patients interested in the study. The investigators may provide the referring physicians a CHR-approved Information Sheet about the study to give to the patients. If interested, the patient will contact the PI. Or, with documented permission from the patient, the PI may be allowed to talk directly with patients about enrollment.	
[ ]	Study investigators provide their colleagues with a "Dear Patient" letter describing the study. This letter can be signed by the treating physicians and would inform the patients how to contact the study investigators. The study investigators may not have access to patient names and addresses for mailing.	
[ ]	Advertisements, notices, and/or media used to recruit subjects. The CHR must first approve the text of these, and interested subjects will initiate contact with study investigators.	
[ ]	Study investigators request a Waiver of Consent/Authorization for recruitment purposes. This waiver is an exception to the policy but may be requested in exceptional circumstances such as:	
	[ ] [ ] [ ]	Minimal risk studies in which subjects will not be contacted (i.e., chart review only); Review of charts is needed to identify prospective subjects who will then be contacted (explain in protocol); Large-scale epidemiological studies and/or other population-based studies when subjects may be contacted by someone other than personal physician (justify in protocol).
[ ]	Direct contact of potential subjects who have previously given consent to be contacted for participation in research. Clinic or program develops a CHR-approved recruitment protocol that asks patients if they agree to be contacted for research (a recruitment database) or consent for future contact was documented using the consent form for another CHR-approved study. ***Provide detail in the space below*** *(i.e., how, when and where potential subjects are approached).*	

[ ]	Study investigators list the study on the UCSF Clinical Trials Seeking Volunteers web page or a similarly managed web site. Interested subjects initiate contact with investigators.
[ ]	Study investigators recruit potential subjects who are unknown to them. Examples include snowball sampling, use of social networks, direct approach in public situations, random digit dialing. *Please explain below:*

PART 8: INFORMED CONSENT PROCESS

Check all that apply:

[x] Signed consent will be obtained from subjects
[ ] Verbal consent will be obtained from subjects, using an
[ ] Information sheet
[ ] Script
[x] Signed consent will be obtained from surrogates
[ ] Informed consent will not be obtained

In the space below, describe *how*, *where*, *when* and *by whom* informed consent will be obtained. How much time will prospective subjects be given to consider study participation? If special subject populations will be included, be sure to describe any additional plans for obtaining consent from particular populations.

Once a blood donor with West Nile virus or a seronegative control has been identified, informed consent will be obtained over the telephone by a trained study coordinator employed by the blood bank at corporate headquarters in Scottsdale, AZ. The study coordinator would have experience in informing blood donors of positive virological testing. Study subjects will be given as much time as they need to consider participation and will sign a consent form at the time of the first blood draw upon enrollment in the study. Hospitalized subjects will be initially contacted by their treating physician for participation in the trial. If agreeable to being approached, consent will be obtained by Dr. Philip Norris at UCSF or Dr. Otto Yang, or their designated assistants. If subjects have severe neurological manifestations of West Nile virus infection and cannot provide informed consent (assessed by the patient's primary physician), a surrogate will be approached for informed consent (see supplement).

How will you make sure subjects understand the information provided to them?

Subjects will demonstrate an understanding of the implications of WNV infection and possible disease manifestations. It will be explicitly stated that there would be no benefit to the study subject from participation in the study, aside from the potential feeling of well-being gained in assisting scientific research.

PART 9: FINANCIAL CONSIDERATIONS

A. Payments to Subjects: Will subjects receive payments or gifts for study participation? If "Yes," please review CHR Subject Payment Guidelines and complete the following:	[x]Yes [ ]No
Payments will be (check all that apply):	[ ] Cash [x] Check [ ] Other (describe below)
Please describe the schedule and amounts of payments, including the total subjects can receive for completing the study. If deviating from recommendations in Subject Payment Guidelines, include specific justification below.	

The subjects will be paid $20 per visit for time and travel expense considerations. The maximum they could receive over the one year study would be $200.

B. Costs to Subjects: Will subjects or their insurance be charged for any study procedures? If "Yes," describe those costs below, and compare subjects' costs to the costs associated with alternative care off-study. Finally, explain why it is appropriate to charge those costs to the subjects.	[ ]Yes [x]No

C. Treatment and Compensation for Injury: The investigators are familiar with and will follow the University of

California policy and (if applicable) Veteran's Affairs policy regarding treatment and compensation for injury. If subjects are injured as a result of being in this study, treatment will be available. The costs of such treatment may be covered by the University of California, by the Department of Veteran's Affairs (for subjects eligible for veteran's benefits, if the SF VAMC is a study site), or by the study sponsor, if any, depending on a number of factors. The University does not normally provide any other form of compensation for injury.

PART 10: BIBLIOGRAPHY

1. Lanciotti RS, Roehrig JT, Deubel V, et al. Origin of the West Nile virus responsible for an outbreak of encephalitis in the northeastern United States. Science 1999;286:2333-7
2. Samuel MA, Diamond MS. Pathogenesis of West Nile Virus infection: a balance between virulence, innate and adaptive immunity, and viral evasion. J Virol 2006;80:9349-60
3. Diamond MS, Sitati EM, Friend LD, Higgs S, Shrestha B and Engle M. A critical role for induced IgM in the protection against West Nile virus infection. J Exp Med 2003;198:1853-62
4. Wang Y, Lobigs M, Lee E and Mullbacher A. CD8+ T cells mediate recovery and immunopathology in West Nile virus encephalitis. J Virol 2003;77:13323-34
5. Kelley TW, Prayson RA, Ruiz AI, Isada CM and Gordon SM. The neuropathology of West Nile virus meningoencephalitis. A report of two cases and review of the literature. Am J Clin Pathol 2003;119:749-53
6. Norris PJ, Moffett HF, Yang OO, et al. Beyond help: direct effector functions of human immunodeficiency virus type 1-specific CD4(+) T cells. J Virol 2004;78:8844-51
7. Norris PJ, Sumaroka M, Brander C, et al. Multiple effector functions mediated by human immunodeficiency virus-specific CD4(+) T-cell clones. J Virol 2001;75:9771-9
8. Altfeld MA, Trocha A, Eldridge RL, et al. Identification of dominant optimal HLA-B60- and HLA-B61-restricted cytotoxic T-lymphocyte (CTL) epitopes: rapid characterization of CTL responses by enzyme-linked immunospot assay. J Virol 2000;74:8541-9
9. Lobigs M, Arthur CE, Mullbacher A and Blanden RV. The flavivirus nonstructural protein NS3 is a dominant source of cytotoxic T cell peptide determinants. Virology 1994;202:195-201
10. Zheng B, Han S, Zhu Q, Goldsby R and Kelsoe G. Alternative pathways for the selection of antigen-specific peripheral T cells. Nature 1996;384:263-6
11. Lanteri MC, Heitman JW, Owen RE, et al. Comprehensive analysis of west nile virus-specific T cell responses in humans. J Infect Dis 2008;197:1296-306
12. Tobler LH, Cameron MJ, Lanteri MC, et al. Interferon and interferon-induced chemokine expression is associated with control of acute viremia in West Nile virus-infected blood donors. J Infect Dis 2008;198:979-83
13. Hill JA, Benoist C and Mathis D. Treg cells: guardians for life. Nat Immunol 2007;8:124-5
14. O'Garra A, Vieira P. Regulatory T cells and mechanisms of immune system control. Nat Med 2004;10:801-5
15. Shevach EM. Certified professionals: CD4(+)CD25(+) suppressor T cells. J Exp Med 2001;193:F41-6
16. Suvas S, Azkur AK, Kim BS, Kumaraguru U and Rouse BT. CD4+CD25+ regulatory T cells control the severity of viral immunoinflammatory lesions. J Immunol 2004;172:4123-32
17. Suvas S, Kumaraguru U, Pack CD, Lee S and Rouse BT. CD4+CD25+ T cells regulate virus-specific primary and memory CD8+ T cell responses. J Exp Med 2003;198:889-901
18. Walker LS, Chodos A, Eggena M, Dooms H and Abbas AK. Antigen-dependent proliferation of CD4+ CD25+ regulatory T cells in vivo. J Exp Med 2003;198:249-58
19. Stockinger B, Veldhoen M. Differentiation and function of Th17 T cells. Curr Opin Immunol 2007;19:281-6
20. Stockinger B, Veldhoen M and Martin B. Th17 T cells: linking innate and adaptive immunity. Semin Immunol 2007;19:353-61
21. Yue FY, Merchant A, Kovacs CM, Loutfy M, Persad D and Ostrowski MA. Virus-specific interleukin-17-producing CD4+ T cells are detectable in early human immunodeficiency virus type 1 infection. J Virol 2008;82:6767-71

PART 11: ATTACHMENTS

Please list Attachments, Supplements and Appendices	Version number(s) or date(s)
Research Subject Information and Consent Forms:	
hospitalized post-2005 subjects	04/30/09
non-hospitalized post-2005 subjects	04/21/08

UCSF
COMMITTEE ON HUMAN RESEARCH
FULL COMMITTEE REVIEW APPLICATION

Please date form: 9/9/09

General Instructions | View Complete Set of Linked Instructions

PART 1: ADMINISTRATIVE REQUIREMENTS

- Eligibility requirements for Principal Investigator, Co-Principal Investigator and Contact Person
- Training requirements

A. Principal Investigator:		
Name and degree Michael P. Busch, M.D., Ph.D.	University Title Adjunct Professor	Department Laboratory Medicine
Campus Mailing Address (Box No.) 270 Masonic Avenue	Phone Number (415) 749-6615	E-mail Address mbusch@bloodsystems.org
Co-Principal Investigator:		
Name and degree Philip J. Norris, M.D.	University Title	Department Blood Systems Research Institute
Campus Mailing Address (Box No.) 270 Masonic Avenue	Phone Number (415) 923-5769	E-mail Address pnorris@bloodsystems.org
Additional Contact Person (if any):		
Name Michelle Quintos	University Title Research Services Mgr.	Department Blood Systems Research Institute
Campus Mailing Address (Box No.) 270 Masonic Avenue	Phone Number (415) 749-6606 x782	E-mail Address mquintos@bloodsystems.org
Send correspondence to (check *one*):	[ ]PI only [ ]PI and Co-PI	[x]PI and Additional Contact Person

Study Title:	Application Type:
Natural history & pathogenesis of WNV in viremic donors	[ ]New Full Committee Application [ ]Response to "Contingent" or "Return" letter [X]Modification [ ]Renewal Current CHR #: H5866-25624-06 Expiration date: 06/19/10

Sites (Check all that apply):

[ ]UCSF [ ]SFGH [ ]VAMC [ ]Fresno [ ]Cancer Center [ ]UC Berkeley
[ ]GCRC (Moffitt/Mt. Zion) [ ]GCRC (SFGH) [ ]PCRC [ ]Foreign Country
[x]Other(s): Blood Systems Research Institute

B. Funding: If this study is eligible for "Just in Time" NIH review, do not submit your application to the CHR until you have received notification from the federal granting agency that your study appears to be in a fundable range. Check all that apply:

Type of funding	Source of funding	Funds will be awarded to/through:
[x]Contract/Grant [ ]Subcontract [ ]Gift [ ]Drug/device donation [ ]Student project **[x]**Other: internal BSRI funding Have funds been awarded? **[ ]**Yes **[X]**Pending [ ]No ***[Blood Systems Inc.]*** Award No.: *RC2HL101632*	[ ]Federal Government [ ]Other Gov. (e.g., State, local) [ ]Industry* [x]Other Private [ ]Campus/UC-Wide program [ ]Departmental Funds [ ]Other: Sponsor Name: *NIH / NHLBI*	Dept./ORU: Blood Systems Research Institute 00006454 *Institution* — *Federal Wide Assurance (FWA) No.* [ ]UCSF ..... 00000068 [ ]Blood Centers of the Pacific ..... 00002111 [ ]Gallo Institute..... 00000304 [ ]Gladstone Institute ..... 00000087 [ ]Goldman Institute on Aging..... 00002525 [ ]NCIRE ..... 00000256 [ ]S.F. Dept. of Public Health ..... 00000162 [ ]VA Research Office 00000280

*UCSF (or affiliate) financial contact person for recharge:	Jerry Michaelson, 415-923-4765
Grant Title and PI (if different from above):	
Secondary sponsors: If there are multiple sources of funding for this study, please describe the additional funding:	

C. Key Personnel: All key personnel must be listed below along with a brief statement of their qualifications. *If the SF VAMC is a study site*, please identify the principal VAMC investigator, unless already listed as PI or CoPI above. For questions regarding the VAMC application process, please contact the VA Clinical Research Office at 221-4810 ext.4655.

Investigator (and institution):	Qualifications:
Michael Busch, M.D., Ph.D. (BSRI)	Adjunct Professor of laboratory medicine with extensive experience in the study of viral infections through the acute phase, with an emphasis on the understanding of viral-immune interactions.
Philip Norris, M.D. (BSRI)	Experienced T cell immunologist with a background in CD4+ T cell immunopathogenesis and HIV infection.
Marion Lanteri, Ph.D. (BSRI)	Staff Scientist experienced in virology with skills related to T cell and immunology assays.
Leslie Tobler, Dr. P.H. (BSRI)	Sr. Scientist and manager of the BSRI Viral Reference Laboratory and Repository responsible for all sample acquisition, processing and storage.
Tzong-Hae Lee, Ph.D.	Sr. Scientist, Molecular Transfusion Laboratory, responsible for some laboratory testing and analyses.

D. Drugs, Devices and Biologics:

Investigational drugs, biologics and IND Numbers:		
Investigational devices and IDE Numbers:		[ ] NSR determination requested
Who holds the IND/IDE?	[ ]Sponsor [ ]Investigator	
Approved Drugs and/or Devices:		
Are investigational drugs, devices, or biologics prepared or manufactured in UCSF research labs?	[ ]Yes [ ]No If "Yes," identify the lab:	

E. Other Approvals/Regulated Materials: Does this study require approval or authorization from any of the following regulatory committees, or involve the use of the regulated materials listed below? Follow the hyperlinks for more information. If "Yes," complete the applicable section(s) below.		[ ]Yes [x]No
[ ]	Biological Safety Committee OSHA compliant on-site safety policy	BUA #:
[ ]	Institutional Animal Care and Use Committee	IACUC #:
[ ]	Controlled Substances	
[ ]	Human Stem Cells	Submit stem cell supplement
[ ]	Radiation Safety Committee	RUA #:

F. Scientific Merit Review: This study has received or will receive scientific merit review from (check all that apply):

[x]NIH [ ]Cancer Center* [ ]GCRC or PCRC [ ]SFVAMC [ ]Dept. Review [x]Other: Blood Systems Blood Systems Foundation Scientific Advisory Board

*Required prior to final CHR approval for oncology studies.

G. Statement of Financial Interest: Do you or the other investigators have a financial interest in the outcome of this study? If "Yes," please describe below and describe briefly in Purpose and Background section of the consent form.	[ ]Yes [x]No

H. Principal Investigator's Certification:

- I certify that the information provided in this application is complete and correct.

- I accept ultimate responsibility for the conduct of this study, the ethical performance of the project, and the protection of the rights and welfare of the human subjects who are directly or indirectly involved in this project.
- I will comply with all policies and guidelines of UCSF and affiliated institutions where this study will be conducted, as well as with all applicable federal, state and local laws regarding the protection of human subjects in research.
- I will ensure that personnel performing this study are qualified, appropriately trained and will adhere to the provisions of the CHR-approved protocol.
- I will not modify this CHR-certified protocol or any attached materials without first obtaining CHR approval for an amendment to the previously approved protocol.
- I assure that the protected health information requested, if any, is the minimum necessary to meet the research objectives.
- I assure that the protected health information I obtain, if any, as part of this research will not be reused or disclosed to any parties other than those described in the CHR-approved protocol, except as required by law.

Michael Busch MD PhD

Principal Investigator's Signature

9/9/09

Date

PART 2: STUDY DESIGN

Complete items A-E using clear, concise, non-technical, lay language (i.e., the type of language used in a newspaper article for the general public) wherever possible. Define all acronyms. Use caution when cutting and pasting from another application or protocol to ensure that information is complete, supplemented where necessary, is pasted in a logical order, and is relevant to the specific section.

Space limits are recommendations and should be adjusted as needed, but the total length for sections A-E should not exceed 5 pages.

For modifications and renewals, please highlight in *italics* all changes from previously approved version.

A. Synopsis (Briefly summarize the study.) Space limit: quarter page

The proposed study would focus on WNV RNA positive donors to perform in-depth virologic and immunologic studies. *The samples collected from a subset of the enrolled WNV+ donors will be stored (plasma and PBMCs aliquots) to build a repository of samples delivered to the NHLBI with linked databases after immunologic and virologic characterization.* Additional cases of severe WNV will be drawn from hospitalized patients identified by clinicians at UCSF and UCLA medical centers and the California Department of Health Services.
Initial studies focused on the prevalence of WNV infection in blood donors. In examining a large cohort of blood donors a number of WNV infected individuals with detectable viremia were identified (about five percent of eligible blood donors in affected areas of the country). Initial follow-up in these individuals has identified the spectrum of symptomatic disease in infected blood donors. The degree to which immune responses correlate with control of infection and contribute to symptomatic disease is not known. The focus of the studies to be conducted under this protocol and consent form is on the interrelationship between virus load, cellular proliferative responses, cytotoxic T lymphocyte responses, and the interplay between master regulators of the inflammation regulatory T cells and Th17 cells. To achieve these research goals, subjects who are WNV RNA+ at the time of blood donation will be identified and enrolled. Study subjects will be followed with blood draws of 75 mL at enrollment, at week one, two, three, six post-enrollment, then at month two, three, six, nine, and twelve post-enrollment. *The samples collected from the WNV+ blood donors enrolled in 2009 and 2010 will be stored temporarily at Blood Systems Research Institute for eventual delivery to the NHLBI Biospecimen repository after characterization for virologic (viral load, transcription mediated assays, infectivity studies) and immunologic parameters (antibody, cytokines, and chemokines testing). This repository of samples will be made available through the NHLBI to the community of scientists interested in the study of WNV infection and pathogenesis.*

B. Purpose (Specify the hypotheses, aims and/or objectives.) Space limit: half page

WNV infection results in variable penetrance of disease manifestations, ranging from asymptomatic infection to severe meningo-encephalitis and death. The immune correlates of protection from disease have not been described, particularly in humans. Previously, human T cell responses were identified for a subset of 8 peptides from the membrane, envelope, nonstructural 3 and 4b proteins of WNV. Further phenotypic studies characterized the WNV-specific T cells as cytotoxic CD8 T cells secreting granzyme A and perforin. The set of peptides identified might be used for T cell stimulation studies and might also be of interest for vaccination studies, relevant to WNV infection as well as Flaviviruses in general. Additionally, the role that the immune system might play in the pathogenesis of WNV infection is not understood but a strong correlation between lower levels of regulatory T cells (T_{reg}) and symptomatic outcome was found comparing PBMCs from asymptomatic versus symptomatic WNV+ donors.
Our specific aims are as follows:
1. To determine if T_{reg} cells expanded in acute WNV infection are WNV-specific and whether they more efficiently suppress WNV-specific immune responses in asymptomatic than in symptomatic individuals.
2. To observe the post-infection dynamics of Th17 cells and to study how the balance between pro-inflammatory Th17 cells and anti-inflammatory T_{reg} cells relates to T cell activation and disease outcomes.
3. To build a repository of samples collected from WNV+ blood donors, at different time-points after positive index donation and characterized for virologic and immunologic parameters. Both the repository and corresponding database will be transferred to the NHLBI Biospecimen Repository.

C. Background (Summarize previous studies. Explain rationale for the proposed investigation.) Space limit: one page

WNV was introduced to the Northeastern United States in late summer of 1999. The virus was determined to be almost identical genetically to strains prevalent in Israel[1]. Since its introduction, WNV has spread relentlessly westward, with large outbreaks in the Midwest and Colorado in 2004 and in Arizona and Southern California in 2005. The transmission period mirrors that of mosquito activity, peaking from May through August. It is expected that WNV will continue its westward expansion in the coming transmission season this spring and summer. During an outbreak of WNV in non-immune populations, approximately 5% of blood donations are positive for WNV IgM. Blood transfusion of WNV contaminated units has resulted in transfusion-associated transmission of the virus, with severe disease and death sometimes resulting. Pooled blood donations are now routinely screened for WNV RNA, though the sensitivity of the screening process is not likely adequate to prevent 100% protection from transfusion associated WNV transmission. Given that WNV will represent an ongoing health problem and threat to the blood supply, greater understanding of the pathogenesis of the virus is required.

Both humoral and cellular immune responses have been implicated in the control of WNV infection. The bulk of the pathogenesis data relating to WNV comes from murine models [2]. Mice deficient in secreted IgM have been shown to be more susceptible to lethal challenge with WNV, and passive transfer of polyclonal IgM can protect against lethal infection with WNV[3]. Additionally, low WNV IgM titer in infected wild type mice is correlated with higher mortality. T cells also likely play a role in control of the virus. CD8+ T cell deficient mice infected with low-dose WNV show increased mortality compared to wild-type controls[4]. Unpublished data suggest that T cell deficient mice initially control WNV replication, but ultimately fail to eradicate the virus, leading to recrudescence of viremia. The role of T cell responses appears not only to be protective, as CD8+ T cells have been isolated in the inflammatory regions of meningoencephalitits in mice and humans [5]. We will assess the role of T cells in neurological manifestations of disease by studying the properties of CSF lymphocytes in hospitalized subjects who undergo diagnostic lumbar puncture.

Our laboratory has traditionally focused on the role of HIV-specific T cell responses in the control of virus replication [6, 7]. In HIV and flaviviruses analogous to WNV, it has been demonstrated that some regions of the viral genome are more susceptible to recognition by T cells than others [8-10]. In collaboration with the Biodefense and Emerging Infections Research Resources Repository we acquired overlapping peptide sets spanning the WNV genome synthesized and coupled with our unique access to patient samples through a large blood donation network, we were able to do comprehensive analysis of WNV-specific T cell responses [11]. We showed several epitopes inducing CD8 T cell responses in humans. We were able to demonstrate that control of acute viremia in WNV-infected blood donors is associated with interferon and interferon-induced chemokine expression [12].

We will be able to monitor the effect T cell responses and especially regulatory T cells [13-18] and Th17 cells [19-21] have on the dynamics of viral infection, the correlation with disease manifestations, and the durability of T cell immune responses to the virus.

The investigators have unparalleled access to viremic blood donors. Over the last four years, they have capitalized on blood bank resources to capture viremic individuals identified during acute WNV infection and established the repository of plasma and PBMC samples they have been using to address immunological studies related to WNV infection and pathogenesis. During the two next years, the investigators will be collecting samples from WNV+ blood donors enrolled in the bleeding protocol described in Part 3 to build a new repository of samples from WNV+ blood donors. The complete pedigree of clinical data and all laboratory data around the virologic and immunologic characterization of the samples will be entered into the WNV repository database that will be transferred to NHLBI.

D. Design (Check all that apply):

[ ]Phase I [ ]Phase II [ ]Phase III [ ]Phase IV [ ]Randomized [x]Blinded

[x]Multicenter: If so, is UCSF the coordinating center? [ ]Yes [x]No

[ ]Open Label Extension: If so, specify CHR Approval Number for original study: __

[ ]Behavioral

Additional description of *general* study design. Attach flow diagram if appropriate. Space limit: half page

The study is designed as an observational study. This is a longitudinal study of cellular and humoral immune function, which will be related to viral load and disease manifestations. A portion of the study will be dedicated to determining the persistence of WNV-specific immune responses over time, so there will be no specified endpoint for the study.

Samples from WNV+ blood donors enrolled in 2009 and 2010 will be used to build a repository of samples characterized for virologic and immunologic parameters to be transferred to the NHLBI.

Subjects will be identified at the time of blood donation by the presence of a WNV RNA positive specimen. Age-matched WNV RNA negative and WNV seronegative control subjects will be derived from the donor population who initially test false-positive for WNV RNA at the time of donation. Samples will be obtained from study subjects at enrollment, then at week one, two, three, and six post-enrollment, and at month two, three, six, nine, and twelve post-enrollment. After that period a subset of donors will be sampled every three months for one year for monitoring of persistence of immune responses. Severe cases of WNV infection with neurological manifestations will be identified by clinicians at UCSF and UCLA medical centers and the California Department of Public Health. In addition to blood samples, hospitalized patients with neurological symptoms will have CSF (5 ml) sampled at the time of diagnostic lumbar puncture.

Study investigators may also provide a portion of the serial samples collected under this protocol to other scientists engaged in WNV research and conversely, may receive specimens from other researchers in an effort to generate additional data to confirm study findings. Samples received will be pre-existing and will not contain any individually identifying information. Additionally, no specimens distributed by BSRI investigators will contain individually identifying information, nor will any key to coded information be shared among investigators. These collaborators include Dr. Bill Kwok at Benaroya Institute in Seattle and Dr. Jonathan Bramson at McMaster University in Hamilton, Ontario. BSRI investigators will also confirm and maintain the appropriate IRB approvals from other institutions before engaging in these activities.

E. Data Analysis (How and by whom will data be analyzed?) Space limit: half page

Data analysis will be performed by the PI in collaboration with statisticians and epidemiologists on site at BSRI. This is a longitudinal study of viral RNA and immune markers following initial detection of a donor as viremic by

nucleic acid testing (NAT). To estimate how rapidly, on average, viral load increased during the ramp-up phase, we first need to select for each panel the RNA+/IgM- bleeds collected when viral load was increasing exponentially. Assuming that the first RNA quantifiable bleed ("time 0" bleed) probably occurred in ramp-up and that viral load increased at a constant rate (i.e., in a linear fashion) on a log scale during ramp-up, the rate of increase in log viral load can be calculated. Information on log viral load and collection days for the selected ramp-up bleeds will be entered in a repeated measures regression model where each panel subject had their own intercept and slope (PROC MIXED, SAS Institute Inc.). The average viral load doubling time during ramp-up will then be calculated as log(2)/average slope. Longitudinal regression analysis will be used to estimate time periods from selected viral RNA thresholds to IgM and IgG seroconversion, as well as the duration of time following IgM or IgG seroconversion that low-level viremia is detectable by replicate TMA. This analysis will use PROC LIFEREG (SAS Institute Inc.), and assume a normal distribution for these window periods.

For the correlation of immune responses and disease outcomes, we expect that for some of the immune measurements (e.g. T cell responses), a sizeable number of participants will have values either below the limit of detection or clustered just above the limit of detection with a rightwards skewed distribution. For these measurements, we will use non-parametric statistical tests (e.g., Wilcoxon rank-sum test) to compare distributions between groups. For measurements for which all participants have observable values and for which distributions are approximately normal, we will use parametric tests (e.g., two-sample t-test).

PART 3: PROCEDURES

Check all that apply.

[x] Biological Specimen Banking (attach supplement) [x] Genetic Testing [ ] HIV Testing

Please list, in sequence, all study procedures, tests, and treatments required for the study. Indicate which would be done even if a subject does not enroll in the study. Include a detailed explanation of any experimental procedures. Attach table if available.

The only procedure to be performed on the outpatient study subjects is phlebotomy and research lab testing. Hospitalized patients will also have CSF sampling performed at the time of diagnostic lumbar puncture (an additional 5 ml will be obtained for research purposes). None of the results will be used for clinical decision making and they will not be relayed to the patient or the patient's physician. The WNV infected subjects would be advised of potential disease manifestations and advised to follow up with their regular physicians in case of symptoms.

The maximum amount of blood obtained from subjects is **375** ml over an 8 week period, and **750** ml over the total one-year period. If a person is hospitalized, we will follow the guidelines for unhealthy individuals and the amount of blood drawn may not exceed the less of 50 ml or 3 ml per kg in an 8 week period. Each phlebotomy will collect 75 ml blood, except in hospitalized patients where 25 ml blood will be collected per phlebotomy. In addition to the phlebotomy schedule described for WNV+ subjects, blood donors' initial donation blood products will be retrieved for study if operationally feasible. This will allow study of the earliest viremic time point.

In addition to phlebotomy, two questionnaires will be administered by telephone. Questionnaire A will be administered within a week of infection being identified. The second will be administered approximately three weeks after infection was identified. Both questionnaires focus on symptoms associated with WNV infection (see questionnaires in Appendix).

Category	Specific Activities
Visit schedule non-hospitalized	1) Index donation (day 0) 2) Enrollment visit (days 2-4) 3) Phlebotomies at weeks one, two, three, six post-enrollment (days 9-46) 4) Phlebotomy at two, three, six, nine, and twelve months post-enrollment.

Visit schedule hospitalized	1) Index donation (25 ml, day 0) 2) Index CSF sample (5ml, day 0 or when first performed) 3) Phlebotomy at week one, two, three, six if still hospitalized (25 ml) 4) Phlebotomy at two and three months post- index donation after hospital discharge (50 ml) 5) Phlebotomy every three months for up to one year after enrollment
Visit procedures	1) Donor consent at enrollment visit 2) Donor risk/symptom questionnaire at enrollment and follow up questionnaire at one month 3) Donor phlebotomy at each visit (draw 7x10mL EDTA plus 1x2.54mL EDTA plus one 2.5 mL PAXgene tube) 4) Anticoagulated whole blood will be shipped using Federal Express to Blood Systems Research Institute.
Tests that may be performed	*1) Complete Blood Count/Platelet count* *2) Plasma and PBMCs separation* 3) WNV TMA (5x) 4) WNV IgM and IgG (Focus) 5) PRNT (CDC protocol) 6) Quantitative WNV PCR (viral load) on index unit and TMA-reactive specimens 7) WNV Viral culture *and infectivity studies* 8) WNV genome sequencing 9) Cytotoxic T cell response 10) CD4 proliferative responses 11) Regulatory T cell frequencies and WNV-specific T cell responses 12) Th17 cells frequencies and WNV-specific responses 13) Cytokine/*chemokines* quantification 14) HLA typing 15) Generation of immortalized B and T cell lines

To provide appropriate experimental controls, the ability of study subjects' cells to combat other viruses such as Epstein Barr virus, hepatitis C virus, HIV, influenza virus, cytomegalovirus, and human herpes virus 8 (HHV-8) may also be tested.

List the clinics and/or other specific locations where study procedures will be performed. Indicate how much time will be required of the subjects, per visit and in total for the study.

Subjects will be recruited from within the network of blood banks in Blood Systems, Inc., concentrated in the West and Midwest of the United States. Candidate sites will be located in regions of the country experiencing outbreaks of West Nile virus, as documented through routine screening of blood donors. Infectious disease physicians at UCSF and UCLA medical centers will also refer patients for contact about enrollment in the study only if the patients are interested. The patients will be identified and initially contacted by their treating physician; the treating physician will document patient consent to be contacted for the study in the patient chart. In order to boost enrollment of symptomatic subject with WNV infection, patients will also be recruited by Dr. Carol Glaser at the California Department of Public Health and those subjects will undergo consent through the state IRB. Blood will be drawn at each study visit. It is not anticipated that more than half an hour per visit will be required, for a total of 4.5 hours over a one year period.

Will any interviews, questionnaires, surveys or focus groups be conducted for the study? If "Yes," please name any standard instruments used for this study and attach any non-standard instruments.	[x]Yes [ ]No

See attached questionnaires.

Will any procedures or tests be done off-site by non-UCSF personnel? If "Yes," please explain.	[x]Yes [ ]No

Consent will be obtained by a study coordinator employed by Blood Systems Inc., and research will be performed at the Blood Systems Research Institute. Initial donor identification will be performed at Blood Systems Laboratories in Scottsdale, AZ. Additional testing will be performed at GenProbe in San Diego, CA Chiron Corporation in Emeryville, CA, *Focus Diagnostics in Cypress, CA, and the CDC in Fort-Collins, CO*.

Will subjects or their health care provider be given the results of any experimental tests that are performed for the study? If "Yes," please describe the tests, provide a rationale for providing subjects with the experimental test results and explain what, how and by whom subjects and their health care provider will be told about the meaning, reliability, and applicability of the test results for health care decisions.	[ ]Yes [x]No

PART 4: ALTERNATIVES

Describe the alternatives to study participation that are available to prospective subjects.

Participation in the study is completely voluntary. The alternative to participation is not to participate. As the study is observational and does not provide therapy, there would be no need to take any alternative action if a subject opts not to participate in the study.

Is study drug or treatment available off-study? If "Yes," discuss this in the consent form.	[ ]Yes [ ]No [x]N/A

PART 5: RISKS AND BENEFITS

A. Risks and Discomforts: Describe the risks and discomforts of any investigational or approved drugs, devices and procedures being used or assigned for study purposes. Describe the expected frequency of particular side effects. If subjects are restricted from receiving standard therapies during the study, please also describe the risks of those restrictions.

Sampling blood may cause a bruise and/or bleeding at the needle site. Occasionally, a person feels faint when their blood is drawn. Rarely, an infection may develop at the needle site. As subjects will have had a full unit (500 mL) phlebotomy immediately prior to enrollment and up to seven 75 ml phlebotomies during the initial three months of the study, they may be deferred from donating blood while participating in the study.

Lumbar puncture carries the rare risk of introduction of infection to the central nervous system (CNS), post-lumbar puncture headache, spinal trauma, and in very rare cases, brainstem herniation and death.

