ISBT WP-TTID
Annual Report for Subgroup on Virology

Drs. Michael Busch, Kurt Roth and Susan Stramer

- Questionnaire on NAT Screening of Blood Donations for an International Forum on 10 years of NAT Screening
- HIV Elite Controllers detected through donor NAT and serology screening
- Performance of 4th generation HCV Ag/Ab-Combo Tests on HCV NAT yield units
- Repository and Characterization of HIV-Infected Plasma Units from acutely infected (NAT yield) donors (Panels Project)
- Donors Viral Load distributions and performance of new (4th gen Ag/Ab-Combo Tests) and rapid serological assays on HIV NAT yield units
- Repository of HIV-Infected Plasma Units from Recently infected Donors for Incidence Assay Development and Calibration
- Dengue viremia in donors and transmission by transfusions

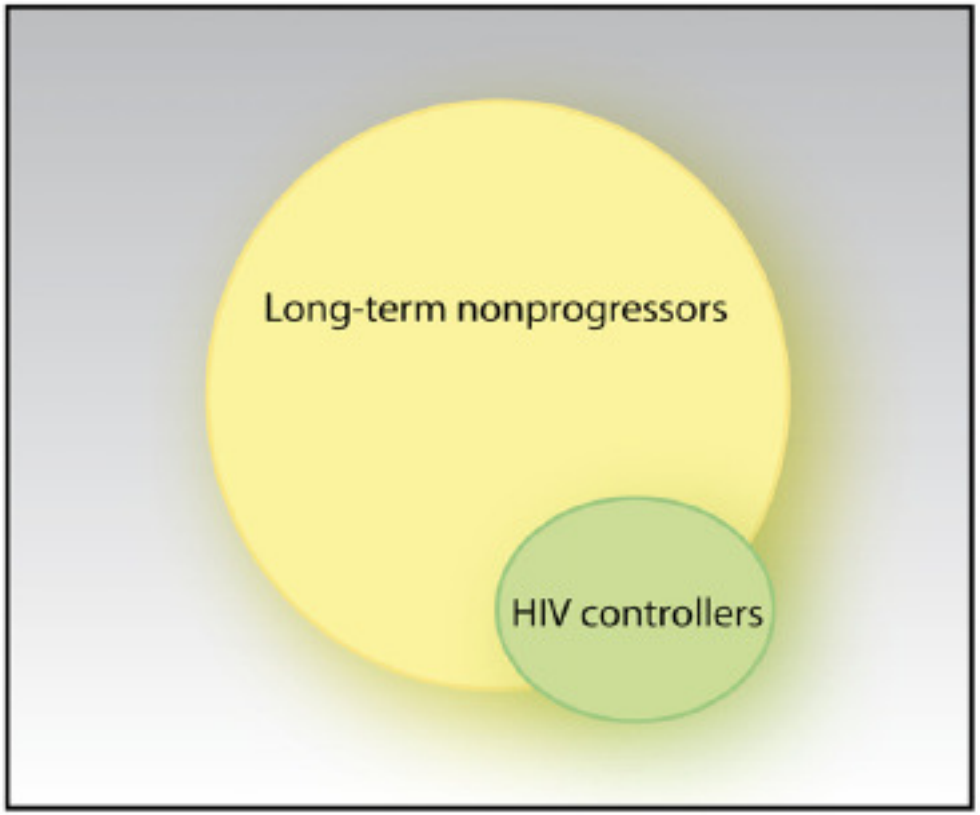
Blood Systems

Immunity
Review

Human Immunodeficiency Virus Controllers: Mechanisms of Durable Virus Control in the Absence of Antiretroviral Therapy

Steven G. Deeks[1,2] and Bruce D. Walker[1,2,*]

Immunity 2007; 27:406-416



"Elite Controllers"

- **HIV seropositive**
- **No detectable HIV RNA (< 50 copies/mL) for > 2 years**
- **Antiretroviral untreated**

The proportion of individuals who become elite controllers estimated at 1-5% but not well established

Blood Systems

Rates and characteristics of HIV "elite controllers" in blood donors

- Routine HIV NAT (Gen-Probe/Chiron TMA; Roche PCR) and antibody screening (3rd generation assays) and confirmatory (western blot, IFA, ImmunoComb, InnoLIPA) data were compiled from blood organizations
  - US - 16 sample minipool (MP)-NAT
  - France - 6-16 sample MP-NAT
  - South Africa - individual donation (ID)-NAT
  - Australia – combination of 16 sample MP and ID-NAT
  - Germany – 96 donation MP-NAT with ultra-centrigation to concentrate virus prior to extraction.
- The analysis was restricted to allogeneic donors to exclude anti-retroviral treated (ART) autologous donors.
- Possible EC cases were evaluated by additional testing and follow-up to exclude cases with false-positive serological results and HIV-2 infections (in France).
- ECs were studied by replicate ID-NAT, and demographic characteristics of ECs compared to HIV-viremic donors.

