**Viral Reference Laboratory and Repository Core (VRLRC)**
**Tobler**
**October 2009**

## I. INTRODUCTION

Dr. Leslie Tobler has continued to oversee the Viral Reference Laboratory and Repository Core (VRLRC). During 2008/2009 (October 2008 – October 2009) this Core has continued to manage the new Freezer Farm as well as maintaining it's responsibilities for the REDS-II Central Laboratory and REDS International.

Major research areas
Hepatitis C Virus infection
West Nile Virus infection
Dengue Virus infection
Chagas infection

Major BSRI collaborations
M. Busch--HCV, WNV, Dengue, Chagas, Microchimerism, REDS-II and REDS International
E. Murphy—HCV and HOST
P. Norris—WNV and Microchimerism
E. Delwart—REDS-II Molecular Surveillance

Major non-BSRI collaborations
Susan Stramer-- American Red Cross, Gaithersburg, MD--HCV and HIV-1
David Krystof--- American Red Cross, Gaithersburg, MD—REDS-II Molecular Surveillance
David Thomas and Chloe Thio—Johns Hopkins University School of Medicine, Baltimore, MD--HCV
P Contestable--Ortho Diagnostics, Rochester, NY—Chagas
Sherri Cyrus and Valerie Winkelman— Molecular Diagnostics Laboratory, Blood Systems Laboratory, Tempe, AZ—Chagas, WNV, HCV, REDS-II and REDS International
Deborah Todd, Stephanie Marshall and Danielle Carrick—Westat (Coordinating Center for REDS-II and REDS International)
Patricia Garrett—SeraCare (Repository Location for the National Heart, Lung and Blood Institute)—HIV
Ester Sabino---REDS International—Chagas and HIV-1 Detuned testing
Mirta Remesar and Ana del Pozo—Argentina—Chagas

Staff
Nelly Gefter, Research Associate
Patrick Loanzon, Research Assistant
Karla Murcia, Research Assistant
Simon Ng, Research Associate
Lubov Pitina, Research Associate
Ingrid Wilson, Research Associate

## II. PROGRAM SUMMARY/ PROGRESS REPORT/PLANS

The VRLRC department was established in recognition of an industry need to rapidly address new and emerging infectious disease issues affecting transfusion medicine. Over the years the focus of this department has changed. The department is now more of a service department and as such the sole recipient and distributor of specimens coming into and leaving Blood Systems Research Institute. This activity has required collaboration with one or more of the following: manufacturers of blood screening assays; blood collection centers through out the United States (ARC and UBS); the Molecular Diagnostics Laboratory (MDL) at Blood Systems Laboratory (BSL); the American Red Cross Reference Laboratory; REDS-II and REDS International Coordinating Center (Westat), REDS International Investigators and the Centers for Disease Control and Prevention (CDC).

MDL Collaborations: The MDL resides within Blood Systems Laboratory in Tempe, Arizona and has two staff members (Val Winkelman and Julie Davis) who are supervised by Sherri Cyrus. Over the past year the number of projects and specimens processed by the MDL staff has increased significantly in comparison to previous years:

- Acquisition of REDS-II HIV-1, HCV and HBV NAT yield cases from United Blood Services (UBS) and the New York Blood Center.
- Collected from UBS centers, subaliquoted and placed in a repository, 2008/2009 WNV RNA Positive index units.
- Collected **900** screening negative EDTA samples for Dr. Eric Delwart.
- Prepared for BSRI, **44** whole blood aliquots from WNV RNA Positive samples.
- Prepared **4 unlinked whole blood aliquots plus 2 plasma aliquots** from **24** HCV antibody confirmed, NAT negative donors (n=10) as well as controls (Ab+/$NAT^{+}$) (n= 14). These aliquots were accompanied by a database of age, sex, ethnicity as well as HCV, HIV and HBV test results. Two whole blood aliquots plus the database were shipped to Dr. Chloe Thio at Johns Hopkins University. It is hoped that these whole blood aliquots can be used instead of peripheral blood mononuclear cells (PBMC) in a continuation of our highly successful hepatitis C joint genetic studies.
- BSRI WNV Study of UBS donors residing in North Dakota--**4,514** aliquots from donors were collected after the conclusion of the 2008 WNV season and sent to Dr. Harry Prince (Focus Diagnostics) for WNV IgG testing followed by IgM testing of the IgG positives. 54 IgG positives were then sent to Dr. Robert Lanciotti (CDC, Fort Collins, CO) for plaque reduction neutralization (PRNT) testing
- BSRI - VRLRC Dept. Summer Intern Project: The intent of this project was to evaluate the effect of shipping either EDTA or ACD anticoagulated blood to the VRLRC department for PBMC isolation. Though the differences were not significant, the ACD anticoagulant seems to gentler.