Describe the steps you have taken to minimize the risks/discomforts to subjects (e.g., stopping rules, special monitoring):

If a person demonstrates any sign of illness, such as fever, malaise, or recent weight loss, we will follow the guidelines for unhealthy individuals and the amount of blood drawn may not exceed the lesser of 50 ml or 3 ml per kg in an 8 week period.

To minimize the risks to hospitalized study subjects, CSF will be obtained at the time of a clinically warranted lumbar puncture, eliminating the need for additional procedures to obtain study CSF samples and making the incremental risk of obtaining the study sample minimal.

B. Data and Safety Monitoring Plan:

Lab tests: Cellular immune function assays will be monitored on a quarterly basis in the laboratory using standardized reagents to monitor for quality control.

Independent monitoring: These are basic immunological studies, which give both qualitative and quantitative readouts. No need for independent monitoring of the data is anticipated.

C. Confidentiality and Privacy: Describe the consequences to subjects of a loss of privacy (e.g., risks to reputation,

insurability, other social risks):

West Nile virus is a self-limited disease in most individuals without social stigma attached, so loss of privacy would not be anticipated to have a major impact on the study subjects.

Identifiers: Please indicate all identifiers that may be included in the research records for the study. Check all that apply.

[ ] Names	[ ] Social Security Numbers	[ ] Device identifiers/Serial numbers
[ ] Dates	[ ] Medical record numbers	[ ] Web URLs
[ ] Postal address	[ ] Health plan numbers	[ ] IP address numbers
[ ] Phone numbers	[ ] Account numbers	[ ] Biometric identifiers
[ ] Fax numbers	[ ] License/Certificate numbers	[ ] Photos and comparable images
[ ] Email address	[ ] Vehicle id numbers	[x] Any other unique identifier
[ ] None of the 18 identifiers listed above		Blood Unit Identifier

Determining Whether HIPAA Regulations Apply to This Study: Please answer the questions below for the items identified in the above section. Check all that apply:

Is any of the study data: [ ] Derived from a medical record? ***Please identify source***: [ ] Added to the hospital or clinical medical record? [ ] Created or collected as part of health care? [ ] Used to make health care decisions?	**HIPAA regulations apply.** The information identified in section B above is PHI
[x] Obtained from the subject, including interviews, questionnaires? [ ] Obtained from a foreign country or countries only? [ ] Obtained from records open to the public? [x] Obtained from existing research records? Blood donor records [ ] None of the above.	**HIPAA regulations do not apply.** The information identified in section B above is not PHI.

If HIPAA regulations apply, you are required to obtain individual subject authorization or a CHR-approved waiver of authorization, or both, to be allowed access to medical records. For the VA, use the SFVAMC authorization. (The one exception to these requirements is the use of a Limited Data Set along with a Data Use Agreement.)

Use and Disclosure of Personal Health Information: Please indicate to whom or where you may disclose any of the identifiers listed above as part of the study process. Check all that apply:

[x] We do not plan to share any of the personally identifying information listed above outside the research team.
[ ] The subject's medical record
[ ] The study sponsor: ***please indicate***:
[ ] The US Food & Drug Administration (FDA)
[ ] Others: ***please indicate***:
[ ] A Foreign Country or Countries

Data Security: Please indicate how study data is kept secure. Check all that apply:

[ ] Data is coded; data key is destroyed at end of study or ***provide date***:
[x] Data is coded; data key is kept separately and securely

[ ] Data is kept in locked file cabinet	[x] Electronic data are protected with a password
[ ] Data is kept in locked office or suite	[x] Data is stored on a secure network

Describe any additional steps taken to assure that identities of subjects and any of their health information which is protected under the law is kept confidential. If video or audio tapes will be made as part of the study, disposition of these tapes should be addressed.

Reportable Information: Is it reasonably foreseeable that the study will collect information that State or Federal law requires to be reported to other officials (e.g., child or elder abuse) or ethically requires action (e.g., suicidal ideation)? If "Yes," please explain below and include a discussion of the reporting requirements in the consent form.	[ ]Yes [x]No

D. Benefits: Are there potential direct benefits to study subjects? If "Yes," please describe below.	[ ]Yes [x]No

What are the potential benefits to society?

The proposed studies will further our knowledge of how the immune system interacts with WNV. These studies will not only lend an understanding of WNV pathogenesis, but also hold the potential to assist in vaccine development and testing. *The repository of WNV samples and linked database transferred to NHLBI for further availability to the scientific community will provide tools to scientists in the field to address WNV infection and pathogenesis.*

E. Risk/Benefit Analysis: How do the benefits of the study outweigh the risks to subjects?

The risks of the study to the subjects are minimal. While there is no direct benefit to the study subjects, the potentially large benefit to society balances the very small risks posed to the study subjects.

PART 6: SUBJECT INFORMATION

A. Number of Subjects: How many subjects will be enrolled at UCSF and affiliated institutions?	0
How many subjects will be enrolled at all sites (i.e., if multicenter study)?	300
How many people do you estimate you will need to consent and screen here (but not necessarily enroll) to get the needed subjects?	0

B. Types of Subjects: Check all that apply. Click on links for additional instructions.	
[ ]	Minors: Complete and attach "Inclusion of Minors" Supplement
[x]	Subjects unable to provide informed consent
[ ]	Subjects unable to read or speak English
[ ]	Pregnant Women
[ ]	Fetuses
[ ]	Neonates
[ ]	Prisoners: Complete and attach "Inclusion of Prisoners" Supplement
[x]	Inpatients
[x]	Outpatients
[x]	Normal Volunteers
[ ]	Staff of UCSF/affiliated institution

C. Eligibility Criteria: General description of subject population(s):

Study subjects will be drawn from the population of volunteer blood donors within the United States population.

Inclusion Criteria:

Subjects will be identified from the pool of WNV+ blood donors. WNV RNA and WNV seronegative individuals will also be included as control subjects. Subjects will range in age from 18 to 100 years.

Exclusion Criteria:

Pregnancy, age less than 18, prisoners

How (chart review, additional tests/exams for study purposes), when and by whom will eligibility be determined?

Subjects will be identified through routine screening of blood donation specimens for WNV RNA. Control subjects will be drawn from age and location matched donors in WNV affected areas of the country.

Are there any inclusion or exclusion criteria based on *gender*, *race* or *ethnicity*? If "Yes," please explain the nature and rationale for the restrictions below.	[ ]Yes [x]No

PART 7: RECRUITMENT

Please review CHR Recruitment Guidelines for more information about acceptable recruitment methods. Note that all advertisements, whether posted or broadcast, and all correspondence used for purposes of recruitment require CHR review and approval before they are used. Check all that apply:

[x]	Study investigators recruit their own patients directly and/or nurses or staff working with researchers approach patients. ***Provide detail in the space below*** *(i.e., how, when and where potential subjects are approached).*

Study subjects will be contacted if their blood donation tests positive for WNV RNA. This group will include WNV infected donors and those who test false-positive for WNV RNA (seronegative control subjects). Initial false positive donors will be identified through negative repeat RNA testing and failure to seroconvert WNV-reactive antibody responses. Both groups of subjects will be invited to enroll in the study upon presentation to their local blood donation center for follow up. In addition, infectious disease physicians at UCSF and UCLA medical centers and Dr. Carol Glaser from the California Department of Health Services will also refer patients with neurological symptoms whom they identify as having possible WNV, for contact about enrollment in the study only if the patients are interested. The patients will be identified and initially contacted by their treating physician; the treating physician will document patient consent to be contacted for the study in the patient chart.

[X]	Study investigators send a CHR-approved letter to colleagues asking for referrals of eligible patients interested in the study. The investigators may provide the referring physicians a CHR-approved Information Sheet about the study to give to the patients. If interested, the patient will contact the PI. Or, with documented permission from the patient, the PI may be allowed to talk directly with patients about enrollment.
[ ]	Study investigators provide their colleagues with a "Dear Patient" letter describing the study. This letter can be signed by the treating physicians and would inform the patients how to contact the study investigators. The study investigators may not have access to patient names and addresses for mailing.
[ ]	Advertisements, notices, and/or media used to recruit subjects. The CHR must first approve the text of these, and interested subjects will initiate contact with study investigators.
[ ]	Study investigators request a Waiver of Consent/Authorization for recruitment purposes. This waiver is an exception to the policy but may be requested in exceptional circumstances such as:
[ ] [ ] [ ]	Minimal risk studies in which subjects will not be contacted (i.e., chart review only); Review of charts is needed to identify prospective subjects who will then be contacted (explain in protocol); Large-scale epidemiological studies and/or other population-based studies when subjects may be contacted by someone other than personal physician (justify in protocol).
[ ]	Direct contact of potential subjects who have previously given consent to be contacted for participation in research. Clinic or program develops a CHR-approved recruitment protocol that asks patients if they agree to be contacted for research (a recruitment database) or consent for future contact was documented using the consent form for another CHR-approved study. ***Provide detail in the space below*** *(i.e., how, when and where potential subjects are approached).*

[ ]	Study investigators list the study on the UCSF Clinical Trials Seeking Volunteers web page or a similarly managed web site. Interested subjects initiate contact with investigators.
[ ]	Study investigators recruit potential subjects who are unknown to them. Examples include snowball sampling, use of social networks, direct approach in public situations, random digit dialing. *Please explain below:*

PART 8: INFORMED CONSENT PROCESS

Check all that apply:

[x] Signed consent will be obtained from subjects

[ ] Verbal consent will be obtained from subjects, using an
[ ] Information sheet
[ ] Script
[x] Signed consent will be obtained from surrogates
[ ] Informed consent will not be obtained

In the space below, describe *how*, *where*, *when* and *by whom* informed consent will be obtained. How much time will prospective subjects be given to consider study participation? If special subject populations will be included, be sure to describe any additional plans for obtaining consent from particular populations.

Once a blood donor with West Nile virus or a seronegative control has been identified, informed consent will be obtained over the telephone by a trained study coordinator employed by the blood bank at corporate headquarters in Scottsdale, AZ. The study coordinator would have experience in informing blood donors of positive virological testing. Study subjects will be given as much time as they need to consider participation and will sign a consent form at the time of the first blood draw upon enrollment in the study. Hospitalized subjects will be initially contacted by their treating physician for participation in the trial. If agreeable to being approached, consent will be obtained by Dr. Philip Norris at UCSF or Dr. Otto Yang, or their designated assistants. If subjects have severe neurological manifestations of West Nile virus infection and cannot provide informed consent (assessed by the patient's primary physician), a surrogate will be approached for informed consent (see supplement).

How will you make sure subjects understand the information provided to them?

Subjects will demonstrate an understanding of the implications of WNV infection and possible disease manifestations. It will be explicitly stated that there would be no benefit to the study subject from participation in the study, aside from the potential feeling of well-being gained in assisting scientific research.

PART 9: FINANCIAL CONSIDERATIONS

A. Payments to Subjects: Will subjects receive payments or gifts for study participation? If "Yes," please review CHR Subject Payment Guidelines and complete the following:	[x]Yes [ ]No
Payments will be (check all that apply):	[ ] Cash [x] Check [ ] Other (describe below)
Please describe the schedule and amounts of payments, including the total subjects can receive for completing the study. If deviating from recommendations in Subject Payment Guidelines, include specific justification below.	

The subjects will be paid $20 per visit for time and travel expense considerations. The maximum they could receive over the one year study would be $200.

B. Costs to Subjects: Will subjects or their insurance be charged for any study procedures? If "Yes," describe those costs below, and compare subjects' costs to the costs associated with alternative care off-study. Finally, explain why it is appropriate to charge those costs to the subjects.	[ ]Yes [x]No

C. Treatment and Compensation for Injury: The investigators are familiar with and will follow the University of California policy and (if applicable) Veteran's Affairs policy regarding treatment and compensation for injury. If subjects are injured as a result of being in this study, treatment will be available. The costs of such treatment may be covered by the University of California, by the Department of Veteran's Affairs (for subjects eligible for veteran's benefits, if the SF VAMC is a study site), or by the study sponsor, if any, depending on a number of factors. The University does not normally provide any other form of compensation for injury.

PART 10: BIBLIOGRAPHY

1. Lanciotti RS, Roehrig JT, Deubel V, et al. Origin of the West Nile virus responsible for an outbreak of encephalitis in the northeastern United States. Science 1999;286:2333-7
2. Samuel MA, Diamond MS. Pathogenesis of West Nile Virus infection: a balance between virulence, innate and adaptive immunity, and viral evasion. J Virol 2006;80:9349-60
3. Diamond MS, Sitati EM, Friend LD, Higgs S, Shrestha B and Engle M. A critical role for induced IgM in the protection against West Nile virus infection. J Exp Med 2003;198:1853-62
4. Wang Y, Lobigs M, Lee E and Mullbacher A. CD8+ T cells mediate recovery and immunopathology in West Nile virus encephalitis. J Virol 2003;77:13323-34
5. Kelley TW, Prayson RA, Ruiz AI, Isada CM and Gordon SM. The neuropathology of West Nile virus meningoencephalitis. A report of two cases and review of the literature. Am J Clin Pathol 2003;119:749-53
6. Norris PJ, Moffett HF, Yang OO, et al. Beyond help: direct effector functions of human immunodeficiency virus type 1-specific CD4(+) T cells. J Virol 2004;78:8844-51
7. Norris PJ, Sumaroka M, Brander C, et al. Multiple effector functions mediated by human immunodeficiency virus-specific CD4(+) T-cell clones. J Virol 2001;75:9771-9
8. Altfeld MA, Trocha A, Eldridge RL, et al. Identification of dominant optimal HLA-B60- and HLA-B61-restricted cytotoxic T-lymphocyte (CTL) epitopes: rapid characterization of CTL responses by enzyme-linked immunospot assay. J Virol 2000;74:8541-9
9. Lobigs M, Arthur CE, Mullbacher A and Blanden RV. The flavivirus nonstructural protein NS3 is a dominant source of cytotoxic T cell peptide determinants. Virology 1994;202:195-201
10. Zheng B, Han S, Zhu Q, Goldsby R and Kelsoe G. Alternative pathways for the selection of antigen-specific peripheral T cells. Nature 1996;384:263-6
11. Lanteri MC, Heitman JW, Owen RE, et al. Comprehensive analysis of west nile virus-specific T cell responses in humans. J Infect Dis 2008;197:1296-306
12. Tobler LH, Cameron MJ, Lanteri MC, et al. Interferon and interferon-induced chemokine expression is associated with control of acute viremia in West Nile virus-infected blood donors. J Infect Dis 2008;198:979-83
13. Hill JA, Benoist C and Mathis D. Treg cells: guardians for life. Nat Immunol 2007;8:124-5
14. O'Garra A, Vieira P. Regulatory T cells and mechanisms of immune system control. Nat Med 2004;10:801-5
15. Shevach EM. Certified professionals: CD4(+)CD25(+) suppressor T cells. J Exp Med 2001;193:F41-6
16. Suvas S, Azkur AK, Kim BS, Kumaraguru U and Rouse BT. CD4+CD25+ regulatory T cells control the severity of viral immunoinflammatory lesions. J Immunol 2004;172:4123-32
17. Suvas S, Kumaraguru U, Pack CD, Lee S and Rouse BT. CD4+CD25+ T cells regulate virus-specific primary and memory CD8+ T cell responses. J Exp Med 2003;198:889-901
18. Walker LS, Chodos A, Eggena M, Dooms H and Abbas AK. Antigen-dependent proliferation of CD4+ CD25+ regulatory T cells in vivo. J Exp Med 2003;198:249-58
19. Stockinger B, Veldhoen M. Differentiation and function of Th17 T cells. Curr Opin Immunol 2007;19:281-6
20. Stockinger B, Veldhoen M and Martin B. Th17 T cells: linking innate and adaptive immunity. Semin Immunol 2007;19:353-61
21. Yue FY, Merchant A, Kovacs CM, Loutfy M, Persad D and Ostrowski MA. Virus-specific interleukin-17-producing CD4+ T cells are detectable in early human immunodeficiency virus type 1 infection. J Virol 2008;82:6767-71

PART 11: ATTACHMENTS

Please list Attachments, Supplements and Appendices	Version number(s) or date(s)
Research Subject Information and Consent Forms:	
hospitalized post-2005 subjects	04/30/09
non-hospitalized post-2005 subjects	09/09/09

UCSF
COMMITTEE ON HUMAN RESEARCH
FULL COMMITTEE REVIEW APPLICATION

Please date form: 12/17/09

General Instructions | View Complete Set of Linked Instructions

PART 1: ADMINISTRATIVE REQUIREMENTS

- Eligibility requirements for Principal Investigator, Co-Principal Investigator and Contact Person
- Training requirements

A. Principal Investigator:		
Name and degree Michael P. Busch, M.D., Ph.D.	University Title Adjunct Professor	Department Laboratory Medicine
Campus Mailing Address (Box No.) 270 Masonic Avenue	Phone Number (415) 749-6615	E-mail Address mbusch@bloodsystems.org
Co-Principal Investigator:		
Name and degree Philip J. Norris, M.D.	University Title	Department Blood Systems Research Institute
Campus Mailing Address (Box No.) 270 Masonic Avenue	Phone Number (415) 923-5769	E-mail Address pnorris@bloodsystems.org
Additional Contact Person (if any):		
Name Michelle Quintos	University Title Research Services Mgr.	Department Blood Systems Research Institute
Campus Mailing Address (Box No.) 270 Masonic Avenue	Phone Number (415) 749-6606 x782	E-mail Address mquintos@bloodsystems.org
Send correspondence to (check *one*):	[ ]PI only [ ]PI and Co-PI [x]PI and Additional Contact Person	
Study Title: Natural history & pathogenesis of WNV in viremic donors	Application Type: [ ]New Full Committee Application [ ]Response to "Contingent" or "Return" letter [X]Modification [ ]Renewal Current CHR #: H5866-25624-06A Expiration date: 06/19/10	
Sites (Check all that apply): [ ]UCSF [ ]SFGH [ ]VAMC [ ]Fresno [ ]Cancer Center [ ]UC Berkeley [ ]GCRC (Moffitt/Mt. Zion) [ ]GCRC (SFGH) [ ]PCRC [ ]Foreign Country [x]Other(s): Blood Systems Research Institute		

B. Funding: If this study is eligible for "Just in Time" NIH review, do not submit your application to the CHR until you have received notification from the federal granting agency that your study appears to be in a fundable range. Check all that apply:

Type of funding	Source of funding	Funds will be awarded to/through:
[x]Contract/Grant [ ]Subcontract [ ]Gift [ ]Drug/device donation [ ]Student project **[x]**Other: internal BSRI funding Have funds been awarded? **[ ]**Yes **[X]**Pending [ ]No ***[Blood Systems Inc.]*** Award No.: *RC2HL101632*	[ ]Federal Government [ ]Other Gov. (e.g., State, local) [ ]Industry* [x]Other Private [ ]Campus/UC-Wide program [ ]Departmental Funds [ ]Other: Sponsor Name: *NIH / NHLBI*	Dept./ORU: Blood Systems Research Institute 00006454 *Institution* — *Federal Wide Assurance (FWA) No.* [ ]UCSF ..... 00000068 [ ]Blood Centers of the Pacific ..... 00002111 [ ]Gallo Institute..... 00000304 [ ]Gladstone Institute ..... 00000087 [ ]Goldman Institute on Aging..... 00002525 [ ]NCIRE ..... 00000256 [ ]S.F. Dept. of Public Health ..... 00000162 [ ]VA Research Office 00000280
*UCSF (or affiliate) financial contact person for recharge:	Jerry Michaelson, 415-923-4765	
Grant Title and PI (if different from above):		
Secondary sponsors: If there are multiple sources of funding for this study, please describe the additional funding:		

C. Key Personnel: All key personnel must be listed below along with a brief statement of their qualifications. *If the SF VAMC is a study site*, please identify the principal VAMC investigator, unless already listed as PI or CoPI above. For questions regarding the VAMC application process, please contact the VA Clinical Research Office at 221-4810 ext.4655.

Investigator (and institution):	Qualifications:
Michael Busch, M.D., Ph.D. (BSRI)	Adjunct Professor of laboratory medicine with extensive experience in the study of viral infections through the acute phase, with an emphasis on the understanding of viral-immune interactions.
Philip Norris, M.D. (BSRI)	Experienced T cell immunologist with a background in CD4+ T cell immunopathogenesis and HIV infection.
Marion Lanteri, Ph.D. (BSRI)	Staff Scientist experienced in virology with skills related to T cell and immunology assays.
Leslie Tobler, Dr. P.H. (BSRI)	Sr. Scientist and manager of the BSRI Viral Reference Laboratory and Repository responsible for all sample acquisition, processing and storage.
Tzong-Hae Lee, Ph.D.	Sr. Scientist, Molecular Transfusion Laboratory, responsible for some laboratory testing and analyses.

D. Drugs, Devices and Biologics:

Investigational drugs, biologics and IND Numbers:		
Investigational devices and IDE Numbers:		[ ] NSR determination requested
Who holds the IND/IDE?	[ ]Sponsor [ ]Investigator	
Approved Drugs and/or Devices:		
Are investigational drugs, devices, or biologics prepared or manufactured in UCSF research labs?	[ ]Yes [ ]No If "Yes," identify the lab:	

E. Other Approvals/Regulated Materials: Does this study require approval or authorization from any of the following regulatory committees, or involve the use of the regulated materials listed below? Follow the hyperlinks for more information. If "Yes," complete the applicable section(s) below.		[ ]Yes [x]No
[ ]	Biological Safety Committee OSHA compliant on-site safety policy	BUA #:
[ ]	Institutional Animal Care and Use Committee	IACUC #:
[ ]	Controlled Substances	
[ ]	Human Stem Cells	Submit stem cell supplement
[ ]	Radiation Safety Committee	RUA #:

F. Scientific Merit Review: This study has received or will receive scientific merit review from (check all that apply):

[x]NIH [ ]Cancer Center* [ ]GCRC or PCRC [ ]SFVAMC [ ]Dept. Review [x]Other: Blood Systems Blood Systems Foundation Scientific Advisory Board

*Required prior to final CHR approval for oncology studies.

G. Statement of Financial Interest: Do you or the other investigators have a financial interest in the outcome of this study? If "Yes," please describe below and describe briefly in Purpose and Background section of the consent form.	[ ]Yes [x]No

H. Principal Investigator's Certification:

- I certify that the information provided in this application is complete and correct.

- I accept ultimate responsibility for the conduct of this study, the ethical performance of the project, and the protection of the rights and welfare of the human subjects who are directly or indirectly involved in this project.
- I will comply with all policies and guidelines of UCSF and affiliated institutions where this study will be conducted, as well as with all applicable federal, state and local laws regarding the protection of human subjects in research.
- I will ensure that personnel performing this study are qualified, appropriately trained and will adhere to the provisions of the CHR-approved protocol.
- I will not modify this CHR-certified protocol or any attached materials without first obtaining CHR approval for an amendment to the previously approved protocol.
- I assure that the protected health information requested, if any, is the minimum necessary to meet the research objectives.
- I assure that the protected health information I obtain, if any, as part of this research will not be reused or disclosed to any parties other than those described in the CHR-approved protocol, except as required by law.

Michael Busch MD PhD

Principal Investigator's Signature

12/29/09

Date

PART 2: STUDY DESIGN

Complete items A-E using clear, concise, non-technical, lay language (i.e., the type of language used in a newspaper article for the general public) wherever possible. Define all acronyms. Use caution when cutting and pasting from another application or protocol to ensure that information is complete, supplemented where necessary, is pasted in a logical order, and is relevant to the specific section.

Space limits are recommendations and should be adjusted as needed, but the total length for sections A-E should not exceed 5 pages.

For modifications and renewals, please highlight in *italics* all changes from previously approved version.

A. Synopsis (Briefly summarize the study.) Space limit: quarter page

The proposed study would focus on WNV RNA positive donors to perform in-depth virologic and immunologic studies. The samples collected from a subset of the enrolled WNV+ donors will be stored (plasma and PBMCs aliquots) to build a repository of samples delivered to the NHLBI with linked databases after immunologic and virologic characterization. Additional cases of severe WNV will be drawn from hospitalized patients identified by clinicians at UCSF and UCLA medical centers and the California Department of Health Services.
Initial studies focused on the prevalence of WNV infection in blood donors. In examining a large cohort of blood donors a number of WNV infected individuals with detectable viremia were identified (about five percent of eligible blood donors in affected areas of the country). Initial follow-up in these individuals has identified the spectrum of symptomatic disease in infected blood donors. The degree to which immune responses correlate with control of infection and contribute to symptomatic disease is not known. The focus of the studies to be conducted under this protocol and consent form is on the interrelationship between virus load, cellular proliferative responses, cytotoxic T lymphocyte responses, and the interplay between master regulators of the inflammation regulatory T cells and Th17 cells. To achieve these research goals, subjects who are WNV RNA+ at the time of blood donation will be identified and enrolled. Study subjects will be followed with blood draws of 75 mL at enrollment, at week one, two, three, six post-enrollment, then at month two, three, six, nine, and twelve post-enrollment. The samples collected from the WNV+ blood donors enrolled in 2009 and 2010 will be stored temporarily at Blood Systems Research Institute for eventual delivery to the NHLBI Biospecimen repository after characterization for virologic (viral load, transcription mediated assays, infectivity studies) and immunologic parameters (antibody, cytokines, and chemokines testing). This repository of samples will be made available through the NHLBI to the community of scientists interested in the study of WNV infection and pathogenesis.

B. Purpose (Specify the hypotheses, aims and/or objectives.) Space limit: half page

WNV infection results in variable penetrance of disease manifestations, ranging from asymptomatic infection to severe meningo-encephalitis and death. The immune correlates of protection from disease have not been described, particularly in humans. Previously, human T cell responses were identified for a subset of 8 peptides from the membrane, envelope, nonstructural 3 and 4b proteins of WNV. Further phenotypic studies characterized the WNV-specific T cells as cytotoxic CD8 T cells secreting granzyme A and perforin. The set of peptides identified might be used for T cell stimulation studies and might also be of interest for vaccination studies, relevant to WNV infection as well as Flaviviruses in general. Additionally, the role that the immune system might play in the pathogenesis of WNV infection is not understood but a strong correlation between lower levels of regulatory T cells (T_{reg}) and symptomatic outcome was found comparing PBMCs from asymptomatic versus symptomatic WNV+ donors.
Our specific aims are as follows:
1. To determine if T_{reg} cells expanded in acute WNV infection are WNV-specific and whether they more efficiently suppress WNV-specific immune responses in asymptomatic than in symptomatic individuals.
2. To observe the post-infection dynamics of Th17 cells and to study how the balance between pro-inflammatory Th17 cells and anti-inflammatory T_{reg} cells relates to T cell activation and disease outcomes.
3. To build a repository of samples collected from WNV+ blood donors, at different time-points after positive index donation and characterized for virologic and immunologic parameters. Both the repository and corresponding database will be transferred to the NHLBI Biospecimen Repository.

C. Background (Summarize previous studies. Explain rationale for the proposed investigation.) Space limit: one page

WNV was introduced to the Northeastern United States in late summer of 1999. The virus was determined to be almost identical genetically to strains prevalent in Israel[1]. Since its introduction, WNV has spread relentlessly westward, with large outbreaks in the Midwest and Colorado in 2004 and in Arizona and Southern California in 2005. The transmission period mirrors that of mosquito activity, peaking from May through August. It is expected that WNV will continue its westward expansion in the coming transmission season this spring and summer. During an outbreak of WNV in non-immune populations, approximately 5% of blood donations are positive for WNV IgM. Blood transfusion of WNV contaminated units has resulted in transfusion-associated transmission of the virus, with severe disease and death sometimes resulting. Pooled blood donations are now routinely screened for WNV RNA, though the sensitivity of the screening process is not likely adequate to prevent 100% protection from transfusion associated WNV transmission. Given that WNV will represent an ongoing health problem and threat to the blood supply, greater understanding of the pathogenesis of the virus is required.

Both humoral and cellular immune responses have been implicated in the control of WNV infection. The bulk of the pathogenesis data relating to WNV comes from murine models [2]. Mice deficient in secreted IgM have been shown to be more susceptible to lethal challenge with WNV, and passive transfer of polyclonal IgM can protect against lethal infection with WNV[3]. Additionally, low WNV IgM titer in infected wild type mice is correlated with higher mortality. T cells also likely play a role in control of the virus. CD8+ T cell deficient mice infected with low-dose WNV show increased mortality compared to wild-type controls[4]. Unpublished data suggest that T cell deficient mice initially control WNV replication, but ultimately fail to eradicate the virus, leading to recrudescence of viremia. The role of T cell responses appears not only to be protective, as CD8+ T cells have been isolated in the inflammatory regions of meningoencephalitits in mice and humans [5]. We will assess the role of T cells in neurological manifestations of disease by studying the properties of CSF lymphocytes in hospitalized subjects who undergo diagnostic lumbar puncture.

Our laboratory has traditionally focused on the role of HIV-specific T cell responses in the control of virus replication [6, 7]. In HIV and flaviviruses analogous to WNV, it has been demonstrated that some regions of the viral genome are more susceptible to recognition by T cells than others [8-10]. In collaboration with the Biodefense and Emerging Infections Research Resources Repository we acquired overlapping peptide sets spanning the WNV genome synthesized and coupled with our unique access to patient samples through a large blood donation network, we were able to do comprehensive analysis of WNV-specific T cell responses [11]. We showed several epitopes inducing CD8 T cell responses in humans. We were able to demonstrate that control of acute viremia in WNV-infected blood donors is associated with interferon and interferon-induced chemokine expression [12].

We will be able to monitor the effect T cell responses and especially regulatory T cells [13-18] and Th17 cells [19-21] have on the dynamics of viral infection, the correlation with disease manifestations, and the durability of T cell immune responses to the virus.

The investigators have unparalleled access to viremic blood donors. Over the last four years, they have capitalized on blood bank resources to capture viremic individuals identified during acute WNV infection and established the repository of plasma and PBMC samples they have been using to address immunological studies related to WNV infection and pathogenesis. During the two next years, the investigators will be collecting samples from WNV+ blood donors enrolled in the bleeding protocol described in Part 3 to build a new repository of samples from WNV+ blood donors. The complete pedigree of clinical data and all laboratory data around the virologic and immunologic characterization of the samples will be entered into the WNV repository database that will be transferred to NHLBI.

D. Design (Check all that apply):

[ ]Phase I [ ]Phase II [ ]Phase III [ ]Phase IV [ ]Randomized [x]Blinded

[x]Multicenter: If so, is UCSF the coordinating center? [ ]Yes [x]No

[ ]Open Label Extension: If so, specify CHR Approval Number for original study: __

[ ]Behavioral

Additional description of *general* study design. Attach flow diagram if appropriate. Space limit: half page

The study is designed as an observational study. This is a longitudinal study of cellular and humoral immune function, which will be related to viral load and disease manifestations. A portion of the study will be dedicated to determining the persistence of WNV-specific immune responses over time, so there will be no specified endpoint for the study.

Samples from WNV+ blood donors enrolled in 2009 and 2010 will be used to build a repository of samples characterized for virologic and immunologic parameters to be transferred to the NHLBI.

Subjects will be identified at the time of blood donation by the presence of a WNV RNA positive specimen. Age-matched WNV RNA negative and WNV seronegative control subjects will be derived from the donor population who initially test false-positive for WNV RNA at the time of donation. Samples will be obtained from study subjects at enrollment, then at week one, two, three, and six post-enrollment, and at month two, three, six, nine, and twelve post-enrollment. After that period a subset of donors will be sampled every three months for one year for monitoring of persistence of immune responses. Severe cases of WNV infection with neurological manifestations will be identified by clinicians at UCSF and UCLA medical centers and the California Department of Public Health. In addition to blood samples, hospitalized patients with neurological symptoms will have CSF (5 ml) sampled at the time of diagnostic lumbar puncture.

Study investigators may also provide a portion of the serial samples collected under this protocol to other scientists engaged in WNV research and conversely, may receive specimens from other researchers in an effort to generate additional data to confirm study findings. Samples received will be pre-existing and will not contain any individually identifying information. Additionally, no specimens distributed by BSRI investigators will contain individually identifying information, nor will any key to coded information be shared among investigators. These collaborators include Dr. Bill Kwok at Benaroya Institute in Seattle and Dr. Jonathan Bramson at McMaster University in Hamilton, Ontario. BSRI investigators will also confirm and maintain the appropriate IRB approvals from other institutions before engaging in these activities.

An additional follow-up study of WNV+ study participants from 2005 will also be conducted. These subjects will be recalled for a one-time blood draw of 75 ml for WNV IgG and PRNT testing. We will then compare these results to their IgG and PRNT levels from the samples obtained during their earlier participation in the study.

E. Data Analysis (How and by whom will data be analyzed?) Space limit: half page

Data analysis will be performed by the PI in collaboration with statisticians and epidemiologists on site at BSRI. This is a longitudinal study of viral RNA and immune markers following initial detection of a donor as viremic by nucleic acid testing (NAT). To estimate how rapidly, on average, viral load increased during the ramp-up phase, we first need to select for each panel the RNA+/IgM- bleeds collected when viral load was increasing exponentially. Assuming that the first RNA quantifiable bleed ("time 0" bleed) probably occurred in ramp-up and that viral load increased at a constant rate (i.e., in a linear fashion) on a log scale during ramp-up, the rate of increase in log viral load can be calculated. Information on log viral load and collection days for the selected ramp-up bleeds will be entered in a repeated measures regression model where each panel subject had their own intercept and slope (PROC MIXED, SAS Institute Inc.). The average viral load doubling time during ramp-up will then be calculated as log(2)/average slope. Longitudinal regression analysis will be used to estimate time periods from selected viral RNA thresholds to IgM and IgG seroconversion, as well as the duration of time following IgM or IgG seroconversion that low-level viremia is detectable by replicate TMA. This analysis will use PROC LIFEREG (SAS Institute Inc.), and assume a normal distribution for these window periods.

