Blood XMRV Scientific Research Working Group

- **Mission - design and coordinate research studies to evaluate whether XMRV poses a threat to blood safety**
- **Working Group includes representatives from transfusion medicine, retrovirology, and CFS scientific communities, as well as representatives from key Federal Agencies including HHS, FDA, NCI, CDC and NHLBI**
- **Evaluation of blood safety risks includes several steps:**
  - Evaluate XMRV nucleic acid and antibody assays
  - Establish prevalence of XMRV in blood donors
  - Determine if XMRV is transfusion-transmitted
  - Determine if transfusions are associated with CFS or prostate cancer (epidemiology studies)

Blood XMRV Scientific Research Working Group

NHLBI - Simone Glynn - Chair
HHS - Jerry Holmberg - Co-chair
NIH - Harvey Alter
ABC - Celso Bianco
CDC - William Bower
BSRI - Michael Busch
ARC - Roger Dodd
FDA - Jay Epstein
FDA - Diane Gubernot
NIH - Eleanor Hanna
CDC- Michael Hendry
MVRBC - Louis Katz
AABB - Steven Kleinman
CDC - Stephan Monroe
NCI - Francis Ruscetti
ARC - Susan Stramer
BSRI - Leslie Tobler
CFIDS - Suzanne Vernon

Testing Labs - Investigators
BSRI - Graham Simmons
CDC - Bill Switzer, Walid Heneine
FDA - Indira Hewlett
FDA - Shyh-Ching Lo
NCI - John Coffin
WPI - Judy Mikovits

Funding for currently launched studies via NHLBI REDS-II Program

XMRV SRWG REDS-II Panel Study Phases

- **Phase I - Analytical Panels**
  - **Evaluate performance of XMRV NAT assays**
- **Phase II - Pilot Clinical Studies**
  - **Whole Blood versus PBMC**
  - **Timing of sample preparation**
- **Phase III - Clinical Sensitivity/Specificity Panel**
  - **Assay performance on pedigreed clinical samples**
- **Phase IV - Blood Donor Clinical Panel**
  - **Initial estimation of XMRV nucleic acids prevalence in blood donors**
  - **Initiation of donor seroprevalence studies**

Phase I - Analytical Panels

- **Analytical performance panels**
  - Comparison of Limit Of Detections and accuracy of Viral Loads of current assays; standardize performance of future XMRV detection assays for blood cells and plasma
  - Whole blood panel - spiked with XMRV positive cells
  - Plasma panel - spiked with supernatant containing XMRV
- **22Rv1 cells**
  - Human prostate cell line chronically infected with XMRV
  - Contain at least 10 XMRV proviral copies each
  - Metzger et al (2010) J Virol 84:1874 (PMID:20007266)
- **Virus supernatant**
  - Supernatant from cultured 22Rv1 cells
  - Approximate Viral Load of 5 x 10^9 RNA copies/ml

Analytical Panel Production

Whole blood	Plasma
Whole Blood (WB) unit from pedigreed (NAT and serology) negative donor	Plasma components from two pedigreed (NAT and serology) negative donors
22Rv1 cells spiked into WB to yield 9,900 22Rv1 cells per ml of WB	22Rv1 supernatant spiked to give approximately 250,000 RNA copies per ml of plasma
Three-fold dilutions in fresh WB	five-fold dilutions in fresh plasma
0.5 ml aliquots to give 15 panels with three replicates at each dilution, plus 6 negative controls	0.5 ml aliquots to give 15 panels with three replicates at each dilution, plus 6 negative controls

Blood Systems Research Institute

Analytical Panel Set-up

Whole blood	Plasma
9,900 cells/ml	250,000 copies/ml
3,300	50,000
1,100	10,000
367	2,000
122	400
41	80
13.6	16
4.5	3.2
1.5	0.64
0.5	0.128
0	0
0	0