HTLV Outcomes Study (HOST)—PI Dr. Ed Murphy: Phase 8 of the HOST Study has ended and the grant will not be renewed. Blood from **147 HOST participants** was processed during the review period, i.e. **serum, whole blood and PBMC aliquots** were made.

West Nile Virus Intensive Study---PI Dr. MP Busch and Co-PI Dr. P Norris: Blood was processed from **169 participants** from either our 2008 or 2009 WNV Intensive Studies. Blood was collected at study approved intervals and processed into **2311 plasma, 338 whole blood and 1935 PBMC aliquots**. TMA aliquots were batched shipped to Val Winkelman in MDL, who then forwarded them to the NAT laboratory for WNV TMA testing. **151** were tested in **singlet**, while **96** samples were tested in **triplicate**. WNV TMA testing still needs to be done on **44** aliquots from 2009 and **5** aliquots from 2008.

Microchimerism Study---PI Dr. MP Busch and Co-PI Dr. P Norris: Blood was processed from **500** participants during the reporting period. **1926 plasma and 2248 PBMC aliquots** were made and frozen away from these trauma patients.

SWAN Study (HCV in intravenous drug users)---PI Dr. Brian Edlin: This study is subcontract. The study is based on prospectively studying intravenous drug user looking for acute hepatitis C infections. The blood is shipped to us from New York. We process the blood making both HCV TMA aliquots and repository aliquots. The TMA aliquots are shipped weekly to Val Winkelman at the MDL, who then forwards them to the NAT laboratory for HCV TMA testing. We have received **969 blood tubes** and the NAT laboratory has generated **1141 dHCV TMA** results and **10 HCV 3.0 EIA** results. The HCV 3.0 EIA testing has just started.

REDS II Studies:

- Molecular Surveillance: During the review period, VRLRC has received **26,796** individual aliquots, primarily from the American Red Cross. All aliquots received have been reviewed with significant errors found and reported back to Westat (REDS II Coordinating Center). Molecular results, i.e. **103** genotype and sequencing results have been reported back to Westat during the review period.
- RISE: During the review period, VRLRC has received **blood from 1082** Blood Centers of the Pacific blood donors enrolled in the RISE study. **3248 aliquots** were made from the blood of these donors. During the same time period **260** results have been reported to Westat.

REDS International:

- Chagas Retrospective Study: VRLRC received **952** aliquots (old plasma, current plasma and serum) from San Paulo. **513 aliquots** (one old plasma and one current plasma and serum aliquot) were released to MDL for Ortho Chagas EIA testing. **512 Chagas EIA** results were received back, processed by Ingrid Wilson and released to Westat (Danielle Carrick).
- Chagas Prospective Study: VRLRC received **1796** aliquots from San Paulo and **1228** aliquots from Montes Claros. Aliquot types were serum, plasma, whole blood lysis, and whole blood aliquots. **352**

**plasma** specimens were released to MDL for Ortho Chagas EIA testing. Results have not been received.

- Chagas PCR testing: **288 whole blood lysis** (two aliquots) were released to Dr. Leiby's laboratory at Holland Laboratories (ARC) and **288 whole blood lysis** Dr. Lee's laboratory at BSRI for comparative PCR testing. Based on the results from both laboratories a blinded panel of **36** members representing the discrepant samples was sent to both laboratories (**HL n=36 aliquots and BSRI n=70**).
- HIV-1 detuned testing: **170** HIV-1 aliquots were received from Recife (n=66), Minas Gerais (n=38) and San Paulo (n=66) in Brazil. All samples were detuned and the results were processed by Ingrid Wilson and then sent to Westat.

Non-REDS Chagas Studies:

- Chagas: **437 Chagas positive specimens**, representing a WHO Chagas panel with specimens from Mexico and 9 countries in Central and South America , were received from Dr. Ester Sabino and then sent on to the MDL laboratory for Ortho Chagas EIA testing. Additionally, **1453 Chagas positive specimens** from sequential blood donors residing in the Chaco region of Northern Argentina (Chagas endemic area) were received and forwarded to the MDL laboratory for triplicate Ortho Chagas EIA testing.