For the correlation of immune responses and disease outcomes, we expect that for some of the immune measurements (e.g. T cell responses), a sizeable number of participants will have values either below the limit of detection or clustered just above the limit of detection with a rightwards skewed distribution. For these measurements, we will use non-parametric statistical tests (e.g., Wilcoxon rank-sum test) to compare distributions between groups. For measurements for which all participants have observable values and for which distributions are approximately normal, we will use parametric tests (e.g., two-sample t-test).

PART 3: PROCEDURES

Check all that apply.

[x] Biological Specimen Banking (attach supplement) [x] Genetic Testing [ ] HIV Testing

Please list, in sequence, all study procedures, tests, and treatments required for the study. Indicate which would be done even if a subject does not enroll in the study. Include a detailed explanation of any experimental procedures. Attach table if available.

The only procedure to be performed on the outpatient study subjects is phlebotomy and research lab testing. Hospitalized patients will also have CSF sampling performed at the time of diagnostic lumbar puncture (an additional 5 ml will be obtained for research purposes). None of the results will be used for clinical decision making and they will not be relayed to the patient or the patient's physician. The WNV infected subjects would be advised of potential disease manifestations and advised to follow up with their regular physicians in case of symptoms.

The maximum amount of blood obtained from subjects is 375 ml over an 8 week period, and 750 ml over the total one-year period. If a person is hospitalized, we will follow the guidelines for unhealthy individuals and the amount of blood drawn may not exceed the less of 50 ml or 3 ml per kg in an 8 week period. Each phlebotomy will collect 75 ml blood, except in hospitalized patients where 25 ml blood will be collected per phlebotomy. In addition to the phlebotomy schedule described for WNV+ subjects, blood donors' initial donation blood products will be retrieved for study if operationally feasible. This will allow study of the earliest viremic time point.

In addition to phlebotomy, two questionnaires will be administered by telephone. Questionnaire A will be administered within a week of infection being identified. The second will be administered approximately three weeks after infection was identified. Both questionnaires focus on symptoms associated with WNV infection (see questionnaires in Appendix).

Category	Specific Activities

Visit schedule non-hospitalized	1) Index donation (day 0) 2) Enrollment visit (days 2-4) 3) Phlebotomies at weeks one, two, three, six post-enrollment (days 9-46) 4) Phlebotomy at two, three, six, nine, and twelve months post-enrollment.
Visit schedule hospitalized	1) Index donation (25 ml, day 0) 2) Index CSF sample (5ml, day 0 or when first performed) 3) Phlebotomy at week one, two, three, six if still hospitalized (25 ml) 4) Phlebotomy at two and three months post- index donation after hospital discharge (50 ml) 5) Phlebotomy every three months for up to one year after enrollment
Visit procedures	1) Donor consent at enrollment visit 2) Donor risk/symptom questionnaire at enrollment and follow up questionnaire at one month 3) Donor phlebotomy at each visit (draw 7x10mL EDTA plus 1x2.54mL EDTA plus one 2.5 mL PAXgene tube) 4) Anticoagulated whole blood will be shipped using Federal Express to Blood Systems Research Institute.
Tests that may be performed	*1) Complete Blood Count/Platelet count* *2) Plasma and PBMCs separation* 3) WNV TMA (5x) 4) WNV IgM and IgG (Focus) 5) PRNT (CDC protocol) 6) Quantitative WNV PCR (viral load) on index unit and TMA-reactive specimens 7) WNV Viral culture *and infectivity studies* 8) WNV genome sequencing 9) Cytotoxic T cell response 10) CD4 proliferative responses 11) Regulatory T cell frequencies and WNV-specific T cell responses 12) Th17 cells frequencies and WNV-specific responses 13) Cytokine*/chemokines* quantification 14) HLA typing 15) Generation of immortalized B and T cell lines

To provide appropriate experimental controls, the ability of study subjects' cells to combat other viruses such as Epstein Barr virus, hepatitis C virus, HIV, influenza virus, cytomegalovirus, and human herpes virus 8 (HHV-8) may also be tested.

For the 2005 recall study, WNV+ donors enrolled in our study in 2005 will be contacted via a letter by our Medical Affairs office and offered the opportunity to participate. Potential participants will phone a donor counselor, as detailed in the letter, given all study information and instructions to have their blood drawn. They will sign their consent forms when they visit their blood centers for the blood draws. Samples will be shipped to BSRI for WNV ELISA and PRNT testing.

List the clinics and/or other specific locations where study procedures will be performed. Indicate how much time will be required of the subjects, per visit and in total for the study.

Subjects will be recruited from within the network of blood banks in Blood Systems, Inc., concentrated in the West and Midwest of the United States. Candidate sites will be located in regions of the country experiencing outbreaks of West Nile virus, as documented through routine screening of blood donors. Infectious disease physicians at UCSF and UCLA medical centers will also refer patients for contact about enrollment in the study only if the patients are interested. The patients will be identified and initially contacted by their treating physician; the treating physician will document patient consent to be contacted for the study in the patient chart. In order to boost enrollment of symptomatic subject with WNV infection, patients will also be recruited by Dr. Carol Glaser at the California Department of Public Health and those subjects will undergo consent through the

state IRB. Blood will be drawn at each study visit. It is not anticipated that more than half an hour per visit will be required, for a total of 4.5 hours over a one year period.

For the recall study, participants will have their blood drawn at their local BSI blood center. The single blood draw visit should not take more than one half hour.

Will any interviews, questionnaires, surveys or focus groups be conducted for the study? If "Yes," please name any standard instruments used for this study and attach any non-standard instruments.	[x]Yes [ ]No

See attached questionnaires.

Will any procedures or tests be done off-site by non-UCSF personnel? If "Yes," please explain.	[x]Yes [ ]No

Consent will be obtained by a study coordinator employed by Blood Systems Inc., and research will be performed at the Blood Systems Research Institute. Initial donor identification will be performed at Blood Systems Laboratories in Scottsdale, AZ. Additional testing will be performed at GenProbe in San Diego, CA Chiron Corporation in Emeryville, CA, *Focus Diagnostics in Cypress, CA, and the CDC in Fort-Collins, CO*.

Will subjects or their health care provider be given the results of any experimental tests that are performed for the study? If "Yes," please describe the tests, provide a rationale for providing subjects with the experimental test results and explain what, how and by whom subjects and their health care provider will be told about the meaning, reliability, and applicability of the test results for health care decisions.	[ ]Yes [x]No

PART 4: ALTERNATIVES

Describe the alternatives to study participation that are available to prospective subjects.

Participation in the study is completely voluntary. The alternative to participation is not to participate. As the study is observational and does not provide therapy, there would be no need to take any alternative action if a subject opts not to participate in the study.

Is study drug or treatment available off-study? If "Yes," discuss this in the consent form.	[ ]Yes [ ]No [x]N/A

PART 5: RISKS AND BENEFITS

A. Risks and Discomforts: Describe the risks and discomforts of any investigational or approved drugs, devices and procedures being used or assigned for study purposes. Describe the expected frequency of particular side effects. If subjects are restricted from receiving standard therapies during the study, please also describe the risks of those restrictions.

Sampling blood may cause a bruise and/or bleeding at the needle site. Occasionally, a person feels faint when their blood is drawn. Rarely, an infection may develop at the needle site. As subjects will have had a full unit (500 mL) phlebotomy immediately prior to enrollment and up to seven 75 ml phlebotomies during the initial three months of the study, they may be deferred from donating blood while participating in the study.

Lumbar puncture carries the rare risk of introduction of infection to the central nervous system (CNS), post-lumbar puncture headache, spinal trauma, and in very rare cases, brainstem herniation and death.

Describe the steps you have taken to minimize the risks/discomforts to subjects (e.g., stopping rules, special monitoring):

If a person demonstrates any sign of illness, such as fever, malaise, or recent weight loss, we will follow the guidelines for unhealthy individuals and the amount of blood drawn may not exceed the lesser of 50 ml or 3 ml per kg in an 8 week period.

To minimize the risks to hospitalized study subjects, CSF will be obtained at the time of a clinically warranted lumbar puncture, eliminating the need for additional procedures to obtain study CSF samples and making the incremental risk of obtaining the study sample minimal.

B. Data and Safety Monitoring Plan:

Lab tests: Cellular immune function assays will be monitored on a quarterly basis in the laboratory using standardized reagents to monitor for quality control.

Independent monitoring: These are basic immunological studies, which give both qualitative and quantitative readouts. No need for independent monitoring of the data is anticipated.

C. Confidentiality and Privacy: Describe the consequences to subjects of a loss of privacy (e.g., risks to reputation, insurability, other social risks):

West Nile virus is a self-limited disease in most individuals without social stigma attached, so loss of privacy would not be anticipated to have a major impact on the study subjects.

Identifiers: Please indicate all identifiers that may be included in the research records for the study. Check all that apply.

[ ] Names	[ ] Social Security Numbers	[ ] Device identifiers/Serial numbers
[ ] Dates	[ ] Medical record numbers	[ ] Web URLs
[ ] Postal address	[ ] Health plan numbers	[ ] IP address numbers
[ ] Phone numbers	[ ] Account numbers	[ ] Biometric identifiers
[ ] Fax numbers	[ ] License/Certificate numbers	[ ] Photos and comparable images
[ ] Email address	[ ] Vehicle id numbers	[x] Any other unique identifier
[ ] None of the 18 identifiers listed above		Blood Unit Identifier

Determining Whether HIPAA Regulations Apply to This Study: Please answer the questions below for the items identified in the above section. Check all that apply:

Is any of the study data: [ ] Derived from a medical record? ***Please identify source***: [ ] Added to the hospital or clinical medical record? [ ] Created or collected as part of health care? [ ] Used to make health care decisions?	**HIPAA regulations apply.** The information identified in section B above is PHI
[x] Obtained from the subject, including interviews, questionnaires? [ ] Obtained from a foreign country or countries only? [ ] Obtained from records open to the public? [x] Obtained from existing research records? Blood donor records [ ] None of the above.	**HIPAA regulations do not apply.** The information identified in section B above is not PHI.

If HIPAA regulations apply, you are required to obtain individual subject authorization or a CHR-approved waiver of authorization, or both, to be allowed access to medical records. For the VA, use the SFVAMC authorization. (The one exception to these requirements is the use of a Limited Data Set along with a Data Use Agreement.)

Use and Disclosure of Personal Health Information: Please indicate to whom or where you may disclose any of the identifiers listed above as part of the study process. Check all that apply:

[x] We do not plan to share any of the personally identifying information listed above outside the research team.
[ ] The subject's medical record
[ ] The study sponsor: ***please indicate***:
[ ] The US Food & Drug Administration (FDA)
[ ] Others: ***please indicate***:
[ ] A Foreign Country or Countries

Data Security: Please indicate how study data is kept secure. Check all that apply:

[ ] Data is coded; data key is destroyed at end of study or ***provide date***:
[x] Data is coded; data key is kept separately and securely
[ ] Data is kept in locked file cabinet
[x] Electronic data are protected with a password

[ ] Data is kept in locked office or suite [x] Data is stored on a secure network

Describe any additional steps taken to assure that identities of subjects and any of their health information which is protected under the law is kept confidential. If video or audio tapes will be made as part of the study, disposition of these tapes should be addressed.

Reportable Information: Is it reasonably foreseeable that the study will collect information that State or Federal law requires to be reported to other officials (e.g., child or elder abuse) or ethically requires action (e.g., suicidal ideation)? If "Yes," please explain below and include a discussion of the reporting requirements in the consent form.	[ ]Yes [x]No

D. Benefits: Are there potential direct benefits to study subjects? If "Yes," please describe below.	[ ]Yes [x]No

What are the potential benefits to society?

The proposed studies will further our knowledge of how the immune system interacts with WNV. These studies will not only lend an understanding of WNV pathogenesis, but also hold the potential to assist in vaccine development and testing. *The repository of WNV samples and linked database transferred to NHLBI for further availability to the scientific community will provide tools to scientists in the field to address WNV infection and pathogenesis.*

E. Risk/Benefit Analysis: How do the benefits of the study outweigh the risks to subjects?

The risks of the study to the subjects are minimal. While there is no direct benefit to the study subjects, the potentially large benefit to society balances the very small risks posed to the study subjects.

PART 6: SUBJECT INFORMATION

A. Number of Subjects: How many subjects will be enrolled at UCSF and affiliated institutions?	0
How many subjects will be enrolled at all sites (i.e., if multicenter study)?	300
How many people do you estimate you will need to consent and screen here (but not necessarily enroll) to get the needed subjects?	0

B. Types of Subjects: Check all that apply. Click on links for additional instructions.	
[ ]	Minors: Complete and attach "Inclusion of Minors" Supplement
[x]	Subjects unable to provide informed consent
[ ]	Subjects unable to read or speak English
[ ]	Pregnant Women
[ ]	Fetuses
[ ]	Neonates
[ ]	Prisoners: Complete and attach "Inclusion of Prisoners" Supplement
[x]	Inpatients
[x]	Outpatients
[x]	Normal Volunteers
[ ]	Staff of UCSF/affiliated institution

C. Eligibility Criteria: General description of subject population(s):

Study subjects will be drawn from the population of volunteer blood donors within the United States population.

Inclusion Criteria:

Subjects will be identified from the pool of WNV+ blood donors. WNV RNA and WNV seronegative individuals will also be included as control subjects. Subjects will range in age from 18 to 100 years.

Inclusion criteria for the recall study are WNV+ blood donors identified in 2005 and enrolled in the study.

Exclusion Criteria:

Pregnancy, age less than 18, prisoners

How (chart review, additional tests/exams for study purposes), when and by whom will eligibility be determined?

Subjects will be identified through routine screening of blood donation specimens for WNV RNA. Control subjects will be drawn from age and location matched donors in WNV affected areas of the country.

Are there any inclusion or exclusion criteria based on *gender*, *race* or *ethnicity*? If "Yes," please explain the nature and rationale for the restrictions below.	[ ]Yes [x]No

PART 7: RECRUITMENT

Please review CHR Recruitment Guidelines for more information about acceptable recruitment methods. Note that all advertisements, whether posted or broadcast, and all correspondence used for purposes of recruitment require CHR review and approval before they are used. Check all that apply:

[x] Study investigators recruit their own patients directly and/or nurses or staff working with researchers approach patients. ***Provide detail in the space below*** *(i.e., how, when and where potential subjects are approached).*

Study subjects will be contacted if their blood donation tests positive for WNV RNA. This group will include WNV infected donors and those who test false-positive for WNV RNA (seronegative control subjects). Initial false positive donors will be identified through negative repeat RNA testing and failure to seroconvert WNV-reactive antibody responses. Both groups of subjects will be invited to enroll in the study upon presentation to their local blood donation center for follow up. In addition, infectious disease physicians at UCSF and UCLA medical centers and Dr. Carol Glaser from the California Department of Health Services will also refer patients with neurological symptoms whom they identify as having possible WNV, for contact about enrollment in the study only if the patients are interested. The patients will be identified and initially contacted by their treating physician; the treating physician will document patient consent to be contacted for the study in the patient chart.

For the 2005 recall study, former WNV study participants enrolled in 2005, will be identified by BSI Medical Affairs and sent a letter (attached) asking them to participate in this follow-up study. These subjects may opt-in by responding to the letter and calling a donor counselor for more information.

[X] Study investigators send a CHR-approved letter to colleagues asking for referrals of eligible patients interested in the study. The investigators may provide the referring physicians a CHR-approved Information Sheet about the study to give to the patients. If interested, the patient will contact the PI. Or, with documented permission from the patient, the PI may be allowed to talk directly with patients about enrollment.

[ ] Study investigators provide their colleagues with a "Dear Patient" letter describing the study. This letter can be signed by the treating physicians and would inform the patients how to contact the study investigators. The study investigators may not have access to patient names and addresses for mailing.

[ ] Advertisements, notices, and/or media used to recruit subjects. The CHR must first approve the text of these, and interested subjects will initiate contact with study investigators.

[ ] Study investigators request a Waiver of Consent/Authorization for recruitment purposes. This waiver is an exception to the policy but may be requested in exceptional circumstances such as:

[ ] Minimal risk studies in which subjects will not be contacted (i.e., chart review only);
[ ] Review of charts is needed to identify prospective subjects who will then be contacted (explain in protocol);
[ ] Large-scale epidemiological studies and/or other population-based studies when subjects may be contacted by someone other than personal physician (justify in protocol).

[ ]	Direct contact of potential subjects who have previously given consent to be contacted for participation in research. Clinic or program develops a CHR-approved recruitment protocol that asks patients if they agree to be contacted for research (a recruitment database) or consent for future contact was documented using the consent form for another CHR-approved study. ***Provide detail in the space below*** *(i.e., how, when and where potential subjects are approached).*

[ ]	Study investigators list the study on the UCSF Clinical Trials Seeking Volunteers web page or a similarly managed web site. Interested subjects initiate contact with investigators.
[ ]	Study investigators recruit potential subjects who are unknown to them. Examples include snowball sampling, use of social networks, direct approach in public situations, random digit dialing. *Please explain below:*

PART 8: INFORMED CONSENT PROCESS

Check all that apply:

[x] Signed consent will be obtained from subjects
[ ] Verbal consent will be obtained from subjects, using an
    [ ] Information sheet
    [ ] Script
[x] Signed consent will be obtained from surrogates
[ ] Informed consent will not be obtained

In the space below, describe *how*, *where*, *when* and *by whom* informed consent will be obtained. How much time will prospective subjects be given to consider study participation? If special subject populations will be included, be sure to describe any additional plans for obtaining consent from particular populations.

Once a blood donor with West Nile virus or a seronegative control has been identified, informed consent will be obtained over the telephone by a trained study coordinator employed by the blood bank at corporate headquarters in Scottsdale, AZ. The study coordinator would have experience in informing blood donors of positive virological testing. Study subjects will be given as much time as they need to consider participation and will sign a consent form at the time of the first blood draw upon enrollment in the study. Hospitalized subjects will be initially contacted by their treating physician for participation in the trial. If agreeable to being approached, consent will be obtained by Dr. Philip Norris at UCSF or Dr. Otto Yang, or their designated assistants. If subjects have severe neurological manifestations of West Nile virus infection and cannot provide informed consent (assessed by the patient's primary physician), a surrogate will be approached for informed consent (see supplement).

The 2005 recall subjects will be given all the study information contained in the informed consent form over the telephone by a trained donor counselor when they respond to the recruitment letter. When subjects visit the blood bank for their blood draw, they will sign the consent form. They will be given opportunities to discuss the study and ask questions during their telephone conversation with the donor counselor and during their visit to the blood bank.

How will you make sure subjects understand the information provided to them?

Subjects will demonstrate an understanding of the implications of WNV infection and possible disease manifestations. It will be explicitly stated that there would be no benefit to the study subject from participation in the study, aside from the potential feeling of well-being gained in assisting scientific research.

PART 9: FINANCIAL CONSIDERATIONS

A. Payments to Subjects: Will subjects receive payments or gifts for study participation? If "Yes," please review CHR Subject Payment Guidelines and complete the following:	[x]Yes [ ]No

Payments will be (check all that apply):	[ ] Cash [x] Check [ ] Other (describe below)
Please describe the schedule and amounts of payments, including the total subjects can receive for completing the study. If deviating from recommendations in Subject Payment Guidelines, include specific justification below.	

The subjects will be paid $20 per visit for time and travel expense considerations. The maximum they could receive over the one year study would be $200.

2005 recall study subjects will be paid $30.

B. Costs to Subjects: Will subjects or their insurance be charged for any study procedures? If "Yes," describe those costs below, and compare subjects' costs to the costs associated with alternative care off-study. Finally, explain why it is appropriate to charge those costs to the subjects.	[ ]Yes [x]No

C. Treatment and Compensation for Injury: The investigators are familiar with and will follow the University of California policy and (if applicable) Veteran's Affairs policy regarding treatment and compensation for injury. If subjects are injured as a result of being in this study, treatment will be available. The costs of such treatment may be covered by the University of California, by the Department of Veteran's Affairs (for subjects eligible for veteran's benefits, if the SF VAMC is a study site), or by the study sponsor, if any, depending on a number of factors. The University does not normally provide any other form of compensation for injury.

PART 10: BIBLIOGRAPHY

1. Lanciotti RS, Roehrig JT, Deubel V, et al. Origin of the West Nile virus responsible for an outbreak of encephalitis in the northeastern United States. Science 1999;286:2333-7
2. Samuel MA, Diamond MS. Pathogenesis of West Nile Virus infection: a balance between virulence, innate and adaptive immunity, and viral evasion. J Virol 2006;80:9349-60
3. Diamond MS, Sitati EM, Friend LD, Higgs S, Shrestha B and Engle M. A critical role for induced IgM in the protection against West Nile virus infection. J Exp Med 2003;198:1853-62
4. Wang Y, Lobigs M, Lee E and Mullbacher A. CD8+ T cells mediate recovery and immunopathology in West Nile virus encephalitis. J Virol 2003;77:13323-34
5. Kelley TW, Prayson RA, Ruiz AI, Isada CM and Gordon SM. The neuropathology of West Nile virus meningoencephalitis. A report of two cases and review of the literature. Am J Clin Pathol 2003;119:749-53
6. Norris PJ, Moffett HF, Yang OO, et al. Beyond help: direct effector functions of human immunodeficiency virus type 1-specific CD4(+) T cells. J Virol 2004;78:8844-51
7. Norris PJ, Sumaroka M, Brander C, et al. Multiple effector functions mediated by human immunodeficiency virus-specific CD4(+) T-cell clones. J Virol 2001;75:9771-9
8. Altfeld MA, Trocha A, Eldridge RL, et al. Identification of dominant optimal HLA-B60- and HLA-B61-restricted cytotoxic T-lymphocyte (CTL) epitopes: rapid characterization of CTL responses by enzyme-linked immunospot assay. J Virol 2000;74:8541-9
9. Lobigs M, Arthur CE, Mullbacher A and Blanden RV. The flavivirus nonstructural protein NS3 is a dominant source of cytotoxic T cell peptide determinants. Virology 1994;202:195-201
10. Zheng B, Han S, Zhu Q, Goldsby R and Kelsoe G. Alternative pathways for the selection of antigen-specific peripheral T cells. Nature 1996;384:263-6
11. Lanteri MC, Heitman JW, Owen RE, et al. Comprehensive analysis of west nile virus-specific T cell responses in humans. J Infect Dis 2008;197:1296-306
12. Tobler LH, Cameron MJ, Lanteri MC, et al. Interferon and interferon-induced chemokine expression is associated with control of acute viremia in West Nile virus-infected blood donors. J Infect Dis 2008;198:979-83

13. Hill JA, Benoist C and Mathis D. Treg cells: guardians for life. Nat Immunol 2007;8:124-5
14. O'Garra A, Vieira P. Regulatory T cells and mechanisms of immune system control. Nat Med 2004;10:801-5
15. Shevach EM. Certified professionals: CD4(+)CD25(+) suppressor T cells. J Exp Med 2001;193:F41-6
16. Suvas S, Azkur AK, Kim BS, Kumaraguru U and Rouse BT. CD4+CD25+ regulatory T cells control the severity of viral immunoinflammatory lesions. J Immunol 2004;172:4123-32
17. Suvas S, Kumaraguru U, Pack CD, Lee S and Rouse BT. CD4+CD25+ T cells regulate virus-specific primary and memory CD8+ T cell responses. J Exp Med 2003;198:889-901
18. Walker LS, Chodos A, Eggena M, Dooms H and Abbas AK. Antigen-dependent proliferation of CD4+ CD25+ regulatory T cells in vivo. J Exp Med 2003;198:249-58
19. Stockinger B, Veldhoen M. Differentiation and function of Th17 T cells. Curr Opin Immunol 2007;19:281-6
20. Stockinger B, Veldhoen M and Martin B. Th17 T cells: linking innate and adaptive immunity. Semin Immunol 2007;19:353-61
21. Yue FY, Merchant A, Kovacs CM, Loutfy M, Persad D and Ostrowski MA. Virus-specific interleukin-17-producing CD4+ T cells are detectable in early human immunodeficiency virus type 1 infection. J Virol 2008;82:6767-71

PART 11: ATTACHMENTS

Please list Attachments, Supplements and Appendices	Version number(s) or date(s)
Main Study Consent form, changes highlighted	12/01/09
Main Study Consent form, clean	12/01/09
2005 Recall Study Consent form	12/17/09
Recall study recruitment letter	12/17/09
Tissue Banking Supplement	12/17/09

1.0 General Information

1.1 *Enter the full title of your study:

Natural History and Pathogenesis of West Nile Virus in Viremic Donors

1.2 *Enter the study number or study alias

WNV

2.0 Add Department(s)

2.1 List of Departments associated with this study:

Primary Dept?	Department Name
◉	**UCSF** - *007940* - LABORATORY MEDICINE

3.0 Assign key study personnel(KSP) access to the study

3.1 *Please add a Principal Investigator for the study:

Michael P Busch

Select if applicable
☐ Fellow
If the Principal Investigator is a Fellow, the name of the Faculty Advisor must be supplied below.

3.2 If applicable, please select the Protocol Staff personnel:

A) Additional Investigators

Norris, Philip J - Other Investigator
Lanteri, Marion C - Other Investigator
Tobler, Leslie H - Other Investigator
Lee, Tzong-Hae - Other Investigator
Custer, Brian - Other Investigator

B) Research Support Staff

Hindes, Daniel A - Research Assistant

3.3 * Please add a Study Contact:

Busch, Michael P
Quintos, Michelle M

The Study Contact(s) will receive all important system notifications along with the Principal Investigator. (e.g. The study contact(s) are typically either the Study Coordinator or the Principal Investigator themselves).

3.4 If applicable, please add a Faculty Advisor:

3.5 If applicable, please select the Designated Department Approval(s):

Add the name of the individual authorized to approve and sign off on this protocol from your Department (e.g. the Department Chair or Dean).

4.0 Qualifications of Key Study Personnel

4.1 List the study responsibilities and qualifications of any individuals who qualify as Key Study Personnel (KSP) by clicking the "Add a new row" button:

KSP Name	Description of Study Responsibilities	Qualifications
Busch, Michael P	Principal Investigator	Adjunct professor of laboratory medicine with extensive experience in the study of viral infections through the acute phase, with an emphasis on the understanding of viral-immune interactions.
Norris, Philip J	Co-investigator	Experienced T-cell immunologist with a background in CD4+ T cell immunology assays.
Lanteri, Marion C	Co-investigator	Staff scientist experienced in virology with skills related to T cell and immunology assays.
Tobler, Leslie H	Co-investigator	Senior scientist and manager of the BSRI Viral Reference Laboratory and Repository responsible for all sample acquisition, processing and storage.
Lee, Tzong-Hae	Co-investigator	Senior scientist, Molecular Transfusion Laboratory, responsible for some laboratory testing and analyses.
Custer, Brian	Co-investigator	Associate Investigator in epidemiology and health policy research.

5.0 Initial Screening Questions

5.1 * This study involves human stem cells, gametes or embryos:

- (•) No
- ( ) Yes, and requires CHR and GESCR review
- ( ) Yes, and requires GESCR review, but NOT CHR review

5.2 * This application involves a Humanitarian Use Device:

- (•) No
- ( ) Yes, and it includes a research component
- ( ) Yes, and it involves clinical care ONLY

5.3 * This is a CIRB study (e.g. the NCI CIRB will be the IRB of record):

( ) Yes (•) No

5.4 * This application includes a request to rely on another UC IRB to be the IRB of record:

( ) Yes (•) No

Note: If this request is approved, the CHR will **NOT** review and approve this study. Another UC campus will be the IRB of record.

6.0 Application Type

6.1 * This research involves:

- (•) Minimal risk
- ( ) Greater than minimal risk

6.2 * This application is:

- (•) Full Committee
- ( ) Expedited
- ( ) Exempt

6.3 If you think this study qualifies for expedited review, select the regulatory category(ies) that the research falls under:

- [ ] Category 1: A very limited number of studies of approved drugs and devices
- [ ] Category 2: Blood sampling
- [ ] Category 3: Noninvasive specimen collection
- [ ] Category 4: Noninvasive clinical procedures
- [ ] Category 5: Research involving materials that were previously collected for either nonresearch or research purposes
- [ ] Category 6: Use of recordings
- [ ] Category 7: Low risk behavioral research
- [ ] Category 8: Renewal of inactive research protocols or protocols that are essentially complete
- [ ] Category 9: Renewal of other minimal risk research protocols

6.4 * This study involves:

- (•) Subject contact (including phone, email or web contact)
- ( ) No subject contact (limited to medical records review, biological specimen analysis, and/or data analysis)

7.0 Funding

7.1 Identify all the funding sources and their roles on the project:

Sponsor List

Sponsor Name:	NIH Natl Heart, Lung & Blood Institute
Sponsor Type:	01
Has the role of Funding?	Yes
Has the role of Protocol Control?	No
Has the role of Data Coordination?	No
Has the role of Monitoring?	No
Has the role of Auditing?	No
Has the role of Passthrough?	No
Awardee Institution:	Blood Systems Research Institute
Is Primary Grant Holder?	Yes
Contract Type:	Grant
UCSF RAS System Proposal Number ("P" + 7 digits):	
UCSF RAS System Award Number ("A" + 6 digits):	
Grant Title:	Viral/immune parameters of Dengue and WNV in donors; blood safety implications
PI Name:	
Significant Discrepancy:	

7.2 If you tried to add the sponsor in the question above and it was not in the list, check here:

( ) Sponsor not in list

Only if your sponsor is not yet in the list, type the sponsor's name:

If the sponsor is not in the system, download the C&G Add Sponsor Form from the Operating Procedures section under the My Assistant tab and attach it to this application. **Your study will not receive CHR approval until the sponsor and funding details have been added to your application.**

7.3 For Federally funded studies only, indicate which portion of your grant you will be attaching:

- [x] The Research Plan, including the Human Subjects Section of your NIH grant
- [ ] For other federal proposals (contracts or grants), the section of the proposal describing human subjects work
- [ ] The section of your progress report if it provides the most current information about your human subjects work

7.4 If this study has no sponsor, check all that apply:

- [ ] Unfunded student project
- [ ] Unfunded (miscellaneous departmental funding)
- [ ] Specific departmental funding

8.0 Statement of Financial Interest

8.1 * The Principal Investigator and/or one or more of the key study personnel has financial interests related to this study:

○ Yes ◉ No

If **Yes**, attach the **Disclosure of Investigators' Financial Interests Supplement** to this application.

9.0 Sites

9.1 Institutions (check all that apply):

- [ ] UCSF
- [ ] Mt. Zion
- [ ] San Francisco General Hospital (SFGH)
- [ ] SF VA Medical Center (SF VAMC)
- [ ] Helen Diller Family Comprehensive Cancer Center
- [ ] Fresno (Community Medical Center)
- [ ] Blood Centers of the Pacific (BCP)
- [x] Blood Systems Research Institute (BSRI)
- [ ] Gallo
- [ ] Gladstone
- [ ] Institute on Aging (IOA)
- [ ] SF Dept of Public Health (DPH)

9.2 Check all the other types of sites not affiliated with UCSF with which you are cooperating or collaborating on this project:

- [ ] Foreign Country

List:

- [ ] Other UC Campus
- [ ] Other institution
- [ ] Other community-based site

9.3 * This is a multicenter study:

◉ Yes ○ No

9.4 Check any research programs this study is associated with:

- [ ] Cancer Center
- [ ] Center for AIDS Prevention Sciences (CAPS)
- [ ] Global Health Sciences
- [ ] Immune Tolerance Network (ITN)
- [ ] Osher Center
- [ ] Positive Health Program

10.0 Studies Involving Other Sites

10.1 UCSF is the coordinating center:

○ Yes ◉ No

If **Yes**, describe the plan for communicating safety updates, interim results, and other information that may impact risks to the subject or others among sites:

If **Yes**, describe the plan for sharing modification(s) to the protocol or consent document(s) among sites:

10.2 Check any other UC campuses with which you are collaborating on this research study:

- [ ] UC Berkeley
- [ ] UC Davis
- [ ] Lawrence Berkeley National Laboratory (LBNL)
- [ ] UC Irvine
- [ ] UC Los Angeles
- [ ] UC Merced
- [ ] UC Riverside
- [ ] UC San Diego
- [ ] UC Santa Barbara
- [ ] UC Santa Cruz

10.3 Are the above UC campuses requesting to rely on UCSF's IRB (check all that apply)?

- [ ] Yes (Attach the Notice of Intent to Rely on One UC IRB form in the Other Study Documents section)
- [ ] No (Complete IRB Approval Certification section)

11.0 Study Design

11.1 Study design:

The study is designed as an observational study. This is a longitudinal study of cellular and humoral immune function, which will be related to viral load and disease manifestations. A portion of the study will be dedicated to determining the persistence of WNV-specific immune responses over time, so there will be no specified endpoint for the study.