- Distributed as two blinded panels of 36 samples each

Participating Lab Assays - Whole Blood

Lab	Extraction method	Blood Volume (μl)	Input DNA (ng)	Assay #	Primer Reference	Target	Assay type	Quant/ Nested
CDC	QIAamp mini blood	400	1000	1	Urisman	gag	PCR southern	Nested
				2	Switzer	pol	PCR southern	Nested
BSRI	QIAamp mini/midi blood	100/400	100/250		Outer - Lombardi Inner - Switzer	gag	PCR	Nested
FDA (Lo)	Qiagen DNeasy blood	200	30-50	1	Outer - Lombardi Inner - Urisman	gag	PCR	Nested
			30-50	2	Outer - Lombardi Inner - Unpublished	gag	PCR	Nested
FDA (Hewlett)	QIAamp mini blood	200	500-1000		Urisman	gag	PCR	Nested
WPI	QIAamp DNA mini blood	250	100		Dong	5Õ UTR of gag	qPCR	Quant
NCI	Promega Wizard Genomic DNA	500	4000		Unpublished	5Õ UTR of gag	PCR SCA	Quant

Participating Lab Assays - Plasma

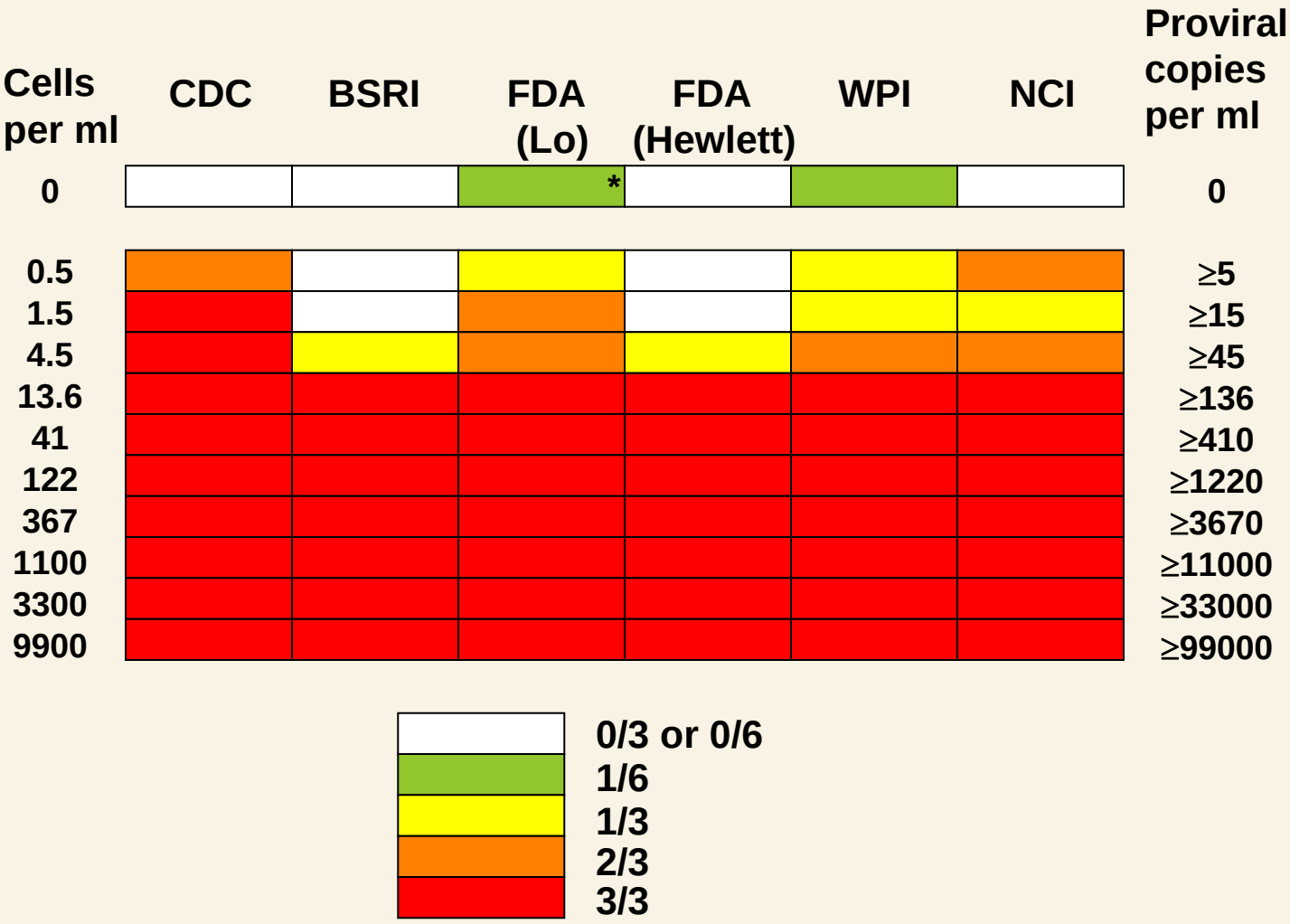
Lab	Extraction method	Volume plasma (μl)	Assay #	Primer Reference	Target	Assay type	Quant/ Nested
CDC	Ultracentrifuge /QIAamp viral RNA mini	500	1	Unpublished	pro	qRT-PCR	Quant
			2	Urisman	gag	RT-PCR southern	Nested
FDA (Lo)	Ultracentrifuge /TRIzol	250	1	Outer - Lombardi Inner - Urisman	gag	RT-PCR	Nested
			2	Outer - Lombardi Inner - Unpublished	gag	RT-PCR	Nested
FDA (Hewlett)	Qiagen Viral RNA mini	140	1	Urisman et al	gag	RT-PCR	Nested
WPI	QIAamp viral RNA mini	140	1	Lombardi et al	pro	qRT-PCR	Quant
NCI	Ultracentrifuge including internal standard Guanidinium Isothicyanate	~ 200-500ul plasma	1	Unpublished	5Õ UTR of gag	RT-PCR SCA	Quant

