

Molecular Transfusion Core

Lee

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I. INTRODUCTION

Major research areas

The Molecular Transfusion Medicine Core's primary technology is real-time Polymerase Chain Reaction (PCR). This technique allows for the quantification and characterization of amplified DNA. PCR is highly specific and, because the target DNA is amplified, highly sensitive. The laboratory develops protocols that facilitate the optimization of quantitative and qualitative PCR assays. The Core lab functions to support BSRI investigators and their collaborators in the detection of viral and genomic DNA or RNA.

Major BSRI collaborations

Immunology, Developmental Biology, Molecular Virology, Virology Core, Epidemiology

Major non-BSRI collaborations

Chiron/Novartis, GenProbe, UCSF, UC Davis, UC Berkeley, NIH, U Michigan

Staff

Lani Montalvo, Research Associate

Daniel Chafets, Research Associate

Li Wen, Research Associate

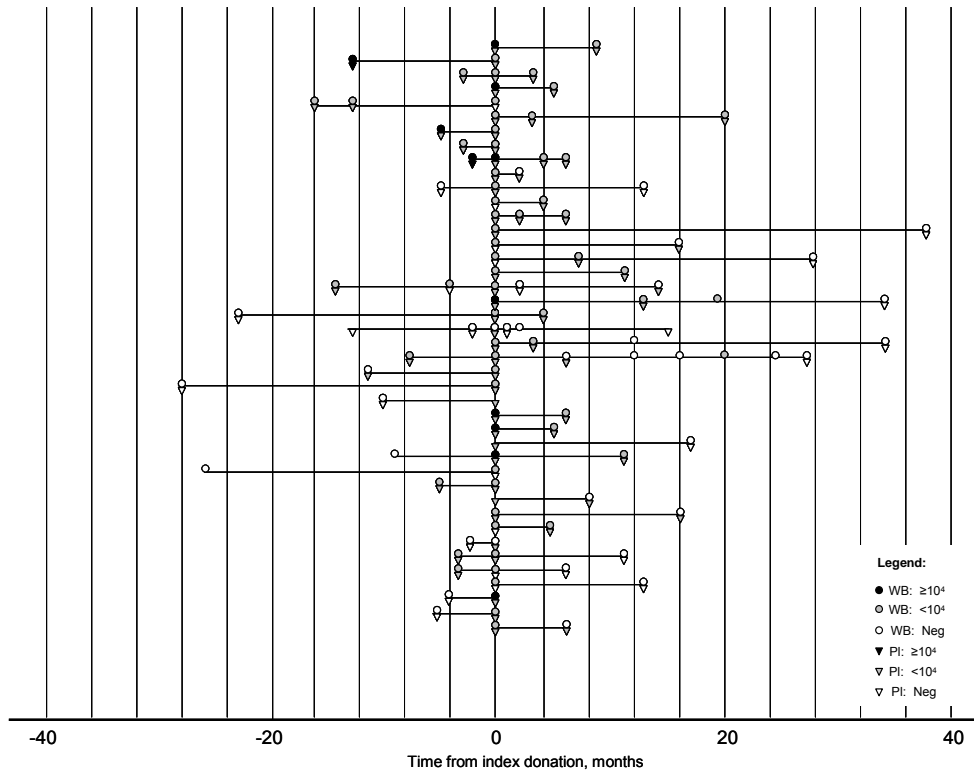
II. PROGRAM SUMMARY/ PROGRESS REPORT/PLANS

REDS

In early 2009, we completed two REDS II projects: RISE and Parvo B-19 persistence study.

For RISE, we tested 2,443 samples for three mutations; HFE 845, H63D and Transferrin 829 using real-time PCR. Last February, we turned in confirmation results of the non wild type PCR data. Samples identified as having homozygous or heterozygous mutations for each of mutation identified in PCR were sequenced for the SNP. We sequenced 248 samples in total for this study. Genotyping results for the three mutations studied largely corroborate already published data from other research groups. More detailed analysis is being done by Dr. Brian Custer.

For the Parvo project, we quantified 131 follow up donor plasma samples and compared viral loads from 111 paired whole blood and plasma donor samples. The figure below summarizes the comparison of viral load from the two compartments of serial donations associated with the 42 blood donors. Quantitative PCR was performed on both whole blood(●) and plasma compartments (▼), with the black symbols denoting viral loads above 10,000 IU/mL, grey symbols denoting viral loads from negative (3IU/mL cut-off) to <10,000IU/mL and empty symbols denoting negative viral load. The x-axis represents time from index donation and time 0 as the donation index. Subjects identified as positive at the index for either whole blood or plasma were included for further characterization. Additional samples going backwards or forward from a positive index donation were identified and tested for quantitative PCR viral load, IgM and IgG antibody testing.