Samples from WNV+ blood donors enrolled in 2009 and 2010 will be used to build a repository of samples characterized for virologic and immunologic parameters to be transferred to the NHLBI.

Subjects will be identified at the time of blood donation by the presence of a WNV RNA positive specimen. Age-matched WNV RNA negative and WNV seronegative control subjects will be derived from the donor population who initially test false-positive for WNV RNA at the time of donation. Samples will be obtained from study subjects at enrollment, then at week one, two, three, and six post-enrollment, and at month two, three, six, nine, and twelve post-enrollment. After that period a subset of donors will be sampled every three months for one year for monitoring of persistence of immune responses. Severe cases of WNV infection with neurological manifestations will be identified by clinicians at UCSF and the California Department of Public Health. In addition to blood samples, hospitalized patients with neurological symptoms will have CSF (5 ml) sampled at the time of diagnostic lumbar puncture.

Study investigators may also provide a portion of the serial samples collected under this protocol to other scientists engaged in WNV research and conversely, may receive specimens from other researchers in an effort to generate additional data to confirm study findings. Samples received will be pre-existing and will not contain any individually identifying information. Additionally, no specimens distributed by BSRI investigators will contain individually identifying information, nor will any key to coded information be shared among investigators. These collaborators include Dr. Bill Kwok at Benaroya Institute in Seattle and Dr. Jonathan Bramson at McMaster University in Hamilton, Ontario. BSRI investigators will also confirm and maintain the appropriate IRB approvals from other institutions before engaging in these activities.

An additional follow-up study of WNV+ study participants from 2005 will also be conducted. These subjects will be recalled for a one-time blood draw of 75 ml for WNV IgG and PRNT testing. We will then compare these results to their IgG and PRNT levels from the samples obtained during their earlier participation in the study.

11.2 Check all that apply:

- ☐ Phase I
- ☐ Phase II
- ☐ Phase III
- ☐ Phase IV

12.0 Scientific Considerations

12.1 Hypothesis:

This study has a hypothesis:
○ Yes ◉ No
If yes, state the hypothesis or hypotheses:

12.2 List the specific aims:

WNV infection results in variable penetrance of disease manifestations, ranging from asymptomatic infection to severe meningo-encephalitis and death. The immune correlates of protection from disease have not been described, particularly in humans. Previously, human T cell responses were identified for a subset of 8 peptides from the membrane, envelope, nonstructural 3 and 4b proteins of WNV. Further phenotypic studies characterized the WNV-specific T cells as cytotoxic CD8 T cells secreting granzyme A and perforin. The set of peptides identified might be used for T cell stimulation studies and might also be of interest for vaccination studies, relevant to WNV infection as well as Flaviviruses in general. Additionally, the role that the immune system might play in the pathogenesis of WNV infection is not understood but a strong correlation between lower levels of regulatory T cells (T_{reg}) and symptomatic outcome was found comparing PBMCs from asymptomatic versus symptomatic WNV+ donors.
Our specific aims are as follows:
1. To determine if T_{reg} cells expanded in acute WNV infection are WNV-specific and whether they more efficiently suppress WNV-specific immune responses in asymptomatic than in symptomatic individuals.
2. To observe the post-infection dynamics of Th17 cells and to study how the balance between pro-inflammatory Th17 cells and anti-inflammatory T_{reg} cells relates to T cell activation and disease outcomes.
3. To build a repository of samples collected from WNV+ blood donors, at different time-points after positive index donation and characterized for virologic and immunologic parameters. Both the repository and corresponding database will be transferred to the NHLBI Biospecimen Repository.

12.3 Statistical analysis:

Data analysis will be performed by the PI in collaboration with statisticians and epidemiologists on site at BSRI. This is a longitudinal study of viral RNA and immune markers following initial detection of a donor as viremic by nucleic acid testing (NAT). To estimate how rapidly, on average, viral load increased during the ramp-up phase, we first need to select for each panel the RNA+/IgM- bleeds collected when viral load was increasing exponentially. Assuming that the first RNA quantifiable bleed ("time 0" bleed) probably occurred in ramp-up and that viral load increased at a constant rate (i.e., in a linear fashion) on a log scale during ramp-up, the rate of increase in log viral load can be calculated. Information on log viral load and collection days for the selected ramp-up bleeds will be entered in a repeated measures regression model where each panel subject had their own intercept and slope (PROC MIXED, SAS Institute Inc.). The average viral load doubling time during ramp-up will then be calculated as log(2)/average slope. Longitudinal regression analysis will be used to estimate time periods from selected viral RNA thresholds to IgM and IgG seroconversion, as well as the duration of time following IgM or IgG seroconversion that low-level viremia is detectable by replicate TMA. This analysis will use PROC LIFEREG (SAS Institute Inc.), and assume a normal distribution for these window periods.

For the correlation of immune responses and disease outcomes, we expect that for some of the immune measurements (e.g. T cell responses), a sizeable number of participants will have values either below the limit of detection or clustered just above the limit of detection with a rightwards skewed distribution. For these measurements, we will use non-parametric statistical tests (e.g., Wilcoxon rank-sum test) to compare distributions between groups. For measurements for which all participants have observable values and for which distributions are approximately normal, we will use parametric tests (e.g., two-sample t-test).

12.4 This is an investigator-initiated study:

◉ Yes ○ No

12.5 This study has received scientific or scholarly review from (check all that apply):

- ☐ Cancer Center Protocol Review Committee (PRC) (Full approval or contingent PRC approval is required prior to final CHR approval for cancer-related protocols.)
- ☐ CTSI Clinical Research Center (CRC) advisory committee
- ☐ Departmental scientific review
- ☑ Other:

Specify **Other**:

National Heart, Lung and Blood Institute

If applicable, attach the **Departmental Scientific Review Form** at the end of the application.

13.0 Background

13.1 Background:

WNV was introduced to the Northeastern United States in late summer of 1999. The virus was determined to be almost identical genetically to strains prevalent in Israel[1]. Since its introduction, WNV has spread relentlessly westward, with large outbreaks in the Midwest and Colorado in 2004 and in Arizona and Southern California in 2005. The transmission period mirrors that of mosquito activity, peaking from May through August. It is expected that WNV will continue its westward expansion in the coming transmission season this spring and summer. During an outbreak of WNV in non-immune populations, approximately 5% of blood donations are positive for WNV IgM. Blood transfusion of WNV contaminated units has resulted in transfusion-associated transmission of the virus, with severe disease and death sometimes resulting. Pooled blood donations are now routinely screened for WNV RNA, though the sensitivity of the screening process is not likely adequate to prevent 100% protection from transfusion associated WNV transmission. Given that WNV will represent an ongoing health problem and threat to the blood supply, greater understanding of the pathogenesis of the virus is required.

Both humoral and cellular immune responses have been implicated in the control of WNV infection. The bulk of the pathogenesis data relating to WNV comes from murine models [2]. Mice deficient in secreted IgM have been shown to be more susceptible to lethal challenge with WNV, and passive transfer of polyclonal IgM can protect against lethal infection with WNV[3]. Additionally, low WNV IgM titer in infected wild type mice is correlated with higher mortality. T cells also likely play a role in control of the virus. CD8+ T cell deficient mice infected with low-dose WNV show increased mortality compared to wild-type controls[4]. Unpublished data suggest that T cell deficient mice initially control WNV replication, but ultimately fail to eradicate the virus, leading to recrudescence of viremia. The role of T cell responses appears not only to be protective, as CD8+ T cells have been isolated in the inflammatory regions of meningoencephalitits in mice and humans [5]. We will assess the role of T cells in neurological manifestations of disease by studying the properties of CSF lymphocytes in hospitalized subjects who undergo diagnostic lumbar puncture.

Our laboratory has traditionally focused on the role of HIV-specific T cell responses in the control of virus replication [6, 7]. In HIV and flaviviruses analogous to WNV, it has been demonstrated that some regions of the viral genome are more susceptible to recognition by T cells than others [8-10]. In collaboration with the Biodefense and Emerging Infections Research Resources Repository we acquired overlapping peptide sets spanning the WNV genome synthesized and coupled with our unique access to patient samples through a large blood donation network, we were able to do comprehensive analysis of WNV-specific T cell responses [11]. We showed several epitopes inducing CD8 T cell responses in humans. We were able to demonstrate that control of acute viremia in WNV-infected blood donors is associated with interferon and interferon-induced chemokine expression [12].

We will be able to monitor the effect T cell responses and especially regulatory T cells [13-18] and Th17 cells [19-21] have on the dynamics of viral infection, the correlation with disease manifestations, and the durability of T cell immune responses to the virus.

The investigators have unparalleled access to viremic blood donors. Over the last four years, they have capitalized on blood bank resources to capture viremic individuals identified during acute WNV infection and established the repository of plasma and PBMC samples they have been using to address immunological studies related to WNV infection and pathogenesis. During the two next years, the investigators will be collecting samples from WNV+ blood donors enrolled in the bleeding protocol described in Part 3 to build a new repository of samples from WNV+ blood donors. The complete pedigree of clinical data and all laboratory data around the virologic and immunologic characterization of the samples will be entered into the WNV repository database that will be transferred to NHLBI.

13.2 Preliminary studies:

See Background.

13.3 References:

1. Lanciotti RS, Roehrig JT, Deubel V, et al. Origin of the West Nile virus responsible for an outbreak of encephalitis in the northeastern United States. Science 1999;286:2333-7
2. Samuel MA, Diamond MS. Pathogenesis of West Nile Virus infection: a balance between virulence, innate and adaptive immunity, and viral evasion. J Virol 2006;80:9349-60
3. Diamond MS, Sitati EM, Friend LD, Higgs S, Shrestha B and Engle M. A critical role for induced IgM in the protection against West Nile virus infection. J Exp Med 2003;198:1853-62
4. Wang Y, Lobigs M, Lee E and Mullbacher A. CD8+ T cells mediate recovery and immunopathology in West Nile virus encephalitis. J Virol 2003;77:13323-34
5. Kelley TW, Prayson RA, Ruiz AI, Isada CM and Gordon SM. The neuropathology of West Nile virus meningoencephalitis. A report of two cases and review of the literature. Am J Clin Pathol 2003;119:749-53
6. Norris PJ, Moffett HF, Yang OO, et al. Beyond help: direct effector functions of human immunodeficiency virus type 1-specific CD4(+) T cells. J Virol 2004;78:8844-51
7. Norris PJ, Sumaroka M, Brander C, et al. Multiple effector functions mediated by human immunodeficiency virus-specific CD4(+) T-cell clones. J Virol 2001;75:9771-9
8. Altfeld MA, Trocha A, Eldridge RL, et al. Identification of dominant optimal HLA-B60- and HLA-B61-restricted cytotoxic T-lymphocyte (CTL) epitopes: rapid characterization of CTL responses by enzyme-linked immunospot assay. J Virol 2000;74:8541-9
9. Lobigs M, Arthur CE, Mullbacher A and Blanden RV. The flavivirus nonstructural protein NS3 is a dominant source of cytotoxic T cell peptide determinants. Virology 1994;202:195-201
10. Zheng B, Han S, Zhu Q, Goldsby R and Kelsoe G. Alternative pathways for the selection of antigen-specific peripheral T cells. Nature 1996;384:263-6
11. Lanteri MC, Heitman JW, Owen RE, et al. Comprehensive analysis of west nile virus-specific T cell responses in humans. J Infect Dis 2008;197:1296-306
12. Tobler LH, Cameron MJ, Lanteri MC, et al. Interferon and interferon-induced chemokine expression is associated with control of acute viremia in West Nile virus-infected blood donors. J Infect Dis 2008;198:979-83
13. Hill JA, Benoist C and Mathis D. Treg cells: guardians for life. Nat Immunol 2007;8:124-5
14. O'Garra A, Vieira P. Regulatory T cells and mechanisms of immune system control. Nat Med 2004;10:801-5
15. Shevach EM. Certified professionals: CD4(+)CD25(+) suppressor T cells. J Exp Med 2001;193:F41-6
16. Suvas S, Azkur AK, Kim BS, Kumaraguru U and Rouse BT. CD4+CD25+ regulatory T cells control the severity of viral immunoinflammatory lesions. J Immunol 2004;172:4123-32
17. Suvas S, Kumaraguru U, Pack CD, Lee S and Rouse BT. CD4+CD25+ T cells regulate virus-specific primary and memory CD8+ T cell responses. J Exp Med 2003;198:889-901
18. Walker LS, Chodos A, Eggena M, Dooms H and Abbas AK. Antigen-dependent proliferation of CD4+ CD25+ regulatory T cells in vivo. J Exp Med 2003;198:249-58
19. Stockinger B, Veldhoen M. Differentiation and function of Th17 T cells. Curr Opin Immunol 2007;19:281-6
20. Stockinger B, Veldhoen M and Martin B. Th17 T cells: linking innate and adaptive immunity. Semin Immunol 2007;19:353-61
21. Yue FY, Merchant A, Kovacs CM, Loutfy M, Persad D and Ostrowski MA. Virus-specific interleukin-17-producing CD4+ T cells are detectable in early human immunodeficiency virus type 1 infection. J Virol 2008;82:6767-71

If you have a separate bibliography, attach it to the submission with your other study documents.

14.0 Sample Size and Eligibility

14.1 Number of subjects that will be enrolled at UCSF and affiliated institutions:

0

14.2 Total number of subjects that will be enrolled at all sites:

300

14.3 Estimated number of people that you will need to consent and screen here (but not necessarily enroll) to get the needed subjects:

0

14.4 Sample size calculation:

Data analysis will be performed by the PI in collaboration with statisticians and epidemiologists on site at BSRI. This is a longitudinal study of viral RNA and immune markers following initial detection of a donor as viremic by nucleic acid testing (NAT). To estimate how rapidly, on average, viral load increased during the ramp-up phase, we first need to select for each panel the RNA+/IgM- bleeds collected when viral load was increasing exponentially. Assuming that the first RNA quantifiable bleed ("time 0" bleed) probably occurred in ramp-up and that viral load increased at a constant rate (i.e., in a linear fashion) on a log scale during ramp-up, the rate of increase in log viral load can be calculated. Information on log viral load and collection days for the selected ramp-up bleeds will be entered in a repeated measures regression model where each panel subject had their own intercept and slope (PROC MIXED, SAS Institute Inc.). The average viral load doubling time during ramp-up will then be calculated as log(2)/average slope. Longitudinal regression analysis will be used to estimate time periods from selected viral RNA thresholds to IgM and IgG seroconversion, as well as the duration of time following IgM or IgG seroconversion that low-level viremia is detectable by replicate TMA. This analysis will use PROC LIFEREG (SAS Institute Inc.), and assume a normal distribution for these window periods.

For the correlation of immune responses and disease outcomes, we expect that for some of the immune measurements (e.g. T cell responses), a sizeable number of participants will have values either below the limit of detection or clustered just above the limit of detection with a rightwards skewed distribution. For these measurements, we will use non-parametric statistical tests (e.g., Wilcoxon rank-sum test) to compare distributions between groups. For measurements for which all participants have observable values and for which distributions are approximately normal, we will use parametric tests (e.g., two-sample t-test).

14.5 * Eligible age range(s):

- [ ] 0-6 years
- [ ] 7-12 years
- [ ] 13-17 years
- [x] 18+ years

14.6 Inclusion criteria:

Subjects will be identified from the pool of WNV+ blood donors. WNV RNA and WNV seronegative individuals will also be included as control subjects. subjects will range in age from 18 to 100 years.

14.7 Exclusion criteria:

Pregnancy, age less than 18, prisoners.

14.8 There are inclusion or exclusion criteria based on gender, race or ethnicity:

○ Yes ◉ No

If **yes**, please explain the nature and rationale for the restrictions:

15.0 Drugs and Devices

15.1 * Drugs or biologics will be studied under this application:

○ Yes ◉ No

15.2 * Medical devices will be studied under this application:

○ Yes ◉ No

15.3 Verification of IND/IDE numbers: If the sponsor's protocol does not list the IND/IDE number, you must submit documentation from the sponsor or FDA identifying the IND/IDE number for this study. Attach this documentation in the Other Study Documents section of the Initial Review Submission Packet.

16.0 Other Approvals and Registrations

16.1 This is a clinical trial:

○ Yes ◉ No

Clinical Trial Registration
"NCT" number for this trial:

16.2 * This study involves human gene transfer or recombinant DNA research:

○ Yes ◉ No

16.3 This study involves other regulated materials and requires approval and/or authorization from the following regulatory committees:

- [ ] Institutional Biological Safety Committee (IBC)

Specify BUA #:

- [ ] Institutional Animal Care and Use Committee (IACUC)

Specify IACUC #:

- [ ] Radiation Safety Committee

Specify RUA #:

- [ ] Radioactive Drug Research Committee (RDRC)

Specify RDRC #:

- [ ] Controlled Substances

17.0 Procedures

17.1 List all study procedures, test and treatments required for this study:

The only procedure to be performed on the outpatient study subjects is phlebotomy and research lab testing. Hospitalized patients will also have CSF sampling performed at the time of diagnostic lumbar puncture (an additional 5 ml will be obtained for research purposes). None of the results will be used for clinical decision making and they will not be relayed to the patient or the patient's physician. The WNV infected subjects would be advised of potential disease manifestations and advised to follow up with their regular physicians in case of symptoms.

The maximum amount of blood obtained from subjects is 375 ml over an 8 week period, and 750 ml over the total one-year period. If a person is hospitalized, we will follow the guidelines for unhealthy individuals and the amount of blood drawn may not exceed the less of 50 ml or 3 ml per kg in an 8 week period. Each phlebotomy will collect 75 ml blood, except in hospitalized patients where 25 ml blood will be collected per phlebotomy. In addition to the phlebotomy schedule described for WNV+ subjects, blood donors' initial donation blood products will be retrieved for study if operationally feasible. This will allow study of the earliest viremic time point.

In addition to phlebotomy, two questionnaires will be administered by telephone. Questionnaire A will be administered within a week of infection being identified. The second will be administered approximately three weeks after infection was identified. Both questionnaires focus on symptoms associated with WNV infection (see questionnaires in Appendix).

Category	Specific Activities
Visit schedule non-hospitalized	1) Index donation (day 0) 2) Enrollment visit (days 2-4) 3) Phlebotomies at weeks one, two, three, six post-enrollment (days 9-46) 4) Phlebotomy at two, three, six, nine, and twelve months post-enrollment.
Visit schedule hospitalized	1) Index donation (25 ml, day 0) 2) Index CSF sample (5ml, day 0 or when first performed) 3) Phlebotomy at week one, two, three, six if still hospitalized (25 ml) 4) Phlebotomy at two and three months post- index donation after hospital discharge (50 ml) 5) Phlebotomy every three months for up to one year after enrollment
Visit procedures	1) Donor consent at enrollment visit 2) Donor risk/symptom questionnaire at enrollment and follow up questionnaire at one month 3) Donor phlebotomy at each visit (draw 7x10mL EDTA plus 1x2.5-4mL EDTA plus one 2.5 mL PAXgene tube) 4) Anticoagulated whole blood will be shipped using Federal Express to Blood Systems Research Institute.
Tests that may be performed	Generation of immortalized B and T cell lines

To provide appropriate experimental controls, the ability of study subjects' cells to combat other viruses such as Epstein Barr virus, hepatitis C virus, HIV, influenza virus, cytomegalovirus, and human herpes virus 8 (HHV-8) may also be tested.

For the 2005 recall study, WNV+ donors enrolled in our study in 2005 will be contacted via a letter by our Medical Affairs office and offered the opportunity to participate. Potential participants will phone a donor counselor, as detailed in the letter, given all study information and instructions to have their blood drawn. They will sign their consent forms when they visit their blood centers for the blood draws. Samples will be shipped to BSRI for WNV ELISA and PRNT testing.

If you have a procedure table, attach it to the submission with your other study documents.

17.2 Interviews, questionnaires, and/or surveys will be administered or focus groups will be conducted:

(•) Yes ( ) No

List any standard instruments used for this study:

Attach any non-standard instruments at the end of the application.

17.3 Conduct of study procedures or tests off-site by non-UCSF personnel:

(•) Yes ( ) No

If yes, explain:

Consent will be obtained by a study coordinator employed by Blood Systems Inc., and research will be performed at the Blood Systems Research Institute. Initial donor identification will be performed at Blood Systems Laboratories in Scottsdale, AZ. Additional testing will be performed at GenProbe in San Diego, CA Chiron Corporation in Emeryville, CA, Focus Diagnostics in Cypress, CA, and the CDC in Fort-Collins, CO.

17.4 Sharing of experimental research test results with subjects or their care providers:

( ) Yes (•) No

If yes, explain:

17.5 * Specimen collection for future research and/or specimen repository/bank administration:

(•) Yes ( ) No

17.6 Time commitment (per visit and in total):

Blood will be drawn at each study visit. It is not anticipated that more than half an hour per visit will be required, for a total of 5 hours over a one year period. for the recall study, participants will have their blood drawn at their local BSI blood center. The single blood draw visit should bot take more than one half hour.

17.7 Locations:

Subjects will be recruited from within the network of blood banks in Blood Systems, Inc., concentrated in the West and Midwest of the United States. Candidate sites will be located in regions of the country experiencing outbreaks of West Nile virus, as documented through routine screening of blood donors. Infectious disease physicians at UCSF will also refer patients for contact about enrollment in the study only if the patients are interested. The patients will be identified and initially contacted by their treating physician; the treating physician will document patient consent to be contacted for the study in the patient chart. In order to boost enrollment of symptomatic subject with WNV infection, patients will also be recruited by Dr. Carol Glaser at the California Department of Public Health and those subjects will undergo consent through the state IRB.

Consent will be obtained by a study coordinator employed by Blood Systems Inc., and research will be performed at the Blood Systems Research Institute. Initial donor identification will be performed at Blood Systems Laboratories in Scottsdale, AZ. Additional testing will be performed at GenProbe in San Diego, CA Chiron Corporation in Emeryville, CA, Focus Diagnostics in Cypress, CA, and the CDC in Fort-Collins, CO.

17.8 Describe the resources in place to conduct this study in a way that assures protection of the rights and welfare of participants:

Blood will be drawn by trained phlebotomists at BSI blood centers conveniently located throughout the US. Subject privacy will be maintained by stringent data security policies at the blood centers, BSRI and NHLBI's BioLLinc repository.

18.0 Specimen Collection for Future Research and/or Specimen Repository/Bank Administration

(Note: This section replaces the old "Human Biologic Specimen Collecting and/or Banking for Future Research" supplement form. Please do not attach the old form to this application.)

18.1 Specimens are (check all that apply):

- ☐ Surplus clinical specimens from a diagnostic or therapeutic procedure
- ☑ Specimens collected for research purposes only
- ☐ Other

If Other, explain:

18.2 Types of specimens:

- ☑ Blood
- ☐ Tissue (describe below):
- ☐ Existing/archival materials (name source below):
- ☐ Other (describe below):

Describe and/or name source:

18.3 Consent will be obtained via:

- ☐ Separate specimen banking consent form
- ☑ Specimen banking section within a main research study consent form
- ☐ Surgical consent form with tissue donation brochure

18.4 Specimens will ultimately be stored (check all that apply):

UCSF

- ☐ UCSF repository/bank being established under this protocol
- ☐ Existing UCSF specimen repository/bank with CHR approval

Provide the name of the bank and CHR approval number (if not being banked at UCSF under this protocol):

Outside Entity

- ☐ Cooperative group bank
- ☑ NIH
- ☐ Other university
- ☐ Industry sponsor
- ☑ Other

Specify to what institution, cooperative group or company specimens will be transferred:

Blood Systems Research Institute, 270 Masonic Avenue, San Francisco, CA 94118 in the BSRI Freezer Farm on the basement level.

The National Heart, Lung, and Blood Institute Biologic Specimen Repository, Bethesda, MD.

18.5 Direct identifiers will be sent with specimens or shared with other researchers and/or outside entities:

- ○ Yes
- ◉ No
- ○ N/A - Specimens will not be shared with others

If **Yes**, which identifiers will be sent with specimens:

- ☐ Name
- ☐ Date of birth
- ☐ Social Security number
- ☐ Medical record number
- ☐ Address
- ☐ Phone number
- ☐ Email address
- ☐ Other dates (surgery date, clinic visit dates, etc.)

If **Yes**, provide a justification for sending direct identifiers with the specimens:

19.0 Alternatives

19.1 Study drug or treatment is available off-study:

- ○ Yes
- ○ No
- ◉ Not applicable

19.2 Describe the usual care or activities at UCSF (or study site) that are available to prospective subjects who do not enroll in this study:

This study is completely voluntary and does not involve any treatments.

19.3 Describe other alternatives to study participation that are available to prospective subjects:

Participation in the study is completely voluntary. The alternative to participation is not to participate. As the study is observational and does not provide therapy, there would be no need to take any alternative action if a subject opts not to participate in the study.

20.0 Risks and Benefits

20.1 Risks and discomforts:

Sampling blood may cause a bruise and/or bleeding at the needle site. Occasionally, a person feels faint when their blood is drawn. Rarely, an infection may develop at the needle site. As subjects will have had a full unit (500 mL) phlebotomy immediately prior to enrollment and up to seven 75 ml phlebotomies during the initial three months of the study, they may be deferred from donating blood while participating in the study.

Lumbar puncture carries the rare risk of introduction of infection to the central nervous system (CNS), post-lumbar puncture headache, spinal trauma, and in very rare cases, brainstem herniation and death.

20.2 Steps taken to minimize risks to subjects:

If a person demonstrates any sign of illness, such as fever, malaise, or recent weight loss, we will follow the guidelines for unhealthy individuals and the amount of blood drawn may not exceed the lesser of 50 ml or 3 ml per kg in an 8 week period.

To minimize the risks to hospitalized study subjects, CSF will be obtained at the time of a clinically warranted lumbar puncture, eliminating the need for additional procedures to obtain study CSF samples and making the incremental risk of obtaining the study sample minimal.

20.3 Benefits to subjects:

○ Yes ◉ No

If yes, describe:

20.4 Benefits to society:

The proposed studies will further our knowledge of how the immune system interacts with WNV.
These studies will not only lend an understanding of WNV pathogenesis, but also hold the potential to assist in vaccine development and testing. The repository of WNV samples and linked database transferred to NHLBI for further availability to the scientific community will provide tools to scientists in the field to address WNV infection and pathogenesis.

20.5 Explain why the risks to subjects are reasonable:

The risks of the study to the subjects are minimal. While there is no direct benefit to the study subjects, the potentially large benefit to society balances the very small risks posed to the study subjects.

21.0 Data and Safety Monitoring Plan

21.1 Describe the plan for monitoring data and safety:

Lab tests: Cellular immune function assays will be monitored on a quarterly basis in the laboratory using standardized reagents to monitor for quality control.

Independent monitoring: These are basic immunological studies, which give both qualitative and quantitative readouts. No need for independent monitoring of the data is anticipated.

21.2 This study requires a Data and Safety Monitoring Board:

○ Yes
◉ No or not sure

If **yes**, press **SAVE and CONTINUE** to move to the next section of the application.

21.3 If No, provide rationale:

○ Social/Behavioral research
○ Phase I trial
○ Treatment IND/Compassionate Use Trial
◉ Other (explain below)

If **Other,** explain:

Study activities covered by this application and approval do not pose greater than minimal risk to subjects.

22.0 Confidentiality and Privacy

22.1 Study data are:

☐ Derived from the Integrated Data Repository (IDR)
☐ Derived from a medical record (identify source below)
☐ Added to the hospital or clinical medical record
☐ Created or collected as part of health care
☐ Used to make health care decisions
☑ Obtained from the subject, including interviews, questionnaires
☐ Obtained from a foreign country or countries only
☐ Obtained from records open to the public
☑ Obtained from existing research records
☐ None of the above

If **derived from a medical record**, identify source:

22.2 Plans for accessing subject information while maintaining privacy:

Specimens and data are coded and the data key is kept separately and securely. Identifiers will not be shared outside of research team.

22.3 Identifiers may be included in research records:

◉ Yes ○ No

If **yes**, check all the identifiers that may be included:

☐ Names
☐ Dates
☐ Postal addresses
☐ Phone numbers
☐ Fax numbers
☐ Email addresses
☐ Social Security Numbers*
☐ Medical record numbers
☐ Health plan numbers
☐ Account numbers
☐ License or certificate numbers
☐ Vehicle ID numbers

- [ ] Device identifiers or serial numbers
- [ ] Web URLs
- [ ] IP address numbers
- [ ] Biometric identifiers
- [ ] Facial photos or other identifiable images
- [x] Any other unique identifier

* Required for studies conducted at the VAMC

22.4 Plans for maintaining privacy in the research setting:

We do not plan to share any personally identifying information outside of the research team.

22.5 Possible consequences to subjects resulting from a loss of privacy:

West Nile virus is a self-limited disease in most individuals without social stigma attached, so loss of privacy would not be anticipated to have a major impact on the study subjects.

22.6 Identifiable information might be disclosed as part of study activities:

○ Yes ◉ No

If **yes**, indicate where identifiable information may be released to:

- [ ] The subject's medical record
- [ ] The study sponsor
- [ ] The US Food & Drug Administration (FDA)
- [ ] Others (Specify below)
- [ ] A Foreign Country or Countries

If **Others**, specify:

22.7 Indicate how data are kept secure (check all that apply):

- [ ] Data are stored securely in My Research
- [ ] Data are coded; data key is destroyed at end of study
- [x] Data are coded; data key is kept separately and securely
- [ ] Data are kept in a locked file cabinet
- [ ] Data are kept in a locked office or suite
- [x] Electronic data are protected with a password
- [x] Data are stored on a secure network
- [ ] Data are collected/stored using REDCap or REDCap Survey

22.8 Additional measures to assure confidentiality:

22.9 This study may collect information that State or Federal law requires to be reported to other officials or ethically requires action:

○ Yes ◉ No

Explain:

22.10 This study will be issued a Certificate of Confidentiality:

○ Yes ◉ No

23.0 Subjects

23.1 Check all types of subjects that may be enrolled:

- [x] Inpatients
- [x] Outpatients
- [x] Healthy volunteers
- [ ] Staff of UCSF or affiliated institutions

23.2 Additional vulnerable populations:

- [ ] Children
- [x] Subjects unable to consent for themselves
- [ ] Subjects unable to consent for themselves (emergency setting)
- [ ] Subjects with diminished capacity to consent
- [ ] Subjects unable to read, speak or understand English
- [ ] Pregnant women
- [ ] Fetuses
- [ ] Neonates
- [ ] Prisoners
- [ ] Economically or educationally disadvantaged persons
- [ ] Investigators' staff
- [ ] Students

Explain why it is appropriate to include the types of subjects checked above in this particular study:

Subjects with severe cases of WNV infection with neurological manifestations may be indentified by clinicians at UCSF and the California Dept. of Public Health. Patients with severe West Nile meningoencephalitis need to be examined to determine the role of the immune system in controlling severe disease.

Describe the additional safeguards that have been included in the study to protect the rights and welfare of these subjects and minimize coercion or undue influence:

Potential study subjects with questionable competence would be assessed by the patient's primary physician. Once a subject has recovered to a mentally competent state, the subject will be approached for consent to the studies. If the subject refuses participation, no further samples will be obtained.

24.0 Recruitment

24.1 * Methods (check all that apply):

- [x] Study investigators (and/or affiliated nurses or staff) recruit their own patients directly in person or by phone.
- [x] Study investigators recruit their own patients by letter. Attach the letter for review.
- [ ] Study investigators send a "Dear Doctor" letter to colleagues asking for referrals of eligible patients. If interested, the patient will contact the PI or the PI may directly recruit the patients (with documented permission from the patient). Investigators may give the referring physicians a study information sheet for the patients.
- [ ] Study investigators provide their colleagues with a "Dear Patient" letter describing the study. This letter can be signed by the treating physicians and would inform the patients how to contact the study investigators. The study investigators may not have access to patient names and addresses for mailing
- [ ] Advertisements, notices, and/or media used to recruit subjects. Interested subjects initiate contact with study investigators. Attach ads, notices, or media text for review. In section below, please explain where ads will be posted.
- [ ] Study investigators identify prospective subjects through chart review. (Study investigators request a Waiver of Authorization for recruitment purposes.)
- [ ] Large-scale epidemiological studies and/or population-based studies: Prospective subjects are identified through a registry or medical records and contacted by someone other than their personal physician. (Study investigators request a Waiver of Authorization for recruitment purposes.)
- [ ] Direct contact of potential subjects who have previously given consent to be contacted for participation in research. Clinic or program develops a CHR-approved recruitment protocol that asks patients if they agree to be contacted for research (a recruitment database) or consent for future contact was documented using the consent form for another CHR-approved study.
- [ ] Study investigators list the study on the School of Medicine list of UCSF Clinical Trials website or a similarly managed site. Interested subjects initiate contact with investigators.
- [ ] Study investigators recruit potential subjects who are unknown to them through methods such as snowball sampling, direct approach, use of social networks, and random digit dialing.
- [ ] Other

If **Other**, explain:

24.2 How, when, and by whom eligibility will be determined:

See below.