Analytical Panel Results - Whole Blood

	Fraction of positive replicates					
Cells per ml of blood	**CDC**	**BSRI**	**FDA (Lo)**	**FDA (Hewlett)**	**WPI**	**NCI**
0	0/6	0/6	1/6*	0/6	1/6	0/6
0.5	2/3	0/3	1/3	0/3	1/3	2/3
1.5	3/3	0/3	2/3	0/3	1/3	1/3
4.5	3/3	1/3	2/3	1/3	2/3	2/3
13.6	3/3	3/3	3/3	3/3	3/3	3/3
41	3/3	3/3	3/3	3/3	3/3	3/3
122	3/3	3/3	3/3	3/3	3/3	3/3
367	3/3	3/3	3/3	3/3	3/3	3/3
1100	3/3	3/3	3/3	3/3	3/3	3/3
3300	3/3	3/3	3/3	3/3	3/3	3/3
9900	3/3	3/3	3/3	3/3	3/3	3/3

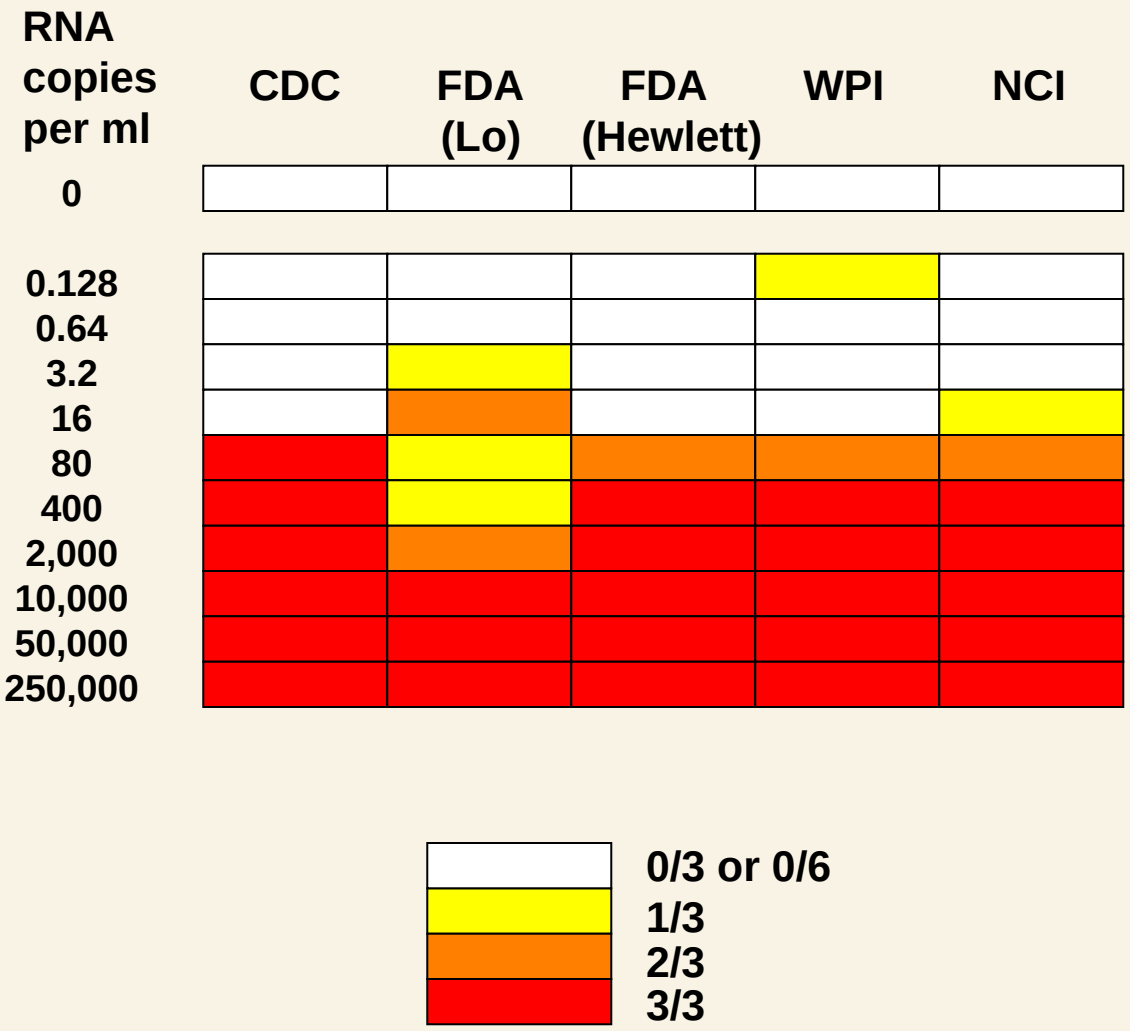
* False positive result identified as non-specific band of human genomic origin by sequencing subsequent to decoding of results

Analytical Panel Results - Whole Blood



* False positive result identified as non-specific band of human genomic origin by sequencing subsequent to decoding of results

Analytical Panel Results - Plasma



Conclusions and Limitations

- **XMRV NAT detection assays were sensitive**
  - **All WB assays detected at least 136 proviral copies/ml and four out of six [CDC, FDA(Lo), WPI and NCI] assays demonstrated even greater limits of detection**
  - **Four out of five plasma RNA assays performed similarly, with limits of detection of at least 80 RNA copies/ml**
- **Limitations**
  - **The study is too small to conduct meaningful statistical comparisons or additional analysis (such as probit to derive confidence intervals around limits of detection)**
  - **WB panel lacked sufficient dilutions to reach endpoints**
  - **XMRV isolate with which 22Rv1 cells are infected may not adequately represent the diversity of XMRV clinical isolates**