In conclusion, the VRLRC department received:

- Blood from **2867 study participants** that was processed into:
  - **8454** plasma aliquots (**3-fold amplification**)
  - **338** whole blood aliquots
  - **4183** PBMC aliquots **(1.5-fold amplification)**
- **32,662 individual aliquots** that were processed (quality control, plus storage at either -80$^0$C or in $LN_2$).
- Of these aliquots, **3,710 (11%) were distributed** for testing. Of the 3,710 sent for testing, **2906 (78%) were sent to MDL** for testing.

## III. GRANTS, CONTRACTS, AND AWARDS

Current

- Acute HCV Infection in Injection Drug Users (NIH NIDA) SUNY (PI: Dr. Brian Edlin and BSRI PI: Dr. Michael Busch)
  - VRLRC processes all incoming specimens from this study
- REDS-II Central Laboratory (PI: Dr. Michael Busch)
  - VRLRC receives, distributes, tests and manages RADAR specimens for the REDS-II Parvo B19 study and RISE
  - VRLRC receives, distributes and manages data from REDS-II Molecular Surveillance specimens tested at BSRI by Dr. Eric Delwart's Department.
- REDS International Central Laboratory (PI: Dr. Michael Busch)
  - VRLRC receives, distributes and manages Chagas and HIV-1 specimens from Brazil
  - VRLRC makes labels on demand for the various studies in Brazil

Pending

- Grand Opportunity grant for West Nile and Dengue viruses

Completed none

Unsuccessful Applications none

## IV. OTHER SIGNIFICANT ACTIVITIES none

## V. ABSTRACTS, PUBLICATIONS, AND PRESENTATIONS

1. Performance of the US-licensed Ortho *Tcruzi* ELISA Test System on 2 large Latin American donor panels and comparison with kits used in Latin America by EC Sabino, M Remesar, **LH Tobler**, MM Otani, DJ Wright, LV Kirchhoff, MP Busch (2009 Oral presentation)
2. Significance of inconclusive results on *T. cruzi* antibody assays in blood donors from a highly endemic region of Argentina by MC Remesar, EC Sabino, C Gamba, MP Busch, **LH Tobler**, M Puppo, MA Ridolfi, S Kupperman (2009 poster presentation)
3. Busch M P, Kleinman SH, **Tobler LH**, Kamel HT, Norris PJ, Walsh I, Matud JL, Prince HE, Lanciotti RS, Wright DJ, Linnen JM, Caglioti S. Virus and antibody dynamics in acute West Nile virus infection. J Infect Dis 2008;**198**: 984-93

4. **Tobler LH**, Cameron MJ, Lanteri MC, Prince HE, Danesh A, Persad D, Lanciotti RS, Norris PJ, Kelvin DJ, Busch MP. 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.
5. Edlin BR, Shu MA, Winkelstein E, Des Jarlais DC, Busch MP, Rehermann B, O'Brien TR, Talal AH, **Tobler LH**, Zeremski M, Beeder AB. More rare birds, and the occasional swan. Gastroenterology 2009; 136: 2412-4.
6. Kleinman SH, Glynn SA, Lee TH, **Tobler LH**, Schlumpf KS Todd DS, Qiao H, Yu M Y, Busch MP. A linked donor-recipient study to evaluate parvovirus B19 transmission by blood component transfusion Blood 2009.
7. Thomas DL, Thio CL, Martin MP, Qi Y, Ge D, O'Huigin C, Kidd J, Kidd K, Khakoo SI, Alexander G, Goedert JJ, ,Kirk GD, Donfield SM, Rosen HR, **Tobler LH**, Busch MP, McHutchison JG, Goldstein DB, Carrington M. Genetic variation in IL28B and spontaneous clearance of hepatitis C virus. Nature 2009; 461: 798-801.
8. Page K, Hahn JA, Evans J, Shiboski S, Lum P, Delwart E, **Tobler L**, Andrews W, Avanesyan L, Cooper S, Busch MP. Acute hepatitis C virus infection in young adult injection drug users: a prospective study of incident infection, resolution, and reinfection. J Infect Dis 2009; 200: 1216-26.