24.3 How, when, where and by whom potential subjects will be approached:

Study subjects will be contacted if their blood donation tests positive for WNV RNA. This group will include WNV infected donors and those who test false-positive for WNV RNA (seronegative control subjects). Initial false positive donors will be identified through negative repeat RNA testing and failure to seroconvert WNV-reactive antibody responses. Both groups of subjects will be invited to enroll in the study upon presentation to their local blood donation center for follow up. In addition, infectious disease physicians at UCSF and Dr. Carol Glaser from the California Department of Health Services will also refer patients with neurological symptoms whom they identify as having possible WNV, for contact about enrollment in the study only if the patients are interested. The patients will be identified and initially contacted by their treating physician; the treating physician will document patient consent to be contacted for the study in the patient chart.

For the 2005 recall study, former WNV study participants enrolled in 2005, will be identified by BSI Medical Affairs and sent a letter (attached) asking them to participate in this follow-up study. These subjects may opt-in by responding to the letter and calling a donor counselor for more information.

24.4 * Protected health information (PHI) will be accessed prior to obtaining consent:

○ Yes ◉ No

25.0 Informed Consent

25.1 * Methods (check all that apply):

- [x] Signed consent will be obtained from subjects and/or parents (if subjects are minors)
- [ ] Verbal consent will be obtained from subjects using an information sheet or script
- [ ] Electronic consent will be obtained from subjects via the web or email
- [ ] Implied consent will be obtained via mail, the web or email
- [x] Signed consent will be obtained from surrogates
- [ ] Emergency waiver of consent is being requested for subjects unable to provide consent
- [ ] Informed consent will not be obtained

25.2 Process for obtaining informed consent:

Once a blood donor with West Nile virus or a seronegative control has been identified, informed consent will be obtained over the telephone by a trained study coordinator employed by the blood bank at corporate headquarters in Scottsdale, AZ. The study coordinator would have experience in informing blood donors of positive virological testing. Study subjects will be given as much time as they need to consider participation and will sign a consent form at the time of the first blood draw upon enrollment in the study. Hospitalized subjects will be initially contacted by their treating physician for participation in the trial. If agreeable to being approached, consent will be obtained by Dr. Philip Norris at UCSF, or his designated assistants. If subjects have severe neurological manifestations of West Nile virus infection and cannot provide informed consent (assessed by the patient's primary physician), a surrogate will be approached for informed consent (see supplement).

The 2005 recall subjects will be given all the study information contained in the informed consent form over the telephone by a trained donor counselor when they respond to the recruitment letter. When subjects visit the blood bank for their blood draw, they will sign the consent form. They will be given opportunities to discuss the study and ask questions during their telephone conversation with the donor counselor and during their visit to the blood bank.

25.3 How investigators will make sure subjects understand the information provided to them:

Subjects will demonstrate an understanding of the implications of WNV infection and possible disease manifestations. It will be explicitly stated that there would be no benefit to the study subject from participation in the study, aside from the potential feeling of well-being gained in assisting scientific research.

26.0 Surrogate Consent

(Note: This section partially replaces the old "Surrogate Consent" supplement form. Please do not attach the old form to this application.)

26.1 Subjects are inpatients on a psychiatric ward or mental health facility, or on psychiatric hold:

◉ No

If **yes**, use of surrogate consent for research is NOT allowed in California.

26.2 This study is related to the cognitive impairment, lack of capacity, or serious or life-threatening diseases and conditions of the research subjects:

◉ Yes

If **no**, use of surrogate consent for research is NOT allowed in California.

26.3 Explain why use of surrogates is necessary for completion of this study:

Patients with severe West Nile meningoencephalitis need to be examined to determine the role of the immune system in controlling severe disease, and severely affected subjects would not be expected to be able to give informed consent.

26.4 Plans for assessing the decision-making capacity of prospective subjects:

Potential study subjects with questionable competence would be assessed by the patient's primary physician. Competence would depend on the subject's understanding of the phlebotomy procedure and the reason for the planned studies.

26.5 Plans for obtaining consent from subjects who regain ability to consent after a surrogate has given initial consent:

Once a subject has recovered to a mentally competent state, the subject will be approached for consent to the studies. If the subject refuses participation, no further smaples will be obtained.

26.6 Requirements for any study involving surrogates for obtaining informed consent. Check to acknowledge:

- [x] Research takes place in California. All surrogates will complete the "Self-Certification of Surrogate Decision Makers for Participation in Research" form.
- [x] Conscious subjects will be notified of the decision to contact a surrogate. If subjects object to study participation, they will be excluded even if their surrogate has given consent.
- [x] Surrogates will not receive any financial compensation for providing consent.
- [x] If a higher-ranking surrogate is identified at any time, the investigators will defer to the higher-ranking surrogate's decision regarding the subject's participation in the research.

For research taking place outside of California, explain how investigators will confirm that surrogates are legally authorized representatives:

26.7 VA Studies Only
Provide any additional information to explain comply with the additional VAMC requirements for use of surrogates in research:

27.0 Financial Considerations

27.1 Subjects payment or compensation method (check all that apply):

Payments will be (check all that apply):

- [ ] Subjects will not be paid
- [ ] Cash
- [x] Check
- [ ] Gift card
- [ ] Other:

Specify **Other**:

27.2 Describe the schedule and amounts of payments, including the total subjects can receive for completing the study. If deviating from recommendations in Subject Payment Guidelines, include specific justification below.

Subjects will be paid $40 after each study visit, comprised of $20 for time spent on study activities and $20 for travel expenses. Additionally, after Visit 1, Visit 7 and Visit 10, subjects will be paid a bonus of $30. Check payments will be mailed to study subjects after each visit. The total amount subjects could receive if they complete all 10 study visits would be $490.

2005 recall study subjects will be paid $30.

27.3 Costs to Subjects: Will subjects or their insurance be charged for any study procedures?

○ Yes ◉ No

If **yes,** describe those costs below, and compare subjects' costs to the costs associated with alternative care off-study. Finally, explain why it is appropriate to charge those costs to the subjects.

28.0 CTSI Screening Questions

28.1 * This study will be carried out at one of the UCSF Clinical Research Centers (CRCs) or will utilize CRC services:

○ Yes ◉ No

28.2 This project involves community-based research:

○ Yes ◉ No

28.3 This project involves practice-based research:

○ Yes ◉ No

28.4 Please check other CTSI services below that you plan to utilize to conduct your research:

Guidance and Services:

- [ ] Biostatistics
- [ ] Study Design and Implementation
- [ ] Data Management
- [ ] Ethics
- [ ] Health Policy
- [ ] Bioinformatics Data Analysis
- [ ] Regulatory Knowledge
- [ ] THREDS The Health Record Data Service
- [ ] Community-Engaged Research
- [ ] Collaborating with Kaiser Researchers

Clinical Research Centers:

- [ ] Community Engagement (CE)

Funds to Innovate:

- [ ] Strategic Opportunity Support (SOS)

Training:

- [ ] Clinical & Translational Sciences Training (CTST)
- [ ] Career Advancement (CA)

CTSI Core Services:

- [ ] Animal/Preclinical
- [ ] Array
- [ ] Bioinformatics
- [ ] Biostatistics

- [ ] Cell Culture
- [ ] Clinical Services
- [ ] Epidemiology
- [ ] Flow Cytometry
- [ ] Human/Clinical
- [ ] Imaging
- [ ] Immunohistochemistry
- [ ] Islet Production
- [ ] Microscopy
- [ ] Molecular/Genomic
- [ ] Monoclonal Antibody
- [ ] Proteomics
- [ ] Resale Products
- [ ] Tissue

1.0 General Information

1.1 *Enter the full title of your study:

Natural History and Pathogenesis of West Nile Virus in Viremic Donors

1.2 *Enter the study number or study alias

WNV

2.0 Add Department(s)

2.1 List of Departments associated with this study:

Primary Dept?	Department Name
◉	**UCSF** - *007940* - LABORATORY MEDICINE

3.0 Assign key study personnel(KSP) access to the study

3.1 *Please add a Principal Investigator for the study:

Busch, Michael P

Select if applicable
☐ Fellow
If the Principal Investigator is a Fellow, the name of the Faculty Advisor must be supplied below.

3.2 If applicable, please select the Protocol Staff personnel:

A) Additional Investigators

Custer, Brian S
Other Investigator
Lanteri, Marion C
Other Investigator
Lee, Tzong-Hae
Other Investigator
Norris, Philip J
Other Investigator
Tobler, Leslie H
Other Investigator

B) Research Support Staff

Hindes, Daniel A - Research Assistant

3.3 *Please add a Study Contact:

1. Busch, Michael P
2. Quintos, Michelle M

The Study Contact(s) will receive all important system notifications along with the Principal Investigator. (e.g. The study contact(s) are typically either the Study Coordinator or the Principal Investigator themselves).

3.4 If applicable, please add a Faculty Advisor:

No Faculty Advisors have been added.

3.5 If applicable, please select the Designated Department Approval(s):

No Department Administrators have been added.

4.0 Qualifications of Key Study Personnel

4.1 List the study responsibilities and qualifications of any individuals who qualify as Key Study Personnel (KSP) at UCSF and affiliated sites ONLY by clicking the "Add a new row" button:

NOTE: This information is required and your application will be considered incomplete without it.

KSP Name	Description of Study Responsibilities	Qualifications
Busch, Michael P	Principal Investigator	Adjunct professor of laboratory medicine with extensive experience in the study of viral infections through the acute phase, with an emphasis on the understanding of viral-immune interactions.
Norris, Philip J	Co-investigator	Experienced T-cell immunologist with a background in CD4+ T cell immunology assays.
Lanteri, Marion C	Co-investigator	Staff scientist experienced in virology with skills related to T cell and immunology assays.
Tobler, Leslie H	Co-investigator	Senior scientist and manager of the BSRI Viral Reference Laboratory and Repository responsible for all sample acquisition, processing and storage.
Lee, Tzong-Hae	Co-investigator	Senior scientist, Molecular Transfusion Laboratory, responsible for some laboratory testing and analyses.
Custer, Brian S	Co-investigator	Associate Investigator in epidemiology and health policy research.

5.0 Initial Screening Questions

5.1 * This study involves human stem cells (including iPS cells and adult stem cells), gametes or embryos:

- (•) No
- ( ) Yes, and requires CHR and GESCR review
- ( ) Yes, and requires GESCR review, but NOT CHR review

5.2 * This application involves a Humanitarian Use Device:

- (•) No
- ( ) Yes, and it includes a research component
- ( ) Yes, and it involves clinical care ONLY

5.3 * This is a CIRB study (e.g. the NCI CIRB will be the IRB of record):

( ) Yes (•) No

5.4 * This application includes a request to rely on another UC IRB to be the IRB of record:

○ Yes ◉ No

Note: If this request is approved, the CHR will **NOT** review and approve this study. Another UC campus will be the IRB of record.

6.0 Application Type

6.1 * This research involves:

◉ Minimal risk
○ Greater than minimal risk

6.2 * This application is:

○ Full Committee
◉ Expedited
○ Exempt

6.3 If you think this study qualifies for expedited review, select the regulatory category(ies) that the research falls under:

☐ Category 1: A very limited number of studies of approved drugs and devices
☑ Category 2: Blood sampling
☐ Category 3: Noninvasive specimen collection (e.g. buccal swabs, urine, hair and nail clippings, etc.)
☐ Category 4: Noninvasive clinical procedures (e.g. physical sensors such as pulse oximeters, MRI, EKG, EEG, ultrasound, moderate exercise testing, etc.)
☐ Category 5: Research involving materials (data, documents, records, or specimens) that were previously collected for either nonresearch or research purposes
☐ Category 6: Use of recordings (voice, video, digital or image)
☐ Category 7: Low risk behavioral research or research employing survey, interview, oral history, focus group, program evaluation, human factors evaluation, or quality assurance methodologies
☐ Category 8: Continuing review of previously approved full committee research that is essentially complete
☐ Category 9: Continuing review of research NOT involving an IND or IDE where the IRB has determined that the research poses no greater than minimal risk

6.4 * This study involves:

◉ Subject contact (including phone, email or web contact)
○ No subject contact (limited to medical records review, biological specimen analysis, and/or data analysis)

7.0 Funding

7.1 Identify all sponsors and provide the funding details:

External Sponsor:

Sponsor List

Sponsor Name:	NIH Natl Heart, Lung & Blood Institute
Sponsor Type:	01
Has the role of Funding?	Yes
Has the role of Protocol Control?	No
Has the role of Data Coordination?	No
Has the role of Monitoring?	No
Has the role of Auditing?	No
Has the role of Passthrough?	No
Awardee Institution:	Blood Systems Research Institute
Is Primary Grant Holder?	Yes
Contract Type:	Grant

UCSF RAS System Proposal Number ("P" + 7 digits):

UCSF RAS System Award Number ("A" + 6 digits):

Grant Title: Viral/immune parameters of Dengue and WNV in donors; blood safety implications

PI Name:

Significant Discrepancy:

Gift, Program, or Internal Funding (check all that apply):

- ☐ Funded by gift (specify source below)
- ☐ Funded by UCSF or UC-wide program (specify source below)
- ☐ Specific departmental funding (specify source below, if applicable)
- ☐ Unfunded (miscellaneous departmental funding)
- ☐ Unfunded student project

List the gift, program, or departmental funding source:

7.2 If you tried to add a sponsor in the question above and it was not in the list, follow these steps:

- **If funding has already been awarded or the contract is being processed by the Contracts and Grants or Industry Contracts unit, your sponsor is already in the system and the project has a UCSF RAS System Proposal or Award number. Check with your department's Research Services Analyst (RSA) to ask how the sponsor is listed in the UC sponsor list and what the Proposal or Award number is.**
- **If you need additional assistance, contact the Contracts and Grants Award Team at CGAwardTeam@ucsf.edu and list the sponsor in the box below.**

◉ Sponsor not in list

Only if your sponsor is not yet in the list, type the sponsor's name:

Novartis Vaccines and Diagnostics, Inc.

If the funding is administered by the UCSF Office of Sponsored Research, your study will not receive CHR approval until the sponsor and funding details have been added to your application.

7.3 * This study is supported in whole or in part by Federal funding:

◉ Yes ○ No

If **yes**, indicate which portion of your grant you will be attaching:

- ☑ The Research Plan, including the Human Subjects Section of your NIH grant or subcontract
- ☐ For other federal proposals (contracts or grants), the section of the proposal describing human subjects work
- ☐ The section of your progress report if it provides the most current information about your human subjects work
- ☐ The grant is not attached. The study is funded by an award that does not describe specific plans for human subjects, such as career development awards (K awards), cooperative agreements, program projects, and training grants (T32 awards)

8.0 Statement of Financial Interest

8.1 * The Principal Investigator and/or one or more of the key study personnel has financial interests related to this study:

○ Yes ◉ No

If **Yes**, attach the **Disclosure of Investigators' Financial Interests Supplement** to this application.

9.0 Sites

9.1 Institutions (check all that apply):

- ☐ UCSF
- ☐ Mt. Zion
- ☐ San Francisco General Hospital (SFGH)
- ☐ SF VA Medical Center (SF VAMC)
- ☐ Helen Diller Family Comprehensive Cancer Center
- ☐ Blood Centers of the Pacific (BCP)
- ☑ Blood Systems Research Institute (BSRI)

- [ ] Fresno (Community Medical Center)
- [ ] Gallo
- [ ] Gladstone
- [ ] Institute on Aging (IOA)
- [ ] Jewish Home
- [ ] SF Dept of Public Health (DPH)

9.2 Check all the other types of sites not affiliated with UCSF with which you are cooperating or collaborating on this project:

- [ ] Other UC Campus
- [ ] Other institution
- [ ] Other community-based site
- [ ] Foreign Country

List the foreign country/ies:

9.3 * This is a multicenter study:

(•) Yes ( ) No

9.4 Check any research programs this study is associated with:

- [ ] Cancer Center
- [ ] Center for AIDS Prevention Sciences (CAPS)
- [ ] Global Health Sciences
- [ ] Immune Tolerance Network (ITN)
- [ ] Osher Center
- [ ] Positive Health Program

10.0 Studies Involving Other Sites

10.1 UCSF is the coordinating center:

(•) Yes ( ) No

If **Yes**, describe the plan for communicating safety updates, interim results, and other information that may impact risks to the subject or others among sites:

BSRI is the coordinating center for this study. Subjects are BSI blood donors found to be positive for WNV during routine donation and subsequently enrolled into the study by BSI donor counselors. Any information that may impact subjects is communicated from study headquarters at BSRI to the donor counselors and blood centers via emails and telephone conferences .

If **Yes**, describe the plan for sharing modification(s) to the protocol or consent document(s) among sites:

All modifications to the protocol or consent forms are distributed from study headquarters to the blood centers and donor counselors after CHR approval .

10.2 Check any other UC campuses with which you are collaborating on this research study:

- [ ] UC Berkeley
- [ ] UC Davis
- [ ] Lawrence Berkeley National Laboratory (LBNL)
- [ ] UC Irvine
- [ ] UC Los Angeles
- [ ] UC Merced
- [ ] UC Riverside
- [ ] UC San Diego
- [ ] UC Santa Barbara
- [ ] UC Santa Cruz

10.3 Are the above UC campuses requesting to rely on UCSF's IRB (check all that apply)?

☐ Yes (Attach the Notice of Intent to Rely on One UC IRB form in the Other Study Documents section)
☐ No (Complete IRB Approval Certification section)

11.0 Study Design

11.1 * Study design:

The study is designed as an observational study. This is a longitudinal study of cellular and humoral immune function, which will be related to viral load and disease manifestations. A portion of the study will be dedicated to determining the persistence of WNV-specific immune responses over time, so there will be no specified endpoint for the study.

Samples from WNV+ blood donors enrolled in 2009, 2010, and 2011 will be used to build a repository of samples characterized for virologic and immunologic parameters to be transferred to the NHLBI.

Subjects will be identified at the time of blood donation by the presence of a WNV RNA positive specimen. Age-matched WNV RNA negative and WNV seronegative control subjects will be derived from the donor population who initially test false-positive for WNV RNA at the time of donation. Samples will be obtained from study subjects at enrollment, then at week one, two, three, four, and six weeks post-enrollment, and at month two, three, six, nine, and twelve post-enrollment.

Study investigators may also provide a portion of the serial samples collected under this protocol to other scientists engaged in WNV research and conversely, may receive specimens from other researchers in an effort to generate additional data to confirm study findings. Samples received will be pre-existing and will not contain any individually identifying information. Additionally, no specimens distributed by BSRI investigators will contain individually identifying information, nor will any key to coded information be shared among investigators. These collaborators include Dr. Bill Kwok at Benaroya Institute in Seattle, Dr. Jonathan Bramson at McMaster University in Hamilton, Ontario, Maria Rios at the FDA and Rob Lanciotti at CDC. BSRI investigators will also confirm and maintain the appropriate IRB approvals from other institutions before engaging in these activities.

An additional follow-up study of WNV+ study participants from 2005 has been conducted. Additionally, we will periodically contact and recall other study participants who have completed their follow-up collection protocol, and ask them to return to provide a one-time blood draw of 75 ml for WNV IgG, IgM, PRNT, and viral load testing. We will then compare these results to their previous levels from the samples obtained during their earlier participation in the study. We may also contact these subjects to offer participation in future WNV studies.

11.2 Check all that apply:

☐ Phase I
☐ Phase II
☐ Phase III
☐ Phase IV

12.0 Scientific Considerations

12.1 Hypothesis:

This study has a hypothesis:
○ Yes ◉ No
If yes, state the hypothesis or hypotheses:

12.2 * List the specific aims:

WNV infection results in variable penetrance of disease manifestations, ranging from asymptomatic infection to severe neurologic disease and death. The immune correlates of protection from disease have not been fully characterized, particularly in humans.
Previously, human T cell responses were identified for a subset of peptides from the membrane, envelope, non-structural protein 3 and 4b proteins of WNV. WNV-specific T cells were characterized as cytotoxic CD8 T cells secreting granzyme A and perforin. This finding might be of interest for vaccination studies and relevant to Flavivirus infection in general. The role for the immunopathogenesis is not understood but the comparison of immune responses between asymptomatic and symptomatic WNV+ donors is the best approach to identify key players involved in an efficient immune response: already a strong correlation between lower levels of regulatory T cells and symptomatic outcome was found. This suggested a protective role for regulatory T cells and a role for inflammation in the development of symptoms. Further studies are ongoing and try to address the dynamic between viral and immune parameters and their impact on WNV disease outcome.

Our specific aims are as follows:

1. To determine the immune parameters of a more efficient response to WNV.
2. To determine the viral dynamic post-infection and the risk for WNV transfusion transmission.
3. To determine the host genetic susceptibility to severe WNV disease outcome.

These specific aims will be addressed using serial samples collected from WNV+ blood donors enrolled in the acute phase of WNV infection and followed up to one year post-infection. Immunologic assays will consist of flow-based assays, ELISPOT, ELISA, Luminex-based assays. Viral dynamic will be addressed by Transcription Mediated amplification Assay, quantitative RT-PCR, infectivity studies, viral sequencing. Genetic susceptibility will be investigated through a whole exome sequencing approach using an Illumina platform comparing WNV+ subjects with neurologic disease to asymptomatic subjects.
This multi-faceted approach will result in a better understanding of the mechanisms underlying the development of symptomatic disease and inform on therapeutic opportunities.

12.3 Statistical analysis:

Data analysis will be performed by the PI in collaboration with statisticians and epidemiologists on site at BSRI. This is a longitudinal study of viral RNA and immune markers following initial detection of a donor as viremic by nucleic acid testing (NAT). To estimate how rapidly, on average, viral load increased during the ramp-up phase, we first need to select for each panel the RNA+/IgM- bleeds collected when viral load was increasing exponentially. Assuming that the first RNA quantifiable bleed ("time 0" bleed) probably occurred in ramp-up and that viral load increased at a constant rate (i.e., in a linear fashion) on a log scale during ramp-up, the rate of increase in log viral load can be calculated. Information on log viral load and collection days for the selected ramp-up bleeds will be entered in a repeated measures regression model where each panel subject had their own intercept and slope (PROC MIXED, SAS Institute Inc.). The average viral load doubling time during ramp-up will then be calculated as log(2)/average slope. Longitudinal regression analysis will be used to estimate time periods from selected viral RNA thresholds to IgM and IgG seroconversion, as well as the duration of time following IgM or IgG seroconversion that low-level viremia is detectable by replicate TMA. This analysis will use PROC LIFEREG (SAS Institute Inc.), and assume a normal distribution for these window periods.

For the correlation of immune responses and disease outcomes, we expect that for some of the immune measurements (e.g. T cell responses), a sizeable number of participants will have values either below the limit of detection or clustered just above the limit of detection with a rightwards skewed distribution. For these measurements, we will use non-parametric statistical tests (e.g., Wilcoxon rank-sum test) to compare distributions between groups. For measurements for which all participants have observable values and for which distributions are approximately normal, we will use parametric tests (e.g., two-sample t-test).

12.4 * This is an investigator-initiated study:

(•) Yes ( ) No

12.5 This study has received scientific or scholarly review from (check all that apply):

- [ ] Cancer Center Protocol Review Committee (PRC) (Full approval or contingent PRC approval is required prior to final CHR approval for cancer-related protocols.)
- [ ] CTSI Clinical Research Center (CRC) advisory committee
- [ ] Departmental scientific review
- [x] Other:

Specify **Other**:

National Heart, Lung and Blood Institute

If applicable, attach the **Departmental Scientific Review Form** at the end of the application.

13.0 Background

13.1 Background:

WNV was introduced to the Northeastern United States in late summer of 1999. The virus was determined to be almost identical genetically to strains prevalent in Israel[1]. Since its introduction, WNV has spread relentlessly westward, with large outbreaks in the Midwest and Colorado in 2004 and in Arizona and Southern California in 2005. The transmission period mirrors that of mosquito activity, peaking from May through August. It is expected that WNV will continue its westward expansion in the coming transmission season this spring and summer. During an outbreak of WNV in non-immune populations, approximately 5% of blood donations are positive for WNV IgM. Blood transfusion of WNV contaminated units has resulted in transfusion-associated transmission of the virus, with severe disease and death sometimes resulting. Pooled blood donations are now routinely screened for WNV RNA, though the sensitivity of the screening process is not likely adequate to prevent 100% protection from transfusion associated WNV transmission. Given that WNV will represent an ongoing health problem and threat to the blood supply, greater understanding of the pathogenesis of the virus is required.

Both humoral and cellular immune responses have been implicated in the control of WNV infection. The bulk of the pathogenesis data relating to WNV comes from murine models [2]. Mice deficient in secreted IgM have been shown to be more susceptible to lethal challenge with WNV, and passive transfer of polyclonal IgM can protect against lethal infection with WNV[3]. Additionally, low WNV IgM titer in infected wild

type mice is correlated with higher mortality. T cells also likely play a role in control of the virus. CD8+ T cell deficient mice infected with low-dose WNV show increased mortality compared to wild-type controls[4]. Unpublished data suggest that T cell deficient mice initially control WNV replication, but ultimately fail to eradicate the virus, leading to recrudescence of viremia. The role of T cell responses appears not only to be protective, as CD8+ T cells have been isolated in the inflammatory regions of meningoencephalitits in mice and humans [5]. We will assess the role of T cells in neurological manifestations of disease by studying the properties of CSF lymphocytes in hospitalized subjects who undergo diagnostic lumbar puncture.

Our laboratory has traditionally focused on the role of HIV-specific T cell responses in the control of virus replication [6, 7]. In HIV and flaviviruses analogous to WNV, it has been demonstrated that some regions of the viral genome are more susceptible to recognition by T cells than others [8-10]. In collaboration with the Biodefense and Emerging Infections Research Resources Repository we acquired overlapping peptide sets spanning the WNV genome synthesized and coupled with our unique access to patient samples through a large blood donation network, we were able to do comprehensive analysis of WNV-specific T cell responses [11]. We showed several epitopes inducing CD8 T cell responses in humans. We were able to demonstrate that control of acute viremia in WNV-infected blood donors is associated with interferon and interferon-induced chemokine expression [12].

We will be able to monitor the effect T cell responses and especially regulatory T cells [13-18] and Th17 cells [19-21] have on the dynamics of viral infection, the correlation with disease manifestations, and the durability of T cell immune responses to the virus.

The investigators have unparalleled access to viremic blood donors. Over the last four years, they have capitalized on blood bank resources to capture viremic individuals identified during acute WNV infection and established the repository of plasma and PBMC samples they have been using to address immunological studies related to WNV infection and pathogenesis. During the two next years, the investigators will be collecting samples from WNV+ blood donors enrolled in the bleeding protocol described in Part 3 to build a new repository of samples from WNV+ blood donors. The complete pedigree of clinical data and all laboratory data around the virologic and immunologic characterization of the samples will be entered into the WNV repository database that will be transferred to NHLBI.

13.2 Preliminary studies:

See Background.

13.3 References:

1. Lanciotti RS, Roehrig JT, Deubel V, et al. Origin of the West Nile virus responsible for an outbreak of encephalitis in the northeastern United States. Science 1999;286:2333-7
2. Samuel MA, Diamond MS. Pathogenesis of West Nile Virus infection: a balance between virulence, innate and adaptive immunity, and viral evasion. J Virol 2006;80:9349-60
3. Diamond MS, Sitati EM, Friend LD, Higgs S, Shrestha B and Engle M. A critical role for induced IgM in the protection against West Nile virus infection. J Exp Med 2003;198:1853-62
4. Wang Y, Lobigs M, Lee E and Mullbacher A. CD8+ T cells mediate recovery and immunopathology in West Nile virus encephalitis. J Virol 2003;77:13323-34
5. Kelley TW, Prayson RA, Ruiz AI, Isada CM and Gordon SM. The neuropathology of West Nile virus meningoencephalitis. A report of two cases and review of the literature. Am J Clin Pathol 2003;119:749-53
6. Norris PJ, Moffett HF, Yang OO, et al. Beyond help: direct effector functions of human immunodeficiency virus type 1-specific CD4(+) T cells. J Virol 2004;78:8844-51
7. Norris PJ, Sumaroka M, Brander C, et al. Multiple effector functions mediated by human immunodeficiency virus-specific CD4(+) T-cell clones. J Virol 2001;75:9771-9
8. Altfeld MA, Trocha A, Eldridge RL, et al. Identification of dominant optimal HLA-B60- and HLA-B61-restricted cytotoxic T-lymphocyte (CTL) epitopes: rapid characterization of CTL responses by enzyme-linked immunospot assay. J Virol 2000;74:8541-9
9. Lobigs M, Arthur CE, Mullbacher A and Blanden RV. The flavivirus nonstructural protein NS3 is a dominant source of cytotoxic T cell peptide determinants. Virology 1994;202:195-201
10. Zheng B, Han S, Zhu Q, Goldsby R and Kelsoe G. Alternative pathways for the selection of antigen-specific peripheral T cells. Nature 1996;384:263-6
11. Lanteri MC, Heitman JW, Owen RE, et al. Comprehensive analysis of west nile virus-specific T cell responses in humans. J Infect Dis 2008;197:1296-306
12. Tobler LH, Cameron MJ, Lanteri MC, et al. Interferon and interferon-induced chemokine expression is associated with control of acute viremia in West Nile virus-infected blood donors. J Infect Dis 2008;198:979-83
13. Hill JA, Benoist C and Mathis D. Treg cells: guardians for life. Nat Immunol 2007;8:124-5
14. O'Garra A, Vieira P. Regulatory T cells and mechanisms of immune system control. Nat Med 2004;10:801-5
15. Shevach EM. Certified professionals: CD4(+)CD25(+) suppressor T cells. J Exp Med 2001;193:F41-6
16. Suvas S, Azkur AK, Kim BS, Kumaraguru U and Rouse BT. CD4+CD25+ regulatory T cells control the severity of viral immunoinflammatory lesions. J Immunol 2004;172:4123-32
17. Suvas S, Kumaraguru U, Pack CD, Lee S and Rouse BT. CD4+CD25+ T cells regulate virus-specific primary and memory CD8+ T cell responses. J Exp Med 2003;198:889-901
18. Walker LS, Chodos A, Eggena M, Dooms H and Abbas AK. Antigen-dependent proliferation of CD4+ CD25+ regulatory T cells in vivo. J Exp Med 2003;198:249-58
19. Stockinger B, Veldhoen M. Differentiation and function of Th17 T cells. Curr Opin Immunol 2007;19:281-6
20. Stockinger B, Veldhoen M and Martin B. Th17 T cells: linking innate and adaptive immunity. Semin Immunol 2007;19:353-61

21. Yue FY, Merchant A, Kovacs CM, Loutfy M, Persad D and Ostrowski MA. Virus-specific interleukin-17-producing CD4+ T cells are detectable in early human immunodeficiency virus type 1 infection. J Virol 2008;82:6767-71

If you have a separate bibliography, attach it to the submission with your other study documents.

14.0 Sample Size and Eligibility

14.1 Number of subjects that will be enrolled at UCSF and affiliated institutions:

0

14.2 Total number of subjects that will be enrolled at all sites:

350

14.3 Estimated number of people that you will need to consent and screen here (but not necessarily enroll) to get the needed subjects:

0

14.4 Sample size calculation:

Data analysis will be performed by the PI in collaboration with statisticians and epidemiologists on site at BSRI. This is a longitudinal study of viral RNA and immune markers following initial detection of a donor as viremic by nucleic acid testing (NAT). To estimate how rapidly, on average, viral load increased during the ramp-up phase, we first need to select for each panel the RNA+/IgM- bleeds collected when viral load was increasing exponentially. Assuming that the first RNA quantifiable bleed ("time 0" bleed) probably occurred in ramp-up and that viral load increased at a constant rate (i.e., in a linear fashion) on a log scale during ramp-up, the rate of increase in log viral load can be calculated. Information on log viral load and collection days for the selected ramp-up bleeds will be entered in a repeated measures regression model where each panel subject had their own intercept and slope (PROC MIXED, SAS Institute Inc.). The average viral load doubling time during ramp-up will then be calculated as log(2)/average slope. Longitudinal regression analysis will be used to estimate time periods from selected viral RNA thresholds to IgM and IgG seroconversion, as well as the duration of time following IgM or IgG seroconversion that low-level viremia is detectable by replicate TMA. This analysis will use PROC LIFEREG (SAS Institute Inc.), and assume a normal distribution for these window periods.

For the correlation of immune responses and disease outcomes, we expect that for some of the immune measurements (e.g. T cell responses), a sizeable number of participants will have values either below the limit of detection or clustered just above the limit of detection with a rightwards skewed distribution. For these measurements, we will use non-parametric statistical tests (e.g., Wilcoxon rank-sum test) to compare distributions between groups. For measurements for which all participants have observable values and for which distributions are approximately normal, we will use parametric tests (e.g., two-sample t-test).

14.5 * Eligible age range(s):

- ☐ 0-6 years
- ☐ 7-12 years
- ☐ 13-17 years
- ☑ 18+ years

14.6 Inclusion criteria:

Subjects will be identified from the pool of WNV+ blood donors. WNV RNA and WNV seronegative individuals will also be included as control subjects. subjects will range in age from 18 to 100 years.

14.7 Exclusion criteria:

Pregnancy, age less than 18, prisoners.