  - **Further work on analytical panel development will need to be performed**

Phase II - Pilot Studies

- **Whole Blood versus PBMC versus plasma**
  - Original Lombardi et al. study performed on isolated PBMC and plasma
  - Large scale studies for prevalence facilitated by using WB or plasma
  - Donor-recipient and other repositories are mainly comprised of frozen WB and/or plasma
- **Timing of Processing**
  - Processing and freezing of donor samples slotted to be included in the blood donor clinical panel (phase IV) vary from 2-4 days due to requirements for completion of ID testing. Available donor repositories include frozen WB and plasma samples processed 1-3 days post-phlebotomy.
  - Studies with other cell-associated viruses (HERVs, HTLV, herpesviruses and anelloviruses), demonstrate that levels of viral nucleic acid in plasma and whole blood vary with time from collection to processing and frozen storage.

Phase II – Pilot Study Setup

- **XMRV-positive samples**
  - WPI has collected blood from four CFS patients previously identified as XMRV positive in the Lombardi et al. study (by PCR, serology and culture)
  - Samples separated into tubes and were processed immediately, or left at 4^{o}C for 2 or 4 days
  - Each sample was processed into PBMC, WB and plasma.
- **Analysis**
  - Panels were distributed by WPI to CDC and NCI for testing; WPI retained one panel for testing. One set of the panel was distributed to BSRI to keep and use for follow-up work as needed.