REDS International: Chagas

We are continuing our Chagas studies, in collaboration with the American Red Cross, BSL and Brazil. Although unfunded for REDS International, we tested a significant number of samples for the presence of *Trypanosoma cruzi*. The *T. cruzi* assay uses the HemoBind protocol developed here at BSRI and was used to detect the parasite in parallel with PCR tests performed by the Holland lab at the American Red Cross in Maryland. It has been shown this assay to be able to detect one parasite per 20mL of whole blood. Specificity was assessed by applying the assay to a coded panel of 100 samples (99 negative donors and one positive control). Both BSRI and ARC picked up the positive control. One sample which tested positive at BSRI and ARC was presumed contaminated at the site of preparation. A second coded panel prepared by Dr. Sabino's group (Brazil) was tested in August. The samples were collected from endemic (Sao Paulo) and non-endemic (Montes Claros) regions of Brazil. The following is a summary of results of 144 samples, comparison of ARC and BSRI results.

Donors from Sao Paulo and Montes Claros

ARC	BSRI	Seronegatives	Seropositives	Total
+	+	0	30	30
+	-	3	3	6
+	IND	0	2	2
-	+	0	16	16
-	-	39	41	80
-	IND	3	7	10
Total		45	99	144
Concordance		86.67%	71.72%	76.39%

This assay was also used to interrogate the presence of the parasite's DNA in the blood of a New England donor and his family members. On going collection of samples from antibody reactive donors from BSL, ARC and Brazil (REDS International collection) will continue to be tested.

Microchimerism

We are in year 4 of our microchimerism grant. We are continuing to receive samples for prospective and retrospective transfusion associated microchimerism studies. We are also continuing with our study Mother-Child Immunogenetics Study. In August, we trained Dr. Yumiko Suto, from the Japanese Red Cross, on techniques used on our microchimerism studies, for the purpose of establishing the assay in Tokyo.

Prospective Microchimerism

Garth Utter at UC Davis recruited 373 subjects to participate in the study. Follow up samples are available for 324 subjects. These samples were genotyped using 12 insertion/deletion panel and 12 HLA-DR panel. The samples were interrogated for presence of microchimerism. Long term microchimerism was detected for 3 subjects and short term for 5 subjects.

Retrospective Microchimerism

There are 48 samples collected. These samples had been genotyped and probed for microchimerism.

Mother-Child Immunogenetics Study

This study is conducted in collaboration with UC Berkeley and BSRI's epidemiology core. There are 66 samples which were collected and processed.

GO Grant

Recent approval of the Go grant would give us the opportunity to develop the Dengue virus genotyping assay for 4 Dengue serotypes and the Dengue viral load. For this study, we will also be importing the West Nile target capture assay from Novartis.

Other Projects

We were involved in several small projects. we examined 114 cord blood dried blood spots (DBS) for possible correlation of HIV in utero transmission with maternal cell contamination of the cord blood. Paired mother and infant DBS were typed using the InDel/DR panels to get the maternal informative allele. Once identified, the informative allele was quantified in the cord dried blood spot and correlated with infant HIV status.

We are continuing our collaboration with Dr. Harvey Alter regarding TRIPS. We test EBV, HHV-8 and microchimerism for all samples sent to us by the NIH.

For the new collaboration with Dr. Pilcher, we leased a barocycler (Pressure Cycling Technology) to determine whether applying pressure for 20-30 cycles would yield DNA or RNA from the hair shaft. The hypothesis is that HIV may be extracted from the hair shaft of HIV positive subjects and incidence may be correlated to the length and position of processed hair shaft. So far, we tried different protocols and different extraction buffers to extract DNA or RNA from hair. More preliminary experiments need to be conducted.

New studies initiated this year include an investigation of fetal-maternal cell trafficking (Tippi McKenzie/UCSF), comparison of viral loads of West Nile Virus in whole blood and plasma compartments (Lori Lai/ Novartis). In October, we imported the HERV-K, Human Endogenous Retro Virus-K assay (Contreras-Galindo/ U Michigan) so that we can adapt the assay here at BSRI. In this project, we will be collaborating with Novartis to correlate HERV-K plasma load to lymphoma and breast cancer. We will be testing a total of 200 subjects collected by the University of Michigan and 200 donor controls.

Last but not least, we filed for the HemoBind utility patent. This protocol will be expanded to include protocols for extraction of RNA virus such as HIV and HCV. We are also developing the assay for HBV. These assays will be in addition to DNA assays currently utilizing this protocol, i.e, Trypanosoma cruzi and Parvo B-19.

III. GRANTS, CONTRACTS, AND AWARDS

Current

Microchimerism: RO1 HIO83388-01A1 (Busch)

HOST: R01HL062235 (sub contract to UCSF)

BSF 5427

Chiron: Grant (Development)

Chiron: Grant (HERV-K)

Go Grant

HIT-IT RFA-AI-08-097 (McCune: UCSF)

SCOPE

Pending

Caridian BCT Contract

Completed

REDS II Central Lab for RISE and Parvo B-19: NO1-HB-57181 (Busch)

Unsuccessful Applications

Maximizing the Sensitivity of Nucleic Acid Testing for Blood Screening (This application addresses broad Challenge Area (06): Enabling Technologies, and specific Challenge Topic 06-HL-106)

IV. OTHER SIGNIFICANT ACTIVITIES

Utility Patent Application: HemoBind

V. ABSTRACTS, PUBLICATIONS, AND PRESENTATIONS

2009 ISBT, Cairo Poster: Long-Term Transfusion Associated Microchimerism (TA-MC) Identified in A Prospective Study