14.8 There are inclusion or exclusion criteria based on gender, race or ethnicity:

○ Yes ◉ No

If **yes**, please explain the nature and rationale for the restrictions:

15.0 Drugs and Devices

15.1 * Drugs or biologics will be studied under this application:

○ Yes ◉ No

15.2 * Investigational medical devices or in vitro diagnostics will be used OR approved medical devices or in vitro diagnostics will be studied under this application:

○ Yes ◉ No

15.3 * A Non-Significant Risk (NSR) determination is being requested for an investigational device:

○ Yes ◉ No

15.4 Verification of IND/IDE numbers: If the sponsor's protocol does not list the IND/IDE number, you must submit documentation from the sponsor or FDA identifying the IND/IDE number for this study. Attach this documentation in the Other Study Documents section of the Initial Review Submission Packet.

16.0 Other Approvals and Registrations

16.1 * This is a clinical trial:

○ Yes ◉ No

Clinical Trial Registration
"NCT" number for this trial:

16.2 * Data from this study will be submitted to NIH for Genome-Wide Association Studies (GWAS):

○ Yes ◉ No

16.3 * This study involves vaccines produced using recombinant DNA technologies:

○ Yes ◉ No

16.4 * This study involves human gene transfer (NOTE: Requires NIH Recombinant DNA Advisory Committee (RAC) review prior to CHR approval):

○ Yes ◉ No

16.5 * The study protocol requires radiological procedures (e.g. CT scans, x-rays) or exposes subjects to radiation:

○ Yes ◉ No

16.6 This study involves other regulated materials and requires approval and/or authorization from the following regulatory committees:

☐ Institutional Biological Safety Committee (IBC)
Specify BUA #:

☐ Institutional Animal Care and Use Committee (IACUC)
Specify IACUC #:

☐ Radiation Safety Committee
Specify RUA #:

☐ Radioactive Drug Research Committee (RDRC)
Specify RDRC #:

☐ Controlled Substances

17.0 Procedures

17.1 * List all study procedures, test and treatments required for this study:

The maximum amount of blood obtained from subjects is 375 ml over an 8 week period, and 750 ml over the total one-year period. Each phlebotomy will collect 75 ml blood. In addition to the phlebotomy schedule described for WNV+ subjects, blood donors' initial donation blood products will be retrieved for study if operationally feasible. This will allow study of the earliest viremic time point.

In addition to phlebotomy, two questionnaires will be administered by telephone. Questionnaire A will be administered within a week of infection being identified. The second will be administered approximately three weeks after infection was identified. Both questionnaires focus on symptoms associated with WNV infection (see questionnaires in Appendix).

Category	Specific Activities
Visit schedule non-hospitalized	1) Index donation (day 0) 2) Enrollment visit (days 2-4) 3) Phlebotomies at weeks one, two, three, six post-enrollment (days 9-46) 4) Phlebotomy at two, three, six, nine, and twelve months post-enrollment.
Visit procedures	1) Donor consent at enrollment visit 2) Donor risk/symptom questionnaire at enrollment and follow up questionnaire at one month 3) Donor phlebotomy at each visit (draw 7x10mL EDTA plus 1x2.5-4mL EDTA plus one 2.5 mL PAXgene tube) 4) Anticoagulated whole blood will be shipped using Federal Express to Blood Systems Research Institute.
Tests that may be performed	1) Complete Blood Count/Platelet count 2) Plasma and PBMCs separation 3) WNV TMA (5x) 4) WNV IgM and IgG (Focus) 5) PRNT (CDC protocol) 6) Quantitative WNV PCR (viral load) on index unit and TMA-reactive specimens 7) WNV Viral culture and infectivity studies 8) WNV genome sequencing 9) Cytotoxic T cell response 10) CD4 proliferative responses 11) Regulatory T cell frequencies and WNV-specific T cell responses 12) Th17 cells frequencies and WNV-specific responses 13) Cytokine/chemokines quantification 14) HLA typing 15) Generation of immortalized B and T cell lines

To provide appropriate experimental controls, the ability of study subjects' cells to combat other viruses such as Epstein Barr virus, hepatitis C virus, HIV, influenza virus, cytomegalovirus, and human herpes virus 8 (HHV-8) may also be tested.

For the recall studies, WNV+ donors enrolled in our study in previous years will be contacted by our Medical Affairs office and offered the opportunity to participate. Potential participants will be given all study information and instructions to have their blood drawn. They will sign their consent forms when they visit their blood centers for the blood draws. Samples will be shipped to BSRI for testing.

If you have a procedure table, attach it to the submission with your other study documents.

17.2 Interviews, questionnaires, and/or surveys will be administered or focus groups will be conducted:

(•) Yes ( ) No

List any standard instruments used for this study:

Attach any non-standard instruments at the end of the application.

17.3 Conduct of study procedures or tests off-site by non-UCSF personnel:

(•) Yes ( ) No

If yes, explain:

Consent will be obtained by a study coordinator employed by Blood Systems Inc., and research will be performed at the Blood Systems Research Institute. Initial donor identification will be performed at Blood Systems Laboratories in Scottsdale, AZ. Additional testing will be performed at GenProbe in San Diego, CA Chiron Corporation in Emeryville, CA, Focus Diagnostics in Cypress, CA, and the CDC in Fort-Collins, CO.

17.4 Sharing of experimental research test results with subjects or their care providers:

( ) Yes (•) No

If yes, explain:

17.5 * Specimen collection for future research and/or specimen repository/bank administration:

(•) Yes ( ) No

17.6 Time commitment (per visit and in total):

Blood will be drawn at each study visit. It is not anticipated that more than half an hour per visit will be required, for a total of 5 hours over a one year period. For any recall studies, participants will have their blood drawn at their local BSI blood center. The single blood draw visit should not take more than one half hour.

17.7 Locations:

Subjects will be recruited from within the network of blood banks in Blood Systems, Inc., concentrated in the West and Midwest of the United States. Candidate sites will be located in regions of the country experiencing outbreaks of West Nile virus, as documented through routine screening of blood donors.

Consent will be obtained by a study coordinator employed by Blood Systems Inc., and research will be performed at the Blood Systems Research Institute. Initial donor identification will be performed at Blood Systems Laboratories in Scottsdale, AZ. Additional testing will be performed at GenProbe in San Diego, CA Chiron Corporation in Emeryville, CA, Focus Diagnostics in Cypress, CA, the CDC in Fort-Collins, CO, and at FDA laboratories.

17.8 Describe the resources in place to conduct this study in a way that assures protection of the rights and welfare of participants:

Blood will be drawn by trained phlebotomists at BSI blood centers conveniently located throughout the US. Subject privacy will be maintained by stringent data security policies at the blood centers, BSRI and NHLBI's BioLLinc repository.

18.0 Specimen Collection for Future Research and/or Specimen Repository/Bank Administration

(Note: This section replaces the old "Human Biologic Specimen Collecting and/or Banking for Future Research" supplement form. Please do not attach the old form to this application.)

18.1 Specimens are (check all that apply):

- ☐ Surplus clinical specimens from a diagnostic or therapeutic procedure
- ☑ Specimens collected for research purposes only
- ☐ Other

If Other, explain:

18.2 Types of specimens:

- ☑ Blood
- ☐ Tissue (describe below):
- ☐ Existing/archival materials (name source below):
- ☐ Other (describe below):

Describe and/or name source:

18.3 Consent will be obtained via:

- ☐ Separate specimen banking consent form
- ☑ Specimen banking section within a main research study consent form
- ☐ Surgical consent form with tissue donation brochure

18.4 Specimens will ultimately be stored (check all that apply):

UCSF

- ☐ UCSF repository/bank being established under this protocol
- ☐ Existing UCSF specimen repository/bank with CHR approval

Provide the name of the bank and CHR approval number (if not being banked at UCSF under this protocol):

Outside Entity

- ☐ Cooperative group bank
- ☑ NIH
- ☐ Other university
- ☐ Industry sponsor
- ☑ Other

Specify to what institution, cooperative group or company specimens will be transferred:

Blood Systems Research Institute, 270 Masonic Avenue, San Francisco, CA 94118 in the BSRI Freezer Farm on the basement level.

The National Heart, Lung, and Blood Institute Biologic Specimen Repository, Bethesda, MD.

18.5 Direct identifiers will be sent with specimens or shared with other researchers and/or outside entities:

- ○ Yes
- ◉ No
- ○ N/A - Specimens will not be shared with others

If **Yes**, which identifiers will be sent with specimens:

- ☐ Name
- ☐ Date of birth
- ☐ Social Security number
- ☐ Medical record number
- ☐ Address
- ☐ Phone number
- ☐ Email address
- ☐ Other dates (surgery date, clinic visit dates, etc.)

If **Yes**, provide a justification for sending direct identifiers with the specimens:

19.0 Alternatives

19.1 Study drug or treatment is available off-study:

- ○ Yes
- ○ No
- ◉ Not applicable

19.2 * Is there a standard of care (SOC) or usual care that would be offered to prospective subjects at UCSF (or the study site) if they did not participate:

○ Yes ◉ No

If yes, describe the SOC or usual care that patients would receive if they choose not to participate:

This study is completely voluntary and does not involve any treatments.

19.3 Describe other alternatives to study participation that are available to prospective subjects:

Participation in the study is completely voluntary. The alternative to participation is not to participate. As the study is observational and does not provide therapy, there would be no need to take any alternative action if a subject opts not to participate in the study.

20.0 Risks and Benefits

20.1 * Risks and discomforts:

Sampling blood may cause a bruise and/or bleeding at the needle site. Occasionally, a person feels faint when their blood is drawn. Rarely, an infection may develop at the needle site. As subjects will have had a full unit (500 mL) phlebotomy immediately prior to enrollment and up to seven 75 ml phlebotomies during the initial three months of the study, they may be deferred from donating blood while participating in the study.

20.2 Steps taken to minimize risks to subjects:

If a person demonstrates any sign of illness, such as fever, malaise, or recent weight loss, we will follow the guidelines for unhealthy individuals and the amount of blood drawn may not exceed the lesser of 50 ml or 3 ml per kg in an 8 week period.

20.3 Benefits to subjects:

○ Yes ◉ No

If yes, describe:

20.4 Benefits to society:

The proposed studies will further our knowledge of how the immune system interacts with WNV.
These studies will not only lend an understanding of WNV pathogenesis, but also hold the potential to assist in vaccine development and testing. The repository of WNV samples and linked database transferred to NHLBI for further availability to the scientific community will provide tools to scientists in the field to address WNV infection and pathogenesis.

20.5 Explain why the risks to subjects are reasonable:

The risks of the study to the subjects are minimal. While there is no direct benefit to the study subjects, the potentially large benefit to society balances the very small risks posed to the study subjects.

21.0 Confidentiality and Privacy

21.1 Plans for maintaining privacy in the research setting:

We do not plan to share any personally identifying information outside of the research team.

21.2 Possible consequences to subjects resulting from a loss of privacy:

West Nile virus is a self-limited disease in most individuals without social stigma attached, so loss of privacy would not be anticipated to have a major impact on the study subjects.

21.3 Study data are:

- [ ] Derived from the Integrated Data Repository (IDR) or The Health Record Data Service (THREDS) at SFGH
- [ ] Derived from a medical record (identify source below)
- [ ] Added to the hospital or clinical medical record
- [ ] Created or collected as part of health care
- [ ] Used to make health care decisions
- [x] Obtained from the subject, including interviews, questionnaires
- [ ] Obtained from a foreign country or countries only
- [ ] Obtained from records open to the public
- [x] Obtained from existing research records
- [ ] None of the above

If **derived from a medical record**, identify source:

21.4 Identifiers may be included in research records:

(•) Yes ( ) No

If **yes**, check all the identifiers that may be included:

- [ ] Names
- [ ] Dates
- [ ] Postal addresses
- [ ] Phone numbers
- [ ] Fax numbers
- [ ] Email addresses
- [ ] Social Security Numbers*
- [ ] Medical record numbers
- [ ] Health plan numbers
- [ ] Account numbers
- [ ] License or certificate numbers
- [ ] Vehicle ID numbers
- [ ] Device identifiers or serial numbers
- [ ] Web URLs
- [ ] IP address numbers
- [ ] Biometric identifiers
- [ ] Facial photos or other identifiable images
- [x] Any other unique identifier

* Required for studies conducted at the VAMC

21.5 Identifiable information might be disclosed as part of study activities:

( ) Yes (•) No

If **yes**, indicate to whom identifiable information may be disclosed:

- [ ] The subject's medical record
- [ ] The study sponsor
- [ ] Collaborators
- [ ] The US Food & Drug Administration (FDA)
- [ ] Others (specify below)
- [ ] A Foreign Country or Countries (specify below)

If **Others**, specify:

21.6 Indicate how data are kept secure and protected from improper use and disclosure (check all that apply):

NOTE: Whenever possible, do not store subject identifiers on laptops, PDAs, or other portable devices. If you collect subject identifiers on portable devices, you MUST encrypt the devices.

- [ ] Data are stored securely in My Research
- [ ] Data are coded; data key is destroyed at end of study
- [x] Data are coded; data key is kept separately and securely
- [ ] Data are kept in a locked file cabinet
- [ ] Data are kept in a locked office or suite
- [x] Electronic data are protected with a password
- [x] Data are stored on a secure network
- [ ] Data are collected/stored using REDCap or REDCap Survey

21.7 Additional measures to assure confidentiality and protect identifiers from improper use and disclosure, if any:

21.8 This study may collect information that State or Federal law requires to be reported to other officials or ethically requires action:

○ Yes ◉ No

Explain:

21.9 This study will be issued a Certificate of Confidentiality:

○ Yes ◉ No

22.0 Subjects

22.1 Check all types of subjects that may be enrolled:

- [ ] Inpatients
- [ ] Outpatients
- [x] Healthy volunteers
- [ ] Staff of UCSF or affiliated institutions

22.2 Additional vulnerable populations:

- [ ] Children
- [ ] Subjects unable to consent for themselves
- [ ] Subjects unable to consent for themselves (emergency setting)
- [ ] Subjects with diminished capacity to consent
- [ ] Subjects unable to read, speak or understand English
- [ ] Pregnant women
- [ ] Fetuses
- [ ] Neonates
- [ ] Prisoners
- [ ] Economically or educationally disadvantaged persons
- [ ] Investigators' staff
- [ ] Students

Explain why it is appropriate to include the types of subjects checked above in this particular study:

Describe the additional safeguards that have been included in the study to protect the rights and welfare of these subjects and minimize coercion or undue influence:

23.0 Recruitment

23.1 * Methods (check all that apply):

- ☐ Study investigators (and/or affiliated nurses or staff) recruit their own patients directly in person or by phone.
- ☐ Study investigators recruit their own patients by letter. Attach the letter for review.
- ☐ Study investigators send a "Dear Doctor" letter to colleagues asking for referrals of eligible patients. If interested, the patient will contact the PI or the PI may directly recruit the patients (with documented permission from the patient). Investigators may give the referring physicians a study information sheet for the patients.
- ☐ Study investigators provide their colleagues with a "Dear Patient" letter describing the study. This letter can be signed by the treating physicians and would inform the patients how to contact the study investigators. The study investigators may not have access to patient names and addresses for mailing
- ☐ Advertisements, notices, and/or media used to recruit subjects. Interested subjects initiate contact with study investigators. Attach ads, notices, or media text for review. In section below, please explain where ads will be posted.
- ☐ Study investigators identify prospective subjects through chart review. (Study investigators request a Waiver of Authorization for recruitment purposes.)
- ☐ Large-scale epidemiological studies and/or population-based studies: Prospective subjects are identified through a registry or medical records and contacted by someone other than their personal physician. (Study investigators request a Waiver of Authorization for recruitment purposes.)
- ☐ Direct contact of potential subjects who have previously given consent to be contacted for participation in research. Clinic or program develops a CHR-approved recruitment protocol that asks patients if they agree to be contacted for research (a recruitment database) or consent for future contact was documented using the consent form for another CHR-approved study.
- ☐ Study investigators list the study on the School of Medicine list of UCSF Clinical Trials website or a similarly managed site. Interested subjects initiate contact with investigators.
- ☐ Study investigators recruit potential subjects who are unknown to them through methods such as snowball sampling, direct approach, use of social networks, and random digit dialing.
- ☑ Other

If **Other**, explain:

BSI Medical Affairs donor counselors will recruit WNV+ positive blood donors over the telephone who have been identified during routine blood donation and testing processes.

23.2 * How, when, and by whom eligibility will be determined:

See below.

23.3 * How, when, where and by whom potential subjects will be approached:

Study subjects will be contacted if their blood donation tests positive for WNV RNA. This group will include WNV infected donors and those who test false-positive for WNV RNA (seronegative control subjects). Initial false positive donors will be identified through negative repeat RNA testing and failure to seroconvert WNV-reactive antibody responses. Both groups of subjects will be invited to enroll in the study by donor counselors and will sign the consent form upon presentation to their local blood donation center for follow up.
For the recall studies, former WNV study participants enrolled in previous years will be identified by BSI Medical Affairs and asked to participate in a follow-up study.

23.4 * Protected health information (PHI) will be accessed prior to obtaining consent:

○ Yes ◉ No

24.0 Informed Consent

24.1 * Methods (check all that apply):

- ☑ Signed consent will be obtained from subjects and/or parents (if subjects are minors)
- ☐ Verbal consent will be obtained from subjects using an information sheet or script
- ☐ Electronic consent will be obtained from subjects via the web or email
- ☐ Implied consent will be obtained via mail, the web or email
- ☐ Signed consent will be obtained from surrogates
- ☐ Emergency waiver of consent is being requested for subjects unable to provide consent
- ☐ Informed consent will not be obtained

24.2 * Process for obtaining informed consent:

Once a blood donor with West Nile virus or a seronegative control has been identified, informed consent will be obtained over the telephone by a trained study coordinator employed by the blood bank at corporate headquarters in Scottsdale, AZ. The study coordinator would have experience in informing blood donors of positive virological testing. Study subjects will be given as much time as they need to consider participation and will sign a consent form at the time of the first blood draw upon enrollment in the study.

Recalled subjects will be given all the study information contained in the informed consent form over the telephone by a trained donor counselor. When subjects visit the blood bank for their blood draw, they will sign the consent form. They will be given opportunities to discuss the study and ask questions during their telephone conversation with the donor counselor and during their visit to the blood bank.

24.3 * How investigators will make sure subjects understand the information provided to them:

Subjects will demonstrate an understanding of the implications of WNV infection and possible disease manifestations. It will be explicitly stated that there would be no benefit to the study subject from participation in the study, aside from the potential feeling of well-being gained in assisting scientific research.

25.0 Financial Considerations

25.1 Subjects payment or compensation method (check all that apply):

Payments will be (check all that apply):

- ☐ Subjects will not be paid
- ☐ Cash
- ☑ Check
- ☐ Gift card
- ☐ Other:

Specify **Other**:

25.2 Describe the schedule and amounts of payments, including the total subjects can receive for completing the study. If deviating from recommendations in Subject Payment Guidelines, include specific justification below.

Subjects will be paid $40 after each study visit, comprised of $20 for time spent on study activities and $20 for travel expenses. Additionally, after Visit 1, Visit 7 and Visit 10, subjects will be paid a bonus of $30. Check payments will be mailed to study subjects after each visit. The total amount subjects could receive if they complete all 10 study visits would be $490.

Recall study subjects will be paid $30.

25.3 Costs to Subjects: Will subjects or their insurance be charged for any study procedures?

○ Yes ◉ No

If **yes,** describe those costs below, and compare subjects' costs to the costs associated with alternative care off-study. Finally, explain why it is appropriate to charge those costs to the subjects.

26.0 CTSI Screening Questions

26.1 * This study will be carried out at one of the UCSF Clinical Research Services (CRS) units or will utilize CRS services:

○ Yes ◉ No

26.2 This project involves community-based research:

○ Yes ◉ No

26.3 This project involves practice-based research:

○ Yes ◉ No

RESEARCH SUBJECT INFORMATION AND CONSENT FORM
(Donors with a positive nucleic acid amplification test result for WNV)

TITLE: Virology and Immunology of West Nile Virus infection

SPONSORS: *Blood Systems, Inc.*

INVESTIGATOR: *Michael P. Busch, M.D., Ph.D.*
Blood Systems Research Institute
270 Masonic Avenue
San Francisco, CA 94118

PURPOSE AND BACKGROUND

In this study, the investigators hope to understand how infected persons clear West Nile virus (WNV) from their bodies and why some people get sick and others don't after being infected by this virus. By participating in our study and giving samples of their blood, a small group of people infected with WNV will allow researchers to work on understanding how the immune system and the virus interact, and how the body fights the virus.

You were selected as a possible subject for this study because we detected WNV in your blood. You have already received information regarding this result, and have been asked to participate in this study.

PROCEDURES

Blood Draw

If you decide to participate, study staff will take a blood sample (no more than 75 ml, or 5 tablespoons) from your arm today. You will also be asked to return once a week for the next three weeks, and again at the following intervals: 6 weeks, 2 months, 3 months, 6 months, 9 months and 12 months. This is a total of 9 more visits with blood draws after today. The amount of blood drawn during the initial eight week period of the study will not exceed 375 ml and the total amount of blood drawn over the course of the whole study (one year) will not exceed 750 ml.

You will not be able to donate blood for eight weeks following conclusion of the first two months of the study and you may need further WNV testing before donating blood. The process for the collection of each sample will only take a few minutes.

Testing

The samples that you provide will be tested in different research experiments. While critical for WNV research, the results of these experiments will not be important to maintaining your health and will not be reported back to you. We will perform the following experiments using your blood:

- Complete blood analysis (white cells, red cells, and platelet count).
- Test for the presence of WNV.
- Measure the amount of WNV in your blood.
- Test to see whether or not your immune system has responded to a WNV infection.
- Develop new tests to detect WNV.
- Grow WNV found in your blood to infect other cells in the laboratory.
- Identify the WNV you were infected with to see how different it is from the virus that has infected other people.
- Measure how some of your white blood cells (T cells) react to WNV.
- See which molecules (named HLA for Human Leukocyte Antigen) are expressed on the surface of your cells.
- Observe the differences found in your genes and how they make infection possible or protect you from the virus
- Grow some of your white blood cells (B and T cells) that will survive for years in the laboratory.

Tissue Banking
A portion of your blood will be frozen and stored and may be used by the researchers of this study or other researchers, for possible future studies on infectious diseases.

Your blood samples will be stored frozen at Blood Systems Research Institute in San Francisco until the end of our study. Then the samples will be transferred to the biorepository at the National Heart, Lung and Blood Institute (NHLBI). The purpose of the biorepository is to collect and store specimens and to make them available to other scientists to do other studies.

The research that may be performed using your blood samples may not be related to WNV. Your samples may be shared with for profit companies working on a specific research project, but your samples will not be sold for profit. If this research leads to the development of a commercial product there will not be any compensation to you or your heirs.

Also, all of the laboratory testing results from this study will be put into a database that will be linked to the stored samples. This database will also be transferred to the biorepository at the NHLBI. The blood samples will be coded. This means that they will not contain any of your personally identifying information, like your name or social security number. There is no scheduled date on which your samples and information in the bank will be destroyed. Your samples may be stored for research until they are "used up."

Since all further testing not related to West Nile Virus studies will be conducted with your personal identifiers removed (also called "unlinked" testing), no further information will be made available to you regarding the results from any of these other research studies.

If you wish to have your specimens removed from frozen storage, you may contact ***Dr. Leslie Tobler at*** the BSRI Viral Reference Laboratory and Repository at 415-749-6606 or via email at ltobler@bloodsystems.org. If your specimens have already been transferred to the biorepository

at the NHLBI, every effort will be made to have your specimens removed, but we cannot guarantee our ability to do so in all circumstances.

RISKS
The risks of giving a blood sample are minor discomfort, bleeding or bruising of your arm and possible infection at the site where the blood was obtained.

Participation in research may cause a loss of privacy, but information about you will be kept as confidential as possible. Your name will not be used in any published reports about this study. We do not think that there will be further risks to your privacy and confidentiality by sharing your samples and study information with the biorepository. Your name or other identifiable information will never be given to the biorepository or other researchers and there are many safeguards in place to protect your information and samples while they are stored and used for research.

Genetic information that results from this study does not have medical or treatment importance at this time. However, there is a risk that information about taking part in a genetic study may influence insurance companies and/or employers regarding your health. To further safeguard your privacy, genetic information or any laboratory test results obtained in this study will not be placed in your medical record.

BENEFITS
There will be no benefit to you from participating in this study.

COSTS
There is no cost to you to participate in this study.

PAYMENT FOR PARTICIPATION
If you had to travel to a center to get your blood drawn, you will be given $20 by check for your time and travel expenses associated with coming to the blood center for study participation today, and for each visit you make for participation in this study in the future.

ALTERNATIVES
This is not a treatment study. Your alternative is not to participate in this study.

VOLUNTARY PARTICIPATION/WITHDRAWAL
Your participation in this study is voluntary. Your decision whether or not to participate will not change or influence your future relations with Blood Systems, Inc. If you decide to participate, you are free to discontinue participation at any time without harm to your rights or future relationships with Blood Systems, Inc. If you decide not to participate, or if you participate and later withdraw from study participation, your decision will involve no penalty or loss of benefits to which you may otherwise be entitled at this site.

Your participation in this study may be stopped at any time by the study doctor or the sponsor without your consent. For example, your participation would end in the event that the sponsor closes the study before the end of your follow-up as described in the Procedures section above.

QUESTIONS
If you have any questions, please ask us. If you have any additional questions later, or if at any time, regarding the research or if you feel you have experienced a research-related injury, contact:

Michael Busch, M.D., Ph.D. at *(415) 749-6615*

If you have questions about your rights as a research subject, you may contact:

Committee on Human Research
Office of Research
3333 California Street, Suite 315
University of California, San Francisco
San Francisco, CA 94118
(415) 476-1814

The Committee on Human Research is concerned with the protection of volunteers in research projects.

Do not sign this consent form unless you have had a chance to ask questions and have received satisfactory answers to all of your questions.

COMPENSATION FOR INJURY
If you are injured as a result of being in this study, treatment will be available. The costs of such treatment may be covered by Blood Systems. Inc., depending on a number of factors. Blood Systems, Inc. does not normally provide any other form of compensation for injury. Further information concerning treatment and payment of medical expenses in the event of any injury may be obtained from *Michael Busch, M.D., Ph.D.* at *(415) 749-6615*.

SOURCE OF FUNDING
Funding for this research study will be provided by Blood Systems Inc. and the National Heart, Lung, and Blood Institute of the National Institutes of Health.

If you agree to participate in this study, you will be given a signed and dated copy of this consent form (and the Experimental Subject's Bill of Rights) to keep for your records.

CONSENT
PARTICIPATION IN RESEARCH IS VOLUNTARY

You have read the information in this consent form (or it has been read to you). All of your questions about the study and your participation in it have been answered. You freely consent to participate in this research study.

By signing this consent form, you have not waived any of the legal rights, which you otherwise would have as a subject in a research study.

By signing this consent form, you agree to allow your blood samples and study data to be stored for future use in the biorepository of the NHLBI.

If you wish to participate you should sign below:

CONSENT SIGNATURE:

_____ _____

Signature of Subject Date

_____

Printed Name of Subject

_____ _____

Signature of Person Conducting Informed Consent Discussion Date

_____ _____

Signature of Investigator (if different from above) Date

[ ] Check and complete for subjects requiring surrogate consent:

_____ _____

Signature of Legally Authorized Representative
(When appropriate) Date

_____

Authority of Subject's Legally Authorized Representative or Relationship to Subject

_____ _____

Signature of Witness Date

----------Use the following only if applicable ----------

If this consent form is read to the subject because the subject (or legally authorized representative) is unable to read the form, an impartial witness not affiliated with the research or investigator must be present for the consent and sign the following statement:

I confirm that the information in the consent form and any other written information was accurately explained to, and apparently understood by, the subject (or the subject's legally authorized representative). The subject (or the subject's legally authorized representative) freely consented to participate in the research study.

_____ _____

Signature of Impartial Witness Date

Note: This signature block cannot be used for translations into another language. A translated consent form is necessary for enrolling subjects who do not speak English.

Blood Systems Research Institute
270 Masonic Avenue / San Francisco, CA 94118
+1 415 923 5771 / FAX +1 415 567 5899 / www.bsrisf.org

Date

Dear Name,

Thank you very much for agreeing to sign the enclosed consent form and for your valued participation in our 2009 West Nile virus study. We are committed to continuing to keep you informed and truly appreciate your efforts on behalf of the study.

Please sign the enclosed consent form and return it to us in the enclosed stamped, self-addressed envelope. If you have any additional questions since discussing this with our donor counselor, please do not hesitate to contact me. My contact information is below and is included on the consent form as well.

Again, thank you, for your help.

Sincerely,

Leslie Tobler, Dr. P.H.
Senior Scientist
ltobler@bloodsystems.org
415-749-6609

UCSF University of California San Francisco
IRB NUMBER: 10-01255
IRB APPROVAL DATE: 6/21/2010
IRB EXPIRATION DATE: 06/19/2011

RESEARCH SUBJECT INFORMATION AND CONSENT FORM
(Donors with a positive nucleic acid amplification test result for WNV)

TITLE: Virology and Immunology of West Nile Virus infection

SPONSORS: *Blood Systems, Inc.*

INVESTIGATOR: *Michael P. Busch, M.D., Ph.D.*
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270 Masonic Avenue
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PURPOSE AND BACKGROUND

In this study, the investigators hope to understand how infected persons clear West Nile virus (WNV) from their bodies and why some people get sick and others don't after being infected by this virus. By participating in our study and giving samples of their blood, a small group of people infected with WNV will allow researchers to work on understanding how the immune system and the virus interact, and how the body fights the virus.

You were selected as a possible subject for this study because we detected WNV in your blood. You have already received information regarding this result, and have been asked to participate in this study.

PROCEDURES

Blood Draw

If you decide to participate, study staff will take a blood sample (no more than 75 ml, or 5 tablespoons) from your arm today. You will also be asked to return once a week for the next three weeks, and again at the following intervals: 6 weeks, 2 months, 3 months, 6 months, 9 months and 12 months. This is a total of 9 more visits with blood draws after today. The amount of blood drawn during the initial eight week period of the study will not exceed 375 ml and the total amount of blood drawn over the course of the whole study (one year) will not exceed 750 ml.

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This is not a treatment study. Your alternative is not to participate in this study.

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If you wish to participate you should sign below:

CONSENT SIGNATURE:

_____ _____
Signature of Subject Date

_____
Printed Name of Subject

_____ _____
Signature of Person Conducting Informed Consent Discussion Date

_____ _____
Signature of Investigator (if different from above) Date

[ ] Check and complete for subjects requiring surrogate consent:

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(When appropriate)

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Authority of Subject's Legally Authorized Representative or Relationship to Subject

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UCSF University of California San Francisco
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RESEARCH SUBJECT INFORMATION AND CONSENT FORM
(Donors with a positive nucleic acid amplification test result for WNV)

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INVESTIGATOR: *Michael P. Busch, M.D., Ph.D.*
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IRB NUMBER: 10-01255
IRB APPROVAL DATE: 06/14/2011
IRB EXPIRATION DATE: 06/19/2012

at the NHLBI, every effort will be made to have your specimens removed, but we cannot guarantee our ability to do so in all circumstances.

Future Contact
We may contact you in the future to ask you to take part in new or ongoing research studies.

RISKS
The risks of giving a blood sample are minor discomfort, bleeding or bruising of your arm and possible infection at the site where the blood was obtained.

Participation in research may cause a loss of privacy, but information about you will be kept as confidential as possible. Your name will not be used in any published reports about this study. We do not think that there will be further risks to your privacy and confidentiality by sharing your samples and study information with the biorepository. Your name or other identifiable information will never be given to the biorepository or other researchers and there are many safeguards in place to protect your information and samples while they are stored and used for research.

Genetic information that results from this study does not have medical or treatment importance at this time. However, there is a risk that information about taking part in a genetic study may influence insurance companies and/or employers regarding your health. To further safeguard your privacy, genetic information or any laboratory test results obtained in this study will not be placed in your medical record.

BENEFITS
There will be no benefit to you from participating in this study.

COSTS
There is no cost to you to participate in this study.

PAYMENT FOR PARTICIPATION
After each study visit, you will be paid $40.00 for your time and travel expenses. In addition, after Visit 1, Visit 7 and Visit 10 you will be paid an additional $30.00 bonus. You will be paid by a check that will be mailed to you after each visit. The total amount you may receive if you complete all 10 study visits would be $490.

ALTERNATIVES
This is not a treatment study. Your alternative is not to participate in this study.

VOLUNTARY PARTICIPATION/WITHDRAWAL
Your participation in this study is voluntary. Your decision whether or not to participate will not change or influence your future relations with Blood Systems, Inc. If you decide to participate, you are free to discontinue participation at any time without harm to your rights or future relationships with Blood Systems, Inc. If you decide not to participate, or if you participate and later withdraw from study participation, your decision will involve no penalty or loss of benefits to which you may otherwise be entitled at this site.

UCSF University of California San Francisco
IRB NUMBER: 10-01255
IRB APPROVAL DATE: 06/14/2011
IRB EXPIRATION DATE: 06/19/2012

Your participation in this study may be stopped at any time by the study doctor or the sponsor without your consent. For example, your participation would end in the event that the sponsor closes the study before the end of your follow-up as described in the Procedures section above.