Phase III - Clinical Sensitivity and Specificity

- **The ability of participant assays to effectively detect clinical XMRV-positive and -negatives will be examined**
  - To attempt to overcome the issues of clinical variation and specificity, larger numbers of pedigreed clinical XMRV-positive and -negative samples are being assembled into coded cell and plasma panels to be distributed to all labs
- **Positives**
  - 25 clinical samples will be collected by WPI. All will be from patients reported in the Lombardi et al. study to be positive for XMRV by PCR, serology and virus culture. Method and timing of processing will be determined based on pilot study data
- **Negatives**
  - PCR detection assays have the potential to amplify non-specific human derived genomic sequences
  - Thus, a larger group of pedigreed negative donors is required in order to introduce generic variability (10 donors, with 3 replicates per donor = 30 negative donor samples in each panel)
  - The donors will be pedigreed negative by PCR at WPI, FDA (Dr. Lo) and CDC labs, and for serology at WPI, CDC and NCI (Dr. Ruscetti) labs

Phase IV - Clinical Panel for Donor Prevalence

Source and coding of donor specimens:

- BSI/CTS processed residual blood in “pilot tubes” (vacutainer tubes used for routine ID screening) from 396 donations given by apheresis and double-RBC donors in the Reno/Tahoe area in Dec 2009 into replicate frozen WB and plasma aliquots.

- Aliquots were anonymized as sequentially coded samples after capturing donor gender, age and zip code of residence

Phase IV - Clinical Panel for Donor Prevalence

- **Blinded panels consisting of approximately 300 blood donor samples, 25 confirmed XMRV-positive clinical samples and about 30 pedigreed-negative samples from 10 independent donors will be created for WB and plasma**
- **Blinded panels will be distributed to at least four of the participating laboratories for testing.**
- **Test results will be analyzed:**
  - Correlation between whole blood and plasma testing will be determined for each lab and between labs.
  - Preliminary XMRV prevalence in blood donors (proportion positive for XMRV DNA or RNA) will be estimated based on compiled results from each lab.

NHLBI specimen repositories* for potential future studies of XMRV prevalence in donors over time and rates of transfusion transmission

Study Name	Time Frame	Population	Specimen Type	Number of Specimens	Major Agents Studied
TTVS	1974 - 79	**Donor - Recip**	Serum	5,655 donat'ns 1533 recip'ts	HCV, HBV
REDS: RADAR	1999 - 2003	**Donor - Recip**	Plasma; **Frozen Whole Blood**	13,201 donat'ns 3,574 recip'ts	Parvovirus B19
TRIPS	2001 - ongoing	**Donor - Recip**	Plasma; **Frozen Whole Blood**	~6,000 donat'ns† ~1,000 recip'ts†	HHV-8, CMV, EBV, Parvo B19
NIH Clinical Center	1968 - 97	**Donor - Recip**	Serum	29,055 donat'ns 3,429 recip'ts	HCV, HGV/GBV-C, TTV, SENV
VATS	1995 - 99	**Donor - Recip**	Plasma; **Frozen Whole Blood**	3,864 donat'ns 531 recip'ts	HIV, CMV, HBV, HCV, HGV, HTLV
FACTS	1985-91	Recip	Serum	11,494 recip'ts	HIV, HTLV, HCV, HHV-8, *T.cruzi*
TSS	1984-85	Donations	Serum	201,212 donat'ns	HIV, HTLV
REDS GSR	1991-94	Donations	Serum	508,151 donat'ns	HBV, CMV, HHV-8
REDS GLPR	1994-95	Donations	Plasma; **Frozen Whole Blood**	147,915 donat'ns	HBV, CMV, HHV-8

*: ISBT International Forum: "Biobanks of blood from donors and recipients of blood products". Vox Sang, 94(2):142-260, 2008: *USA*: Kleinman S, Bianco C, Stramer SL, Dodd RY, Busch MP. pp 258-260. † Numbers updated as of June 2010.