QUESTIONS
If you have any questions, please ask us. If you have any additional questions later, or if at any time, regarding the research or if you feel you have experienced a research-related injury, contact:

Michael Busch, M.D., Ph.D. at *(415) 749-6615*

If you have questions about your rights as a research subject, you may contact:

Committee on Human Research
Office of Research
3333 California Street, Suite 315
University of California, San Francisco
San Francisco, CA 94118
(415) 476-1814

The Committee on Human Research is concerned with the protection of volunteers in research projects.

Do not sign this consent form unless you have had a chance to ask questions and have received satisfactory answers to all of your questions.

COMPENSATION FOR INJURY
If you are injured as a result of being in this study, treatment will be available. The costs of such treatment may be covered by Blood Systems. Inc., depending on a number of factors. Blood Systems, Inc. does not normally provide any other form of compensation for injury. Further information concerning treatment and payment of medical expenses in the event of any injury may be obtained from *Michael Busch, M.D., Ph.D.* at *(415) 749-6615*.

SOURCE OF FUNDING
Funding for this research study will be provided by Blood Systems Inc., Novartis Vaccines and diagnostics, Inc., and the National Heart, Lung, and Blood Institute of the National Institutes of Health.

If you agree to participate in this study, you will be given a signed and dated copy of this consent form (and the Experimental Subject's Bill of Rights) to keep for your records.

CONSENT

UCSF University of California San Francisco
IRB NUMBER: 10-01255
IRB APPROVAL DATE: 06/14/2011
IRB EXPIRATION DATE: 06/19/2012

PARTICIPATION IN RESEARCH IS VOLUNTARY

You have read the information in this consent form (or it has been read to you). All of your questions about the study and your participation in it have been answered. You freely consent to participate in this research study.

By signing this consent form, you have not waived any of the legal rights, which you otherwise would have as a subject in a research study.

By signing this consent form, you agree to allow your blood samples and study data to be stored for future use in the biorepository of the NHLBI.

If you wish to participate you should sign below:

CONSENT SIGNATURE:

_____ _____
Signature of Subject Date

_____
Printed Name of Subject

_____ _____
Signature of Person Conducting Informed Consent Discussion Date

_____ _____
Signature of Investigator (if different from above) Date

----------**Use the following only if applicable** ----------
If this consent form is read to the subject because the subject (or legally authorized representative) is unable to read the form, an impartial witness not affiliated with the research or investigator must be present for the consent and sign the following statement:

I confirm that the information in the consent form and any other written information was accurately explained to, and apparently understood by, the subject (or the subject's legally authorized representative). The subject (or the subject's legally authorized representative) freely consented to participate in the research study.

_____ _____
Signature of Impartial Witness Date

Note: This signature block cannot be used for translations into another language. A translated consent form is necessary for enrolling subjects who do not speak English.

4018

Blood Systems Research Institute

West Nile Virus Initial Questionnaire (Questionnaire A)

Index Donation Number: | | | | | | | | | | | | | | |

Donor Id Number: | | | | | | | | | | | | | | |

Date of Index Donation (MM / DD / YYYY): | | / | | / | | | | |

Blood Center: | | | | | | | | | | | | | | |

A. Donor located for interview, if not select reason: Donor Located ○ Refused ○ Unable to Locate ○ Died ○ Other: ○

If OTHER please specify:_____

B. Date of Interview (MM / DD / YYYY): | | / | | / | | | | |

C. Donor Properly Identified: Yes ○ No ○ D. Interviewer initials: | | | |

Notes to interviewer:
*1. Complete this survey **AS EARLY AS POSSIBLE** after the date of the index donation.*
*2. **Complete Part II (Pages 7-8) of survey before contacting donor for interview. IF donor consents to interview (below) complete Part I (pages 1-6).(For Part II, If information is not complete in the donor record, please complete by asking donor at the time of interview.)***
3. Write the day and date of the index donation in the blanks preceding questions 5 and 12 before you begin the interview.
4. Identify donor by name, date of birth, or social security number.

Read:
We are asking you to be part of an investigation about West Nile virus infection in blood donors. Blood Systems Research Institute, in association with your local blood center, is in charge of this investigation. We are contacting you because a test has shown that you probably had West Nile virus the last time you donated blood. We want to study how you caught the virus and whether you have or had symptoms of West Nile virus infection. If you agree to participate, then I will ask you some questions about your medical history. The interview should only take about 15 minutes. We will keep your personal information confidential to the extent possible by law. Your participation is voluntary. You may refuse to answer any of the questions. If you have questions, you may ask me now. You may also call _____at your blood center at _____ or Dr. Hany Kamel at Blood Systems headquarters at 480-675-5659. Do you have any questions for me before we begin?

1. Do you agree to participate in this investigation? Yes ○ No ○ **If YES**, verbal consent obtained.

Signature of Medical Affairs staff:_____ Date:_____
Interviewer:
If **NO**, *then stop the interview.*
If **YES**, *then continue with the survey on next page and* **complete all pages 1 through 8.**

Continues on next page

4018

West Nile Virus Initial Questionnaire (Questionnaire A) Page 2 of 8

PART I Donor Reported Medical History

Read:

I am going to ask you some questions about whether you got sick around the time of your blood donation. If you have a calendar available, it may help you answer the questions more accurately. Do you have a calendar?

2. What is your **county** of residence? [ ]

*Note to Interviewer: Be sure to record **county** of residence so that we can relate information to CDC WNV databases. Zip code is not sufficient*

3. How many years have you lived at your current address? [ ] years Don't Know ○ Refused ○

4. What is your occupation? ***Interviewer write in response and then select closest match from list below:***

Occupation: [ ]

- ○ Business management or professional service
- ○ Farming, fishing, or forestry
- ○ Sales or office administration
- ○ Construction, building, or grounds maintenance
- ○ Health care services or support
- ○ Military service
- ○ Food preparation or restaurant industry
- ○ Transportation or material transport
- ○ Education or teaching
- ○ Other: _____

You donated blood on (MM / DD / YYYY): [ ][ ] / [ ][ ] / [ ][ ][ ][ ]

In the week before your donation, did you have any of the following symptoms?

5. Fever

Yes ○ No ○ Don't Know ○ Refused ○

If YES to 5: Yes No

5a. Was your temperature measured with a thermometer? ○ ○

If YES to 5a:

5b. What was the highest measured temperature? Don't Remember ○

[ ][ ][ ] . [ ] degrees F or [ ][ ] . [ ] degrees C

6. Headache

Yes ○ No ○ Don't Know ○ Refused ○

7. Eye Pain

Yes ○ No ○ Don't Know ○ Refused ○

8. Body aches (including stiff neck or neck pain)

Yes ○ No ○ Don't Know ○ Refused ○

9. New skin rash

Yes ○ No ○ Don't Know ○ Refused ○

Continues on next page

4018

West Nile Virus Initial Questionnaire (Questionnaire A) Page 3 of 8

10. Swollen lymph nodes

Yes ○ No ○ Don't Know ○ Refused ○

11. Nausea or vomiting

Yes ○ No ○ Don't Know ○ Refused ○

12. Muscle weakness

Yes ○ No ○ Don't Know ○ Refused ○

13. Confusion

Yes ○ No ○ Don't Know ○ Refused ○

14. Disorientation

Yes ○ No ○ Don't Know ○ Refused ○

15. Memory problems

Yes ○ No ○ Don't Know ○ Refused ○

16. **In the week before your donation**, did you develop any other symptoms?

Yes ○ No ○ Don't Know ○ Refused ○

If YES, What other symptoms?

16a. [ ]

16b. [ ]

You donated blood on (MM / DD / YYYY): [ ][ ] / [ ][ ] / [ ][ ][ ][ ]

On the day that you donated blood, after your donation did you have any of the following symptoms?

17. Fever

Yes ○ No ○ Don't Know ○ Refused ○

If YES to 17: Yes No

17a. Was your temperature measured with a thermometer? ○ ○

If YES to 17a:

17b. What was the highest measured temperature?

[ ][ ][ ] . [ ] degrees F or [ ][ ] . [ ] degrees C Don't Remember ○

18. Headache

Yes ○ No ○ Don't Know ○ Refused ○

19. Eye Pain

Yes ○ No ○ Don't Know ○ Refused ○

Continues on next page

4018

West Nile Virus Initial Questionnaire (Questionnaire A)

20. Body aches (including stiff neck or neck pain)

Yes ○ No ○ Don't Know ○ Refused ○

21. New skin rash

Yes ○ No ○ Don't Know ○ Refused ○

22. Swollen lymph nodes

Yes ○ No ○ Don't Know ○ Refused ○

23. Nausea or vomiting

Yes ○ No ○ Don't Know ○ Refused ○

24. Muscle weakness

Yes ○ No ○ Don't Know ○ Refused ○

25. Confusion

Yes ○ No ○ Don't Know ○ Refused ○

26. Disorientation

Yes ○ No ○ Don't Know ○ Refused ○

27. Memory problems

Yes ○ No ○ Don't Know ○ Refused ○

28. **On the day that you donated blood**, after your donation did you develop any other symptoms?

Yes ○ No ○ Don't Know ○ Refused ○

If YES, What other symptoms?

28a. | | | | | | | | | | | | | | | |

28b. | | | | | | | | | | | | | | | |

We want to know if West Nile Virus is more dangerous for people with certain medical conditions.

Has your doctor previously diagnosed you as having any of the following conditions ?

29. High blood pressure

Yes ○ No ○ Don't Know ○ Refused ○

30. Diabetes

Yes ○ No ○ Don't Know ○ Refused ○

31. Heart disease

Yes ○ No ○ Don't Know ○ Refused ○

32. Previous seizures

Yes ○ No ○ Don't Know ○ Refused ○

Continues on next page

4018

West Nile Virus Initial Questionnaire (Questionnaire A)

33. Previous stroke

Yes ○ No ○ Don't Know ○ Refused ○

34. Previous meningitis (inflammation of the lining of the brain or spinal cord)

Yes ○ No ○ Don't Know ○ Refused ○

35. Previous encephalitis (inflammation or infection of the brain)

Yes ○ No ○ Don't Know ○ Refused ○

36. At the time that you donated blood, were you taking any medications?

Yes ○ No ○ Don't Know ○ Refused ○

If YES, what medications were you taking?

36a. | | | | | | | | | | | | | | |

36b. | | | | | | | | | | | | | | |

36c. | | | | | | | | | | | | | | |

36d. | | | | | | | | | | | | | | |

37. At time you donated blood, were you a cigarette smoker?

Yes ○ No ○ Don't Know ○ Refused ○

IF YES, On average how many cigarettes do you smoke in one day?

37a. | | | cigarettes per day Don't Know ○ Refused ○

Now we would like to ask about some of your PAST medical history.

Have you ever been diagnosed by a doctor with any of the following diseases?

38. Any previous illness due to West Nile virus infection:

Yes ○ What Year? | | | | | No ○ Don't Know ○ Refused ○

39. St. Louis encephalitis:

Yes ○ What Year? | | | | | No ○ Don't Know ○ Refused ○

40. Dengue ("deng gee") fever:

Yes ○ What Year? | | | | | No ○ Don't Know ○ Refused ○

41. Japanese encephalitis:

Yes ○ What Year? | | | | | No ○ Don't Know ○ Refused ○

Continues on next page

4018

West Nile Virus Initial Questionnaire (Questionnaire A) Page 6 of 8

Have you ever been vaccinated against any of the following diseases?

42. Yellow fever:

Yes ○ What Year? [ ][ ][ ][ ] No ○ Don't Know ○ Refused ○

43. Japanese encephalitis:

Yes ○ What Year? [ ][ ][ ][ ] No ○ Don't Know ○ Refused ○

44. Tick-borne encephalitis:

Yes ○ What Year? [ ][ ][ ][ ] No ○ Don't Know ○ Refused ○

45. Have you ever served in the military?

Yes ○ No ○ Don't Know ○ Refused ○

If YES, please provide the dates and locations of service.

45a. Entered military service (MM / DD / YYYY): [ ][ ] / [ ][ ] / [ ][ ][ ][ ]

45b. Left military service (MM / DD / YYYY): [ ][ ] / [ ][ ] / [ ][ ][ ][ ]

Where were you stationed?

45c. [ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ]

45d. [ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ]

45e. [ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ]

46. Have you traveled outside your current state of residence in the last 3 weeks?

Yes ○ No ○ Don't Know ○ Refused ○

If YES, please list all of the states you have traveled in:

46a. [ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ]

46b. [ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ]

46c. [ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ]

46d. [ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ]

Continues on next page

4018

West Nile Virus Initial Questionnaire (Questionnaire A) Page 7 of 8

PART II Index Donation Record

47. In the past 5 years, have you traveled to or lived in a country outside of the United States?

Yes ○ No ○ Don't Know ○ Refused ○

If YES, please list all of the countries you have traveled or lived in and the dates you were there starting with the most recent and working backward:

47a. Country: | | | | | | | | | | | | | Dates in country: _____

47b. Country: | | | | | | | | | | | | | Dates in country: _____

47c. Country: | | | | | | | | | | | | | Dates in country: _____

47d. Country: | | | | | | | | | | | | | Dates in country: _____

47e. Country: | | | | | | | | | | | | | Dates in country: _____

Interviewer if the next section was completed using the index donation record, **Please Read:** This concludes the survey today. Thank you very much for your participation. We will contact you in a few weeks for follow-up surveys.

Interviewer or Blood Center Staff:
1. Complete this part of the survey for all donors whether they have participated in the survey or not.
*2. Obtain the information from the Donation Records of the Blood Center. (IF Information is not complete in the donor record **and** donor consents to be interviewed, please complete by asking donor at the time of interview.)*

48. Index donation phlebotomy type (enter letter code from record): | |

Donor Demographics

49. Zip code of donor's residence on date of index donation: | | | | | |

50. Donor initials: | | | |

51. Date of birth: (MM / DD / YYYY) | | / | | / | | | | |

52. Gender: Male ○ Female ○

53. Race:

- ○ White or Caucasian
- ○ Black or African-American
- ○ Asian
- ○ Native Hawaiian or Other Pacific Islander
- ○ American Indian or Native Alaskan
- ○ Other:_____
- ○ Don't Know
- ○ Refused

Continues on next page

4018

West Nile Virus Initial Questionnaire (Questionnaire A)

54. Ethnicity

Hispanic or Latino ○ **NOT** Hispanic or Latino ○ Don't Know ○ Refused ○

55. Educational attainment:

○ 8th Grade or Less

○ Some High School but **NO** Diploma

○ High school graduate (for example, Diploma or GED)

○ Some College or Technical School

○ Bachelor Degree (for example, BA, BS, or AB)

○ Master or Professional Degree (for example, MA, MS, MD, PhD, or JD)

Physical Findings

56. Donor temperature? ☐☐☐.☐ degrees F or ☐☐.☐ degrees C

57. Pulse? ☐☐

58. Blood pressure? ☐☐☐ systolic / ☐☐☐ diastolic

Responses to Health History Questionnaire

	Yes	No
59. Feeling well and health today:	○	○
60. Fever with headache in past 7 days:	○	○
61. Pills or medications in past 4 weeks:	○	○
62. Shots or vaccinations in past 4 weeks:	○	○

If YES, which shots or vaccinations:

62a. ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

62b. ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

62c. ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

62d. ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

Interviewer, if the previous section was completed at the time of interview, **Please Read:** This concludes the survey today. Thank you very much for your participation. We will contact you in a few weeks for follow-up surveys.

End of Questionnaire

15574

Blood Systems Research Institute
West Nile Virus Follow-up Questionnaire (Questionnaire B)

Index Donation Number: | | | | | | | | | | | | | | | |

Donor ID Number: | | | | | | | | | | | | | | | |

Date of Index Donation (MM / DD / YYYY): | | | / | | | / | | | | |

Blood Center: | | | | | | | | | | | | | | | |

A. Donor located for interview, if not select reason: Donor Located ○ Refused ○ Unable to Locate ○ Died ○ Other: ○

If OTHER please specify:_____

B. Date of Interview (MM / DD / YYYY): | | / | | / | | | | |

C. Donor Properly Identified: Yes ○ No ○

D. Interviewer initials: | | | |

Note to interviewer:
*1. Complete this survey **AT LEAST 14 DAYS AFTER** the date of the index donation*
2. Write the day and date of the index donation in the blanks preceding question 2 before you begin the interview.
3. Identify donor by name, date of birth, or social security number

Read:
We are asking you to be part of an investigation about West Nile virus infection in blood donors. Blood Systems Research Institute, in association with your local blood center, is in charge of this investigation. We are contacting you because a test has shown that you probably had West Nile virus the last time you donated blood. We want to study how you caught the virus and whether you have or had symptoms of West Nile virus infection. If you agree to participate, then I will ask you some questions about your medical history. The interview should only take about 5-10 minutes. We will keep your personal information confidential to the extent possible by law. Your participation is voluntary. You may refuse to answer any of the questions. If you have questions, you may ask me now. You may also call _____at your blood center at _____ or Dr. Hany Kamel at Blood Systems headquarters at 480-675-5659. Do you have any questions for me before we begin?

1. Do you agree to participate in this investigation? Yes ○ No ○ **If YES**, verbal consent obtained.

Signature of Medical Affairs staff:_____ Date:_____

Interviewer:
If **NO***, then stop the interview***.**
If **YES***, then continue with the survey on next page and complete all pages***.**

Continues on next page

15574

West Nile Virus Follow-up Questionnaire (Questionnaire B)

Read:

I am going to ask you some questions about whether you got sick in the 14 days after your blood donation. If you have a calendar available, it may help you answer the questions more accurately. Do you have a calendar?

You donated blood on (MM / DD / YYYY): ☐☐ / ☐☐ / ☐☐☐☐

In the 14 days after your donation, did you have any of the following symptoms?

2. Fever

Yes ○ No ○ Don't Know ○ Refused ○

If YES to 2: Yes No

2a. Was your temperature measured with a thermometer? ○ ○

If YES to 2a:

2b. What was the highest measured temperature?

☐☐☐.☐ degrees F or ☐☐.☐ degrees C Don't Remember ○

3. Headache

Yes ○ No ○ Don't Know ○ Refused ○

4. Eye Pain

Yes ○ No ○ Don't Know ○ Refused ○

5. Body aches (including stiff neck or neck pain)

Yes ○ No ○ Don't Know ○ Refused ○

6. New skin rash

Yes ○ No ○ Don't Know ○ Refused ○

7. Swollen lymph nodes

Yes ○ No ○ Don't Know ○ Refused ○

8. Nausea or vomiting

Yes ○ No ○ Don't Know ○ Refused ○

9. Muscle weakness

Yes ○ No ○ Don't Know ○ Refused ○

10. Confusion

Yes ○ No ○ Don't Know ○ Refused ○

Continues on next page

15574

West Nile Virus Follow-up Questionnaire (Questionnaire B) Page 3 of 4

11. Disorientation

Yes ○ No ○ Don't Know ○ Refused ○

12. Memory problems

Yes ○ No ○ Don't Know ○ Refused ○

13. **In the 14 days after your donation**, did you develop any other symptoms?

Yes ○ No ○ Don't Know ○ Refused ○

If YES, What other symptoms?

13a. ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

13b. ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

13c. ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

13d. ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

Interviewer:
*1. If the respondent answered **NO** to every Symptom Question 2 through 13, **then stop and conclude the interview by reading the text in the middle of Page 4.***

*2. If the respondent answered **YES** to **ANY** Symptom Question 2 through 13, **then continue with Questions 14 through 19.***

14. What was the date of onset of your illness? (MM / DD / YYYY)

☐☐ / ☐☐ / ☐☐☐☐ Don't Know ○ Refused ○

If respondent answered Don't Know:

14a. How many days after donation did you get sick? ☐☐ days Don't Know ○ Refused ○

15. What was the date of resolution of your illness or symptom(s)? (MM / DD / YYYY)

☐☐ / ☐☐ / ☐☐☐☐ Not Yet Resolved ○ Don't Know ○ Refused ○

16. Did you go to a doctor or clinic for this illness?

Yes ○ No ○ Don't Know ○ Refused ○

Continues on next page

15574

West Nile Virus Follow-up Questionnaire (Questionnaire B) Page 4 of 4

17. Did you spend at least one night in the hospital because of this illness?

Yes ○ No ○ Don't Know ○ Refused ○

If YES, 17a. What was the date of your admission to the hospital? (MM / DD / YYYY)

☐☐ / ☐☐ / ☐☐☐☐ Don't Know ○ Refused ○

18. Did your doctor or other health care professional tell you had West Nile fever?

Yes ○ No ○ Don't Know ○ Refused ○

19. Did your doctor or other health care professional tell you had West Nile meningitis or encephalitis?

Yes ○ No ○ Don't Know ○ Refused ○

Read:
This concludes this survey. You may be contacted in a few months for a final follow-up survey.

End of questionnaire

8738

2010 - Blood Systems Research Institute
West Nile Virus Initial Questionnaire (Questionnaire A)

Index Donation Number: | | | | | | | | | | | | | | | |

Donor Id Number: | | | | | | | | | | | | | | | |

Date of Index Donation (MM / DD / YYYY): | | | / | | | / | | | | |

Blood Center: | | | | | | | | | | | | | | | |

A. Donor located for interview, if not select reason: Donor Located ○ Refused ○ Unable to Locate ○ Died ○ Other: ○

If OTHER please specify:_____

B. Date of Interview (MM / DD / YYYY): | | | / | | | / | | | | |

C. Donor Properly Identified: Yes ○ No ○ D. Interviewer initials: | | | |

Notes to interviewer:
*1. Complete this survey **AS EARLY AS POSSIBLE** after the date of the index donation.*
*2. **Complete Part II (Pages 7-8) of survey before contacting donor for interview. IF donor consents to interview (below) complete Part I (pages 1-6).(For Part II, If information is not complete in the donor record, please complete by asking donor at the time of interview.)***
3. Write the day and date of the index donation in the blanks preceding questions 5 and 12 before you begin the interview.
4. Identify donor by name, date of birth, or social security number.

Read:
We are asking you to be part of an investigation about West Nile virus infection in blood donors. Blood Systems Research Institute, in association with your local blood center, is in charge of this investigation. We are contacting you because a test has shown that you probably had West Nile virus the last time you donated blood. We want to study how you caught the virus and whether you have or had symptoms of West Nile virus infection. If you agree to participate, then I will ask you some questions about your medical history. The interview should only take about 15 minutes. We will keep your personal information confidential to the extent possible by law. Your participation is voluntary. You may refuse to answer any of the questions. If you have questions, you may ask me now. You may also call _____at your blood center at _____ or Dr. Hany Kamel at Blood Systems headquarters at 480-675-5659. Do you have any questions for me before we begin?

1. Do you agree to participate in this investigation? Yes ○ No ○ **If YES**, verbal consent obtained.

Signature of Medical Affairs staff:_____ Date:_____
Interviewer:
If **NO**, *then stop the interview.*
If **YES**, *then continue with the survey on next page and* **complete all pages 1 through 8.**

Continues on next page

8738

West Nile Virus Initial Questionnaire (Questionnaire A) Page 2 of 8

PART I Donor Reported Medical History

Read:

I am going to ask you some questions about whether you got sick around the time of your blood donation. If you have a calendar available, it may help you answer the questions more accurately. Do you have a calendar?

2. What is your **county** of residence? [ ]

*Note to Interviewer: Be sure to record **county** of residence so that we can relate information to CDC WNV databases. Zip code is not sufficient*

3. How many years have you lived at your current address? [ ] years Don't Know ○ Refused ○

4. What is your occupation? ***Interviewer write in response and then select closest match from list below:***

Occupation: [ ]

- ○ Business management or professional service
- ○ Farming, fishing, or forestry
- ○ Sales or office administration
- ○ Construction, building, or grounds maintenance
- ○ Health care services or support
- ○ Military service
- ○ Food preparation or restaurant industry
- ○ Transportation or material transport
- ○ Education or teaching
- ○ Other: _____

You donated blood on (MM / DD / YYYY): [ ][ ] / [ ][ ] / [ ][ ][ ][ ]

In the week before your donation, did you have any of the following symptoms?

5. Fever

Yes ○ No ○ Don't Know ○ Refused ○

If YES to 5: Yes No

5a. Was your temperature measured with a thermometer? ○ ○

If YES to 5a:

5b. What was the highest measured temperature?

[ ][ ][ ] . [ ] degrees F or [ ][ ] . [ ] degrees C Don't Remember ○

6. Headache

Yes ○ No ○ Don't Know ○ Refused ○

7. Eye Pain

Yes ○ No ○ Don't Know ○ Refused ○

8. Body aches (including stiff neck or neck pain)

Yes ○ No ○ Don't Know ○ Refused ○

9. New skin rash

Yes ○ No ○ Don't Know ○ Refused ○

Continues on next page

8738

West Nile Virus Initial Questionnaire (Questionnaire A) Page 3 of 8

10. Swollen lymph nodes

Yes ○ No ○ Don't Know ○ Refused ○

11. Nausea or vomiting

Yes ○ No ○ Don't Know ○ Refused ○

12. Muscle weakness

Yes ○ No ○ Don't Know ○ Refused ○

13. Confusion

Yes ○ No ○ Don't Know ○ Refused ○

14. Disorientation

Yes ○ No ○ Don't Know ○ Refused ○

15. Memory problems

Yes ○ No ○ Don't Know ○ Refused ○

16. **In the week before your donation**, did you develop any other symptoms?

Yes ○ No ○ Don't Know ○ Refused ○

If YES, What other symptoms?

16a. [ ]

16b. [ ]

You donated blood on (MM / DD / YYYY): [ ][ ] / [ ][ ] / [ ][ ][ ][ ]

On the day that you donated blood, after your donation did you have any of the following symptoms?

17. Fever

Yes ○ No ○ Don't Know ○ Refused ○

If YES to 17: Yes No

17a. Was your temperature measured with a thermometer? ○ ○

If YES to 17a:

17b. What was the highest measured temperature?

[ ][ ][ ] . [ ] degrees F or [ ][ ] . [ ] degrees C Don't Remember ○

18. Headache

Yes ○ No ○ Don't Know ○ Refused ○

19. Eye Pain

Yes ○ No ○ Don't Know ○ Refused ○

Continues on next page

West Nile Virus Initial Questionnaire (Questionnaire A)

20. Body aches (including stiff neck or neck pain)

Yes ○ No ○ Don't Know ○ Refused ○

21. New skin rash

Yes ○ No ○ Don't Know ○ Refused ○

22. Swollen lymph nodes

Yes ○ No ○ Don't Know ○ Refused ○

23. Nausea or vomiting

Yes ○ No ○ Don't Know ○ Refused ○

24. Muscle weakness

Yes ○ No ○ Don't Know ○ Refused ○

25. Confusion

Yes ○ No ○ Don't Know ○ Refused ○

26. Disorientation

Yes ○ No ○ Don't Know ○ Refused ○

27. Memory problems

Yes ○ No ○ Don't Know ○ Refused ○

28. **On the day that you donated blood**, after your donation did you develop any other symptoms?

Yes ○ No ○ Don't Know ○ Refused ○

If YES, What other symptoms?

28a. | | | | | | | | | | | | | | |

28b. | | | | | | | | | | | | | | |

We want to know if West Nile Virus is more dangerous for people with certain medical conditions.

Has your doctor previously diagnosed you as having any of the following conditions ?

29. High blood pressure

Yes ○ No ○ Don't Know ○ Refused ○

30. Diabetes

Yes ○ No ○ Don't Know ○ Refused ○

31. Heart disease

Yes ○ No ○ Don't Know ○ Refused ○

32. Previous seizures

Yes ○ No ○ Don't Know ○ Refused ○

Continues on next page

8738

West Nile Virus Initial Questionnaire (Questionnaire A) Page 5 of 8

33. Previous stroke

Yes ○ No ○ Don't Know ○ Refused ○

34. Previous meningitis (inflammation of the lining of the brain or spinal cord)

Yes ○ No ○ Don't Know ○ Refused ○

35. Previous encephalitis (inflammation or infection of the brain)

Yes ○ No ○ Don't Know ○ Refused ○

36. At the time that you donated blood, were you taking any medications?

Yes ○ No ○ Don't Know ○ Refused ○

If YES, what medications were you taking?

36a. ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

36b. ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

36c. ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

36d. ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

37. At time you donated blood, were you a cigarette smoker?

Yes ○ No ○ Don't Know ○ Refused ○

IF YES, On average how many cigarettes do you smoke in one day?

37a. ☐☐ cigarettes per day Don't Know ○ Refused ○

Now we would like to ask about some of your PAST medical history.

Have you ever been diagnosed by a doctor with any of the following diseases?

38. Any previous illness due to West Nile virus infection:

Yes ○ What Year? ☐☐☐☐ No ○ Don't Know ○ Refused ○

39. St. Louis encephalitis:

Yes ○ What Year? ☐☐☐☐ No ○ Don't Know ○ Refused ○

40. Dengue ("deng gee") fever:

Yes ○ What Year? ☐☐☐☐ No ○ Don't Know ○ Refused ○

41. Japanese encephalitis:

Yes ○ What Year? ☐☐☐☐ No ○ Don't Know ○ Refused ○

Continues on next page

8738

West Nile Virus Initial Questionnaire (Questionnaire A) Page 6 of 8

Have you ever been vaccinated against any of the following diseases?

42. Yellow fever:

Yes ○ What Year? [ ][ ][ ][ ] No ○ Don't Know ○ Refused ○

43. Japanese encephalitis:

Yes ○ What Year? [ ][ ][ ][ ] No ○ Don't Know ○ Refused ○

44. Tick-borne encephalitis:

Yes ○ What Year? [ ][ ][ ][ ] No ○ Don't Know ○ Refused ○

45. Have you ever served in the military?

Yes ○ No ○ Don't Know ○ Refused ○

If YES, please provide the dates and locations of service.

45a. Entered military service (MM / DD / YYYY): [ ][ ] / [ ][ ] / [ ][ ][ ][ ]

45b. Left military service (MM / DD / YYYY): [ ][ ] / [ ][ ] / [ ][ ][ ][ ]

Where were you stationed?

45c. [ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ]

45d. [ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ]

45e. [ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ]

46. Have you traveled outside your current state of residence in the last 3 weeks?

Yes ○ No ○ Don't Know ○ Refused ○

If YES, please list all of the states you have traveled in:

46a. [ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ]

46b. [ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ]

46c. [ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ]

46d. [ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ]

Continues on next page

8738

West Nile Virus Initial Questionnaire (Questionnaire A) Page 7 of 8

PART II Index Donation Record

47. In the past 5 years, have you traveled to or lived in a country outside of the United States?

Yes ○ No ○ Don't Know ○ Refused ○

If YES, please list all of the countries you have traveled or lived in and the dates you were there starting with the most recent and working backward:

47a. Country: | | | | | | | | | | | | | Dates in country: _____

47b. Country: | | | | | | | | | | | | | Dates in country: _____

47c. Country: | | | | | | | | | | | | | Dates in country: _____

47d. Country: | | | | | | | | | | | | | Dates in country: _____

47e. Country: | | | | | | | | | | | | | Dates in country: _____

***Interviewer if the next section was completed using the index donation record,* Please Read:** This concludes the survey today. Thank you very much for your participation. We will contact you in a few weeks for follow-up surveys.

Interviewer or Blood Center Staff:
1. Complete this part of the survey for all donors whether they have participated in the survey or not.
*2. Obtain the information from the Donation Records of the Blood Center. (IF Information is not complete in the donor record **and** donor consents to be interviewed, please complete by asking donor at the time of interview.)*

48. Index donation phlebotomy type (enter letter code from record): | |

Donor Demographics

49. Zip code of donor's residence on date of index donation: | | | | | |

50. Donor initials: | | | |

51. Date of birth: (MM / DD / YYYY) | | / | | / | | | | |

52. Gender: Male ○ Female ○

53. Race:

- ○ White or Caucasian
- ○ Black or African-American
- ○ Asian
- ○ Native Hawaiian or Other Pacific Islander
- ○ American Indian or Native Alaskan
- ○ Other:_____
- ○ Don't Know
- ○ Refused

Continues on next page

8738

West Nile Virus Initial Questionnaire (Questionnaire A)

54. Ethnicity

Hispanic or Latino ○ **NOT** Hispanic or Latino ○ Don't Know ○ Refused ○

55. Educational attainment:

○ 8th Grade or Less

○ Some High School but **NO** Diploma

○ High school graduate (for example, Diploma or GED)

○ Some College or Technical School

○ Bachelor Degree (for example, BA, BS, or AB)

○ Master or Professional Degree (for example, MA, MS, MD, PhD, or JD)

Physical Findings

56. Donor temperature? ☐☐☐.☐ degrees F or ☐☐.☐ degrees C

57. Pulse? ☐☐

58. Blood pressure? ☐☐☐ systolic / ☐☐☐ diastolic

Responses to Health History Questionnaire

	Yes	No
59. Feeling well and health today:	○	○
~~60. Fever with headache in past 7 days:~~	○	○
61. Pills or medications in past 4 weeks:	○	○
62. Shots or vaccinations in past 4 weeks:	○	○

If YES, which shots or vaccinations:

62a. ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

62b. ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

62c. ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

62d. ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

Interviewer, if the previous section was completed at the time of interview, **Please Read:** This concludes the survey today. Thank you very much for your participation. We will contact you in a few weeks for a follow-up survey.

End of Questionnaire

11968

2010 - Blood Systems Research Institute
West Nile Virus Follow-up Questionnaire (Questionnaire B)

Index Donation Number: | | | | | | | | | | | | | | | |

Donor ID Number: | | | | | | | | | | | | | | | |

Date of Index Donation (MM / DD / YYYY): | | | / | | | / | | | | |

Blood Center: | | | | | | | | | | | | | | | |

A. Donor located for interview, if not select reason: Donor Located ○ Refused ○ Unable to Locate ○ Died ○ Other: ○

If OTHER please specify:_____

B. Date of Interview (MM / DD / YYYY): | | / | | / | | | | |

C. Donor Properly Identified: Yes ○ No ○

D. Interviewer initials: | | | |

Note to interviewer:
*1. Complete this survey **AT LEAST 14 DAYS AFTER** the date of the index donation*
2. Write the day and date of the index donation in the blanks preceding question 2 before you begin the interview.
3. Identify donor by name, date of birth, or social security number

Read:
We are asking you to be part of an investigation about West Nile virus infection in blood donors. Blood Systems Research Institute, in association with your local blood center, is in charge of this investigation. We are contacting you because a test has shown that you probably had West Nile virus the last time you donated blood. We want to study how you caught the virus and whether you have or had symptoms of West Nile virus infection. If you agree to participate, then I will ask you some questions about your medical history. The interview should only take about 5-10 minutes. We will keep your personal information confidential to the extent possible by law. Your participation is voluntary. You may refuse to answer any of the questions. If you have questions, you may ask me now. You may also call _____at your blood center at _____ or Dr. Hany Kamel at Blood Systems headquarters at 480-675-5659. Do you have any questions for me before we begin?

1. Do you agree to participate in this investigation? Yes ○ No ○ **If YES**, verbal consent obtained.

Signature of Medical Affairs staff:_____ Date:_____

Interviewer:
If **NO***, then stop the interview***.**
If **YES***, then continue with the survey on next page and complete all pages***.**

Continues on next page

11968

West Nile Virus Follow-up Questionnaire (Questionnaire B)

Read:

I am going to ask you some questions about whether you got sick in the 14 days after your blood donation. If you have a calendar available, it may help you answer the questions more accurately. Do you have a calendar?

You donated blood on (MM / DD / YYYY): ☐☐ / ☐☐ / ☐☐☐☐

In the 14 days after your donation, did you have any of the following symptoms?

2. Fever

Yes ○ No ○ Don't Know ○ Refused ○

If YES to 2: Yes No

2a. Was your temperature measured with a thermometer? ○ ○

If YES to 2a:

2b. What was the highest measured temperature?

☐☐☐.☐ degrees F or ☐☐.☐ degrees C Don't Remember ○

3. Headache

Yes ○ No ○ Don't Know ○ Refused ○

4. Eye Pain

Yes ○ No ○ Don't Know ○ Refused ○

5. Body aches (including stiff neck or neck pain)

Yes ○ No ○ Don't Know ○ Refused ○

6. New skin rash

Yes ○ No ○ Don't Know ○ Refused ○

7. Swollen lymph nodes

Yes ○ No ○ Don't Know ○ Refused ○

8. Nausea or vomiting

Yes ○ No ○ Don't Know ○ Refused ○

9. Muscle weakness

Yes ○ No ○ Don't Know ○ Refused ○

10. Confusion

Yes ○ No ○ Don't Know ○ Refused ○

Continues on next page

11968

West Nile Virus Follow-up Questionnaire (Questionnaire B) Page 3 of 4

11. Disorientation

Yes ○ No ○ Don't Know ○ Refused ○

12. Memory problems

Yes ○ No ○ Don't Know ○ Refused ○

13. **In the 14 days after your donation**, did you develop any other symptoms?

Yes ○ No ○ Don't Know ○ Refused ○

If YES, What other symptoms?

13a. ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

13b. ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

13c. ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

13d. ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

Interviewer:
*1. If the respondent answered **NO** to every Symptom Question 2 through 13, **then stop and conclude the interview by reading the text in the middle of Page 4.***

*2. If the respondent answered **YES** to **ANY** Symptom Question 2 through 13, **then continue with Questions 14 through 19.***

14. What was the date of onset of your illness? (MM / DD / YYYY)

☐☐ / ☐☐ / ☐☐☐☐ Don't Know ○ Refused ○

If respondent answered Don't Know:

14a. How many days after donation did you get sick? ☐☐ days Don't Know ○ Refused ○

15. What was the date of resolution of your illness or symptom(s)? (MM / DD / YYYY)

☐☐ / ☐☐ / ☐☐☐☐ Not Yet Resolved ○ Don't Know ○ Refused ○

16. Did you go to a doctor or clinic for this illness?

Yes ○ No ○ Don't Know ○ Refused ○

Continues on next page

11968

West Nile Virus Follow-up Questionnaire (Questionnaire B)

17. Did you spend at least one night in the hospital because of this illness?

Yes ○ No ○ Don't Know ○ Refused ○

If YES, 17a. What was the date of your admission to the hospital? (MM / DD / YYYY)

☐☐ / ☐☐ / ☐☐☐☐ Don't Know ○ Refused ○

18. Did your doctor or other health care professional tell you had West Nile fever?

Yes ○ No ○ Don't Know ○ Refused ○

19. Did your doctor or other health care professional tell you had West Nile meningitis or encephalitis?

Yes ○ No ○ Don't Know ○ Refused ○

Read:

This concludes this survey. Thank you again for your participation.

62725

2011 - Blood Systems Research Institute
West Nile Virus Initial Questionnaire (Questionnaire A)

Index Donation Number: | | | | | | | | | | | | | | | |

Donor Id Number: | | | | | | | | | | | | | | | |

Date of Index Donation (MM / DD / YYYY): | | | / | | | / | | | | |

Blood Center: | | | | | | | | | | | | | | | |

A. Donor located for interview, if not select reason: Donor Located ○ Refused ○ Unable to Locate ○ Died ○ Other: ○

If OTHER please specify:_____

B. Date of Interview (MM / DD / YYYY): | | / | | / | | | | |

C. Donor Properly Identified: Yes ○ No ○ D. Interviewer initials: | | |

Notes to interviewer:
*1. Complete this survey **AS EARLY AS POSSIBLE** after the date of the index donation.*
*2. **Complete Part II (Pages 7-8) of survey before contacting donor for interview. IF donor consents to interview (below) complete Part I (pages 1-6).(For Part II, If information is not complete in the donor record, please complete by asking donor at the time of interview.)***
3. Write the day and date of the index donation in the blanks preceding questions 5 and 12 before you begin the interview.
4. Identify donor by name, date of birth, or social security number.

Read:
We are asking you to be part of an investigation about West Nile virus infection in blood donors. Blood Systems Research Institute, in association with your local blood center, is in charge of this investigation. We are contacting you because a test has shown that you probably had West Nile virus the last time you donated blood. We want to study how you caught the virus and whether you have or had symptoms of West Nile virus infection. If you agree to participate, then I will ask you some questions about your medical history. The interview should only take about 15 minutes. We will keep your personal information confidential to the extent possible by law. Your participation is voluntary. You may refuse to answer any of the questions. If you have questions, you may ask me now. You may also call _____at your blood center at _____ or Dr. Hany Kamel at Blood Systems headquarters at 480-675-5659. Do you have any questions for me before we begin?

1. Do you agree to participate in this investigation? Yes ○ No ○ **If YES**, verbal consent obtained.

Signature of Medical Affairs staff:_____ Date:_____
Interviewer:
If **NO**, *then stop the interview.*
If **YES**, *then continue with the survey on next page and* **complete all pages 1 through 8.**

Continues on next page

62725

West Nile Virus Initial Questionnaire (Questionnaire A) Page 2 of 8

PART I Donor Reported Medical History

Read:

I am going to ask you some questions about whether you got sick around the time of your blood donation. If you have a calendar available, it may help you answer the questions more accurately. Do you have a calendar?

2. What is your **county** of residence? [ ]

*Note to Interviewer: Be sure to record **county** of residence so that we can relate information to CDC WNV databases. Zip code is not sufficient*

3. How many years have you lived at your current address? [ ] years Don't Know ○ Refused ○

4. What is your occupation? ***Interviewer write in response and then select closest match from list below:***

Occupation: [ ]

- ○ Business management or professional service
- ○ Farming, fishing, or forestry
- ○ Sales or office administration
- ○ Construction, building, or grounds maintenance
- ○ Health care services or support
- ○ Military service
- ○ Food preparation or restaurant industry
- ○ Transportation or material transport
- ○ Education or teaching
- ○ Other: _____

You donated blood on (MM / DD / YYYY): [ ][ ] / [ ][ ] / [ ][ ][ ][ ]

In the week before your donation, did you have any of the following symptoms?

5. Fever

Yes ○ No ○ Don't Know ○ Refused ○

If YES to 5: Yes No

5a. Was your temperature measured with a thermometer? ○ ○

If YES to 5a:

5b. What was the highest measured temperature?

[ ][ ][ ] . [ ] degrees F or [ ][ ] . [ ] degrees C Don't Remember ○

6. Headache

Yes ○ No ○ Don't Know ○ Refused ○

7. Eye Pain

Yes ○ No ○ Don't Know ○ Refused ○

8. Body aches (including stiff neck or neck pain)

Yes ○ No ○ Don't Know ○ Refused ○

9. New skin rash

Yes ○ No ○ Don't Know ○ Refused ○

Continues on next page

62725

West Nile Virus Initial Questionnaire (Questionnaire A) Page 3 of 8

10. Swollen lymph nodes

Yes ○ No ○ Don't Know ○ Refused ○

11. Nausea or vomiting

Yes ○ No ○ Don't Know ○ Refused ○

12. Muscle weakness

Yes ○ No ○ Don't Know ○ Refused ○

13. Confusion

Yes ○ No ○ Don't Know ○ Refused ○

14. Disorientation

Yes ○ No ○ Don't Know ○ Refused ○

15. Memory problems

Yes ○ No ○ Don't Know ○ Refused ○

16. **In the week before your donation**, did you develop any other symptoms?

Yes ○ No ○ Don't Know ○ Refused ○

If YES, What other symptoms?

16a. [ ]

16b. [ ]

You donated blood on (MM / DD / YYYY): [ ][ ] / [ ][ ] / [ ][ ][ ][ ]

On the day that you donated blood, after your donation did you have any of the following symptoms?

17. Fever

Yes ○ No ○ Don't Know ○ Refused ○

If YES to 17: Yes No

17a. Was your temperature measured with a thermometer? ○ ○

If YES to 17a:

17b. What was the highest measured temperature?

[ ][ ][ ] . [ ] degrees F or [ ][ ] . [ ] degrees C Don't Remember ○

18. Headache

Yes ○ No ○ Don't Know ○ Refused ○

19. Eye Pain

Yes ○ No ○ Don't Know ○ Refused ○

Continues on next page

62725

West Nile Virus Initial Questionnaire (Questionnaire A)

20. Body aches (including stiff neck or neck pain)

Yes ○ No ○ Don't Know ○ Refused ○

21. New skin rash

Yes ○ No ○ Don't Know ○ Refused ○

22. Swollen lymph nodes

Yes ○ No ○ Don't Know ○ Refused ○

23. Nausea or vomiting

Yes ○ No ○ Don't Know ○ Refused ○

24. Muscle weakness

Yes ○ No ○ Don't Know ○ Refused ○

25. Confusion

Yes ○ No ○ Don't Know ○ Refused ○

26. Disorientation

Yes ○ No ○ Don't Know ○ Refused ○

27. Memory problems

Yes ○ No ○ Don't Know ○ Refused ○

28. **On the day that you donated blood**, after your donation did you develop any other symptoms?

Yes ○ No ○ Don't Know ○ Refused ○

If YES, What other symptoms?

28a. | | | | | | | | | | | | | | |

28b. | | | | | | | | | | | | | | |

We want to know if West Nile Virus is more dangerous for people with certain medical conditions.

Has your doctor previously diagnosed you as having any of the following conditions ?

29. High blood pressure

Yes ○ No ○ Don't Know ○ Refused ○

30. Diabetes

Yes ○ No ○ Don't Know ○ Refused ○

31. Heart disease

Yes ○ No ○ Don't Know ○ Refused ○

32. Previous seizures

Yes ○ No ○ Don't Know ○ Refused ○

Continues on next page

62725

West Nile Virus Initial Questionnaire (Questionnaire A) Page 5 of 8

33. Previous stroke

Yes ○ No ○ Don't Know ○ Refused ○

34. Previous meningitis (inflammation of the lining of the brain or spinal cord)

Yes ○ No ○ Don't Know ○ Refused ○

35. Previous encephalitis (inflammation or infection of the brain)

Yes ○ No ○ Don't Know ○ Refused ○

36. At the time that you donated blood, were you taking any medications?

Yes ○ No ○ Don't Know ○ Refused ○

If YES, what medications were you taking?

36a. ☐☐☐☐☐☐☐☐☐☐☐☐☐☐

36b. ☐☐☐☐☐☐☐☐☐☐☐☐☐☐

36c. ☐☐☐☐☐☐☐☐☐☐☐☐☐☐

36d. ☐☐☐☐☐☐☐☐☐☐☐☐☐☐

37. At time you donated blood, were you a cigarette smoker?

Yes ○ No ○ Don't Know ○ Refused ○

IF YES, On average how many cigarettes do you smoke in one day?

37a. ☐☐ cigarettes per day Don't Know ○ Refused ○

Now we would like to ask about some of your PAST medical history.

Have you ever been diagnosed by a doctor with any of the following diseases?

38. Any previous illness due to West Nile virus infection:

Yes ○ What Year? ☐☐☐☐ No ○ Don't Know ○ Refused ○

39. St. Louis encephalitis:

Yes ○ What Year? ☐☐☐☐ No ○ Don't Know ○ Refused ○

40. Dengue ("deng gee") fever:

Yes ○ What Year? ☐☐☐☐ No ○ Don't Know ○ Refused ○

41. Japanese encephalitis:

Yes ○ What Year? ☐☐☐☐ No ○ Don't Know ○ Refused ○

Continues on next page

62725

West Nile Virus Initial Questionnaire (Questionnaire A) Page 6 of 8

Have you ever been vaccinated against any of the following diseases?

42. Yellow fever:

Yes ○ What Year? [ ][ ][ ][ ] No ○ Don't Know ○ Refused ○

43. Japanese encephalitis:

Yes ○ What Year? [ ][ ][ ][ ] No ○ Don't Know ○ Refused ○

44. Tick-borne encephalitis:

Yes ○ What Year? [ ][ ][ ][ ] No ○ Don't Know ○ Refused ○

45. Have you ever served in the military?

Yes ○ No ○ Don't Know ○ Refused ○

If YES, please provide the dates and locations of service.

45a. Entered military service (MM / DD / YYYY): [ ][ ] / [ ][ ] / [ ][ ][ ][ ]

45b. Left military service (MM / DD / YYYY): [ ][ ] / [ ][ ] / [ ][ ][ ][ ]

Where were you stationed?

45c. [ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ]

45d. [ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ]

45e. [ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ]

46. Have you traveled outside your current state of residence in the last 3 weeks?

Yes ○ No ○ Don't Know ○ Refused ○

If YES, please list all of the states you have traveled in:

46a. [ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ]

46b. [ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ]

46c. [ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ]

46d. [ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ]

Continues on next page

62725

West Nile Virus Initial Questionnaire (Questionnaire A) Page 7 of 8

PART II Index Donation Record

47. In the past 5 years, have you traveled to or lived in a country outside of the United States?

Yes ○ No ○ Don't Know ○ Refused ○

If YES, please list all of the countries you have traveled or lived in and the dates you were there starting with the most recent and working backward:

47a. Country: | | | | | | | | | | | | | Dates in country: _____

47b. Country: | | | | | | | | | | | | | Dates in country: _____

47c. Country: | | | | | | | | | | | | | Dates in country: _____

47d. Country: | | | | | | | | | | | | | Dates in country: _____

47e. Country: | | | | | | | | | | | | | Dates in country: _____

***Interviewer if the next section was completed using the index donation record,* Please Read:** This concludes the survey today. Thank you very much for your participation. We will contact you in a few weeks for follow-up surveys.

Interviewer or Blood Center Staff:
1. Complete this part of the survey for all donors whether they have participated in the survey or not.
*2. Obtain the information from the Donation Records of the Blood Center. (IF Information is not complete in the donor record **and** donor consents to be interviewed, please complete by asking donor at the time of interview.)*

48. Index donation phlebotomy type (enter letter code from record): | |

Donor Demographics

49. Zip code of donor's residence on date of index donation: | | | | | |

50. Donor initials: | | | |

51. Date of birth: (MM / DD / YYYY) | | / | | / | | | | |

52. Gender: Male ○ Female ○

53. Race:

- ○ White or Caucasian
- ○ Black or African-American
- ○ Asian
- ○ Native Hawaiian or Other Pacific Islander
- ○ American Indian or Native Alaskan
- ○ Other:_____
- ○ Don't Know
- ○ Refused

Continues on next page

62725

West Nile Virus Initial Questionnaire (Questionnaire A)

54. Ethnicity

Hispanic or Latino ○ **NOT** Hispanic or Latino ○ Don't Know ○ Refused ○

55. Educational attainment:

○ 8th Grade or Less

○ Some High School but **NO** Diploma

○ High school graduate (for example, Diploma or GED)

○ Some College or Technical School

○ Bachelor Degree (for example, BA, BS, or AB)

○ Master or Professional Degree (for example, MA, MS, MD, PhD, or JD)

Physical Findings

56. Donor temperature? ☐☐☐.☐ degrees F or ☐☐.☐ degrees C

57. Pulse? ☐☐

58. Blood pressure? ☐☐☐ systolic / ☐☐☐ diastolic

Responses to Health History Questionnaire

	Yes	No
59. Feeling well and health today:	○	○
~~60. Fever with headache in past 7 days:~~	○	○
61. Pills or medications in past 4 weeks:	○	○
62. Shots or vaccinations in past 4 weeks:	○	○

If YES, which shots or vaccinations:

62a. ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

62b. ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

62c. ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

62d. ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

Interviewer, if the previous section was completed at the time of interview, **Please Read:** This concludes the survey today. Thank you very much for your participation. We will contact you in a few weeks for a follow-up survey.

End of Questionnaire

37943

2011 - Blood Systems Research Institute
West Nile Virus Follow-up Questionnaire (Questionnaire B)

Index Donation Number: | | | | | | | | | | | | | | | |

Donor ID Number: | | | | | | | | | | | | | | | |

Date of Index Donation (MM / DD / YYYY): | | | / | | | / | | | | |

Blood Center: | | | | | | | | | | | | | | | |

A. Donor located for interview, if not select reason: Donor Located ○ Refused ○ Unable to Locate ○ Died ○ Other: ○

If OTHER please specify:_____

B. Date of Interview (MM / DD / YYYY): | | / | | / | | | | |

C. Donor Properly Identified: Yes ○ No ○

D. Interviewer initials: | | | |

Note to interviewer:
*1. Complete this survey **AT LEAST 14 DAYS AFTER** the date of the index donation*
2. Write the day and date of the index donation in the blanks preceding question 2 before you begin the interview.
3. Identify donor by name, date of birth, or social security number

Read:
We are asking you to be part of an investigation about West Nile virus infection in blood donors. Blood Systems Research Institute, in association with your local blood center, is in charge of this investigation. We are contacting you because a test has shown that you probably had West Nile virus the last time you donated blood. We want to study how you caught the virus and whether you have or had symptoms of West Nile virus infection. If you agree to participate, then I will ask you some questions about your medical history. The interview should only take about 5-10 minutes. We will keep your personal information confidential to the extent possible by law. Your participation is voluntary. You may refuse to answer any of the questions. If you have questions, you may ask me now. You may also call _____at your blood center at _____ or Dr. Hany Kamel at Blood Systems headquarters at 480-675-5659. Do you have any questions for me before we begin?

1. Do you agree to participate in this investigation? Yes ○ No ○ **If YES**, verbal consent obtained.

Signature of Medical Affairs staff:_____ Date:_____

Interviewer:
If **NO***, then stop the interview***.**
If **YES***, then continue with the survey on next page and complete all pages***.**

Continues on next page

37943

West Nile Virus Follow-up Questionnaire (Questionnaire B)

Read:

I am going to ask you some questions about whether you got sick in the 14 days after your blood donation. If you have a calendar available, it may help you answer the questions more accurately. Do you have a calendar?

You donated blood on (MM / DD / YYYY): ☐☐ / ☐☐ / ☐☐☐☐

In the 14 days after your donation, did you have any of the following symptoms?

2. Fever

Yes ○ No ○ Don't Know ○ Refused ○

If YES to 2: Yes No

2a. Was your temperature measured with a thermometer? ○ ○

If YES to 2a:

2b. What was the highest measured temperature?

☐☐☐.☐ degrees F or ☐☐.☐ degrees C Don't Remember ○

3. Headache

Yes ○ No ○ Don't Know ○ Refused ○

4. Eye Pain

Yes ○ No ○ Don't Know ○ Refused ○

5. Body aches (including stiff neck or neck pain)

Yes ○ No ○ Don't Know ○ Refused ○

6. New skin rash

Yes ○ No ○ Don't Know ○ Refused ○

7. Swollen lymph nodes

Yes ○ No ○ Don't Know ○ Refused ○

8. Nausea or vomiting

Yes ○ No ○ Don't Know ○ Refused ○

9. Muscle weakness

Yes ○ No ○ Don't Know ○ Refused ○

10. Confusion

Yes ○ No ○ Don't Know ○ Refused ○

Continues on next page

37943

West Nile Virus Follow-up Questionnaire (Questionnaire B) Page 3 of 4

11. Disorientation

Yes ○ No ○ Don't Know ○ Refused ○

12. Memory problems

Yes ○ No ○ Don't Know ○ Refused ○

13. **In the 14 days after your donation**, did you develop any other symptoms?

Yes ○ No ○ Don't Know ○ Refused ○

If YES, What other symptoms?

13a. ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

13b. ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

13c. ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

13d. ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

Interviewer:
*1. If the respondent answered **NO** to every Symptom Question 2 through 13, **then stop and conclude the interview by reading the text in the middle of Page 4.***

*2. If the respondent answered **YES** to **ANY** Symptom Question 2 through 13, **then continue with Questions 14 through 19.***

14. What was the date of onset of your illness? (MM / DD / YYYY)

☐☐ / ☐☐ / ☐☐☐☐ Don't Know ○ Refused ○

If respondent answered Don't Know:

14a. How many days after donation did you get sick? ☐☐ days Don't Know ○ Refused ○

15. What was the date of resolution of your illness or symptom(s)? (MM / DD / YYYY)

☐☐ / ☐☐ / ☐☐☐☐ Not Yet Resolved ○ Don't Know ○ Refused ○

16. Did you go to a doctor or clinic for this illness?

Yes ○ No ○ Don't Know ○ Refused ○

Continues on next page

37943

West Nile Virus Follow-up Questionnaire (Questionnaire B) Page 4 of 4

17. Did you spend at least one night in the hospital because of this illness?

Yes ○ No ○ Don't Know ○ Refused ○

If YES, 17a. What was the date of your admission to the hospital? (MM / DD / YYYY)

☐☐ / ☐☐ / ☐☐☐☐ Don't Know ○ Refused ○

18. Did your doctor or other health care professional tell you had West Nile fever?

Yes ○ No ○ Don't Know ○ Refused ○

19. Did your doctor or other health care professional tell you had West Nile meningitis or encephalitis?

Yes ○ No ○ Don't Know ○ Refused ○

Read:

This concludes this survey. Thank you again for your participation.

End of questionnaire

18. BSRI FACILITIES

18.1 General

The BSRI repository has been designed to ensure safety of employees, specimens and equipment.

18.2 Heating, Ventilation and Air Conditioning

Ambient temperature based on return air is within range of 15oC and 22oC (65oF – 72oF) in order to ensure optimal life of refrigeration equipment.

Irwin Repository HVAC Specs

- Dedicated Airhandler (S3-A)
  - Installed 2008
  - 5000 CFM Supply Fan
- Dedicated Exhaust Fan
  - Installed 2008
  - 5000 CFM Exhaust
- Heat Exchange Cooling Coil Supplied by:
  - Building Central 130 Ton Two Circuit Chiller Plant (scheduled for replacement in 2010)
  - New - Building Central 160 Ton 4 Circuit Chiller Plant (greater capacity w/double the redundancy)

18.3 Air Flow and Circulation

The HVAC system provides for 12 times per hour exchange of 100% outside air. Relative humidity is maintained at 35% to 40% at all times. Room is monitored by two oxygen sensors. HVAC has Web Based controls that record temperature and system performance. Alarms are sent by email notification when outside normal specifications.

18.4 Maintenance

Substantial plant operations support is available on site, including carpentry and machine shop for minor repair/fabrication. Freezers are maintained by an outside company on a regular basis.

18.5 Temperature monitoring system

There is a secondary temperature monitoring system (Mack Information Systems) for all temperature-critical equipment. Environmental Controls, Temperature Monitoring and one Oxygen monitor are on UPS.

All freezers within the Biorepository are connected to a centralized temperature monitoring system from Mack Information Systems. If a unit's temperature falls outside the set range, key Biorepository personnel will be phoned and asked to initiate corrective action.

18.6 Lighting

General fluorescent lighting (does not create a source of heat) provides 150/500 lux through out the repository.

Currently only 1 of the 10 fluorescent lights is on battery backup.

18.7 Flooring

The repository floor is a sealed concrete floor providing easy cleaning and movement of equipment.

18.8 Backup Power

Utility Power is provided by PG&E

- Dedicated 2500 AMP power feed (upgraded in 1994)
- Listed as a high priority critical medical/commercial account
- Participate in Energy curtailment program that provides 24 hour notice of possible high demand and transfer generator power from 7AM to 6PM to avoid possible drops in power.

The building is equipped with a Onan 350KW generator that has a 4- to 5-day fuel load. Appropriately trained facilities staff inspect and test the generator weekly for automatic starting and power generation (30 minutes with 15 minute coll down), and monthly for load testing (inspected and cold start + 30% load). Backup power is automatically initiated upon power interruption. Repository freezers are linked into this system. The Generator is maintained and repaired by Cummins West, who perform quarterly inspections/maintenance. There is an annual inspection/maintenance with 100% capacity load bank test.

Generator Specifications:

- Cummins 350KW Diesel Generator Set
- Installed new in 1994
- 120/208V, 3Ø, Wire
- 60HZ @ 180
- Automatic Transfer Switch
- 1 second activation delay
- Replacement installed in 2009
- 1200AMP, 120/208V, 3Ø, Wire
- Generator Fuel Tank Capacity 900 Gallons Diesel (About 3 days to 4 days worth of fuel)
- Generator load is about 35% of 350 KW capacity

Diesel Fuel contracted delivery is by Mansfield Fuel Systems with 24/7/356 Service. Guaranteed National "Warm Backup" Supply should primary supplier be unavailable. Fuel is tested/treated annually for quality, stability and purity.

During a power outage, the emergency standby generator will maintain power for all freezers contained in the Biorepository.

18.9 Access

Building access is controlled by DSX card key system. Per Institutional policy, All visitors are required to register, have visible dated, Picture ID Visitors Badge and be with an employee escort at all times. Visitors are check out when leaving using the same automated system.

Repository access is restricted 24/7/365 to authorized staff only and further limited during non-business hours. Security of non-public access areas consists of a Card Key Access system, which allows only authorized personnel into controlled areas. Entry access reports are available going back 1 year. Individual freezers are locked

18.10 Security

Although the repository does not have an intrusion detection system, there is 24-7-365 video surveillance of entries and access corridors, with on-site security personnel.

18.11 Fire prevention Systems

The building, including the repository, is equipped with smoke detectors, a sprinkler system that sprays water upon activation, and appropriate stand-alone fire extinguishers.

18.12 Safety program

Blood Systems Research Institute (BSRI) is a division of Blood Systems, Inc. and is housed within Blood Centers of the Pacific in San Francisco, CA. BSRI has a Safety and Loss Control Program which includes Standard Operating Procedures (SOPs) for general and specific safety, training, inspections, exposure control, and regulatory compliance. There is a trained full-time Safety Officer who oversees the goals and objectives of the safety program and is responsible for training and compliance with both company policies and regulatory requirements.

Safety training is an integral part of medical research at Blood Systems Research Institute. Blood System's supplies an annual training program to all employees. Safety orientation regarding all applicable sections of the BSI safety manual must be performed prior to employees starting his/her duties.

Periodically, the Safety Officer and/or designee(s) will conduct site inspections to identify and evaluate hazards, and for OSHA and other regulatory compliance. At least two inspections are conducted each year. Reports include physical as well as behavioral observations, and are directed to Risk Management and the Safety Committee. The Safety Officer is responsible for hazard resolution in a timely manner.

18.13 Emergency Preparedness

The Irwin building as a whole, has a Disaster Recovery and Operational Continuity Plan. As a Blood Center, Irwin is on both the SF City high-priority user list and the power (PG&E) high-priority user list.
Key individuals have been identified to respond to an emergency at the repository. Leave and vacations schedules are monitored to ensure coverage. Emergency contact numbers are posted in prominent locations in the repository and carried by on-call staff. On call staff have a check list of activities to follow during an emergency, and are familiar with the location and operation of certain key equipment and controls. Telephone numbers for professional services are clearly posted.

18.14 Hazard Analysis

18.14.1 Blood-borne pathogens

The Institute's Exposure Control Plan (ECP) outlines federal regulations and

general policy with respect to compliance with OSHA Bloodborne Pathogens Standard, code 29 CFR 1910.1030 to reduce occupational exposure to Hepatitis B Virus (HBV), Human Immunodeficiency Virus (HIV) and other bloodborne pathogens. The Safety Officer or designee is responsible for overall management of the ECP. Department Directors, Managers and Supervisors are responsible for exposure control in their respective areas.

Employees who have a potential for exposure must participate or decline participation in the Hepatitis B Vaccination program. All employees are required to complete the Employee Protection Program Informed Consent for Hepatitis B Vaccination, BSI 124.

The ECP incorporates SOPs for hand washing, receptacle cleaning, infectious biohazardous waste handling, and personal protective equipment. The ECP also incorporates the following methods:

a) Universal Precautions: All human blood and certain human body fluids are treated as if known to be infectious for HIV, HBV, HCV and other bloodborne pathogens.

b) Engineering Controls: These include, but not limited to readily accessible handwashing facilites; puncture-resistant, color-coded or labeled, leak-proof containers for sharps; splash guards or covers for areas/procedures where aerosolization of blood or body fluids might be anticipated; red bag waste disposal for biohazardous or potentially infectious waste; eye wash stations; blood spill kits; proper labeling.

c) Work Practice Controls: These include methods that reduce the likelihood of exposure to bloodborne pathogens by altering the manner in which a task is performed. These include, but are not limited to handwashing upon contact with blood, body fluids, non-intact skin, or other potentially infectious materials; prohibiting eating, drinking, smoking, gum chewing etc. in work areas where there is potential for exposure to bloodborne pathogens; storage of food and drink in appropriate areas only; procedures that minimize splashing, spraying or other actions generating droplets of blood or other infectious materials.

d) Personal Protective Equipment (PPE): Employees are provided with the PPE deemed necessary to perform job tasks which might involve a potential exposure to blood, body fluids or other potentially infectious materials. PPE includes, but is not limited to, gloves, gowns, laboratory coats/aprons, face shields/masks, safety glasses/goggles and mouthpiece.

e) Needlestick protection: Employees are required to use available sharps with engineered protections features to reduce risk.

f) Environmental Sanitation/Housekeeping: These include measures to maintain each center or facility in a clean and sanitary condition, protective coverings, cleaning of major equipment, disposal of hazardous materials.
g) Biohazardous or Infectious Waste: Specific policies and procedures exist for the labeling, storing, and handling of biohazardous waste, including contaminated laundry.
h) Monitoring: The Safety Officer is responsible for monitoring the effectiveness of work practices, engineering controls and PPE used.

18.14.2 Occupational Exposures in Laboratories

The Institute has established a Chemical Hygiene Plan to ensure employees are protected from health hazards associated with hazardous materials in the laboratory and to ensure exposures are minimized. A review and evaluation of the Chemical Hygiene Plan is conducted at least annually and is updated as necessary. The Corporate Safety and Loss Control Manager is responsible for implementation of the plan. The Chemical Hygiene Plan has specific SOPs for minor spills (both liquids and solids), radiation spills, emergency chemical spills, and cryogenic liquid.

The Institute provides online Material Safety Data Sheets (MSDS) for immediate viewing and printing.

18.14.3 Training

This profile is required for all BSRI employees, regardless of job title (see Training & Education Policy Manual, Appendix C, Program Summaries for specifics on programs). Training events must be documented on a Training Event Attendance Record, TED 100.

Table 18.2 List of training provided to BSRI employees

Training	General BSRI	Go Grant	Topic
GMP: Blood Banking Essentials	√		Blood Basics Workbook accompanies.
New Employee Orientation	√		BSRI specific.
Basic Safety Fundamentals	√		Replaced Safety Orientation Checklist and Biological & Job General Safety

Slips, Trips and Falls	√		
Welcome to Blood Systems	√		
Anti-Harassment Policy Training	√		
Diversity: Recognizing the Talents of Everyone	√		Replaced Partners Helping Partners: Diversity Training Program
Customer Service – "Holy Mackerel! What Great Customer Service!"	√		Replaced Quality Customer Service as of June 2004.
What is Quality?	√		Replaced BSI Quality Manual – Quality Coupe
The R.I.T.E. Values	√		Replaced Values & Principles as of June 2003.
Blood Born Pathogen	√		