Blood Systems

Rates of HIV "elite controllers" in blood donors

Country	Period of screening	NAT (MP/ID) 50% LOD (cps/mL)	# allogeneic donations	# (%) HIV Ab+	# (%) Ab+ that tested NAT-Neg
US	1/99-5/08	MP: ~222	62,044,407	**1692 (0.0027)**	**58 (3.2)**
France	7/01-12/07	MP: 50-75	16,400,000	**226 (0.0014)**	**6 (2.6)**
South Africa	10/05-9/07	ID: ~8.5	1,461,211	**1705 (0.12)**	**12 (0.7)**
Germany	2003-2007	MP: 600-3000	3,752,309	**45 (0.0012)**	**1 (2.2)**
Australia	6/00-9/08	MP: ~222	8,910,863	**35 (0.0004)**	**0 (0.0)**

Blood Systems

Estimated Viral Loads and Antibody Reactivity in SANBS Elite Controllers

EC for 2 year period	S/CO on Prism	No. of replicates reactive	Follow up confirmed	W Blot pattern	Estimated Viral load Cps/ml	(95% CI)	DT S/CO
2847074	85.35	0/33	No	p24, p31, gp120, gp41	<1		0.135
2888634	67.76	3/10	No	p24, wk gp120, wk gp41	4.2	2.79-5.93	0.014
2916316	89.24	0/11	Yes	p24, p31, gp120, gp41	<1		0.598
2972539	73.16	0/32	Yes	p24, wk gp120, gp41	<1		0.043
2845047	69.84	7/14	No	p24, wk p31, gp120, gp41	8.3	5.91-11.83	0.61
2934818	87.07	8/9	No	p24, p31, gp120, gp41	43.97	27.94-84.99	6.76
2957480	31.13	4/11	Yes	p24, p31, gp120, gp41	5.05	3.43-7.07	0.004
18985235	139.63	6/31	No		2.79	1.71-4.04	ND
19354914	132.18	11/40	Yes	GP160, GP120, p66, p55, p51 GP41, p31, p24, p17(W)	4.21	2.78-5.93	ND
19836282*	100.6	0/2	Yes		<1		ND
20235369	148.47	4/7	No	All bands present	9.8	6.99-14.13	ND

VL estimated using replicate dHIV TMA and probit analysis

Blood Systems

Estimated Viral Loads and Antibody Reactivity in French Elite Controllers

	Gender / Age	BD category	Risk factor	NAT	S/CO on Prism	No. of replicates reactive on pool	No. of replicates reactive on single	Follow up confirmed	W Blot pattern	Viral load Cps/ml
1-2002	F/51	FTBD	?	TMA x 8	88/91	2/5	2/2	No	All bands present	27 [1]
2-2004	F/62	RBD	Hetero	TMA x 8	8	0/1	3/6	Yes (1 month)	p24, p31, p55, p68, gp160	33 [1]
3-2004	M/43	FTBD	MSM	Roche x24	160	0/1	1/1	Yes (16 days)	All bands present	13 [1]
4-2004	M/47	FTBD	Hetero	TMA x 8	83	0/1	1/1	no	GP160, GP120, GP41, p24, p17(W)	11 [1]
5-2005	M/36	FTBD	Africa	Roche x24	pos	0/2	0/2	no	GP160, GP120, p24, p17(W	< 50 [2]
6-2006	F/25	FTBD	Hetero	TMA x 8	pos	0/2	0/2	Yes (3 months)	All bands present	Neg [1]

VL : [1] Monitor HIV Roche US method or [2] Quantiplex bDNA Bayer

Blood Systems

RNA Detection in US Elite Controllers

- 65 ARC ECs (MP-NAT-neg/Ab-confirmed pos) tested by PCR at NGI ---

  17 (26%) had detectable RNA
- 8 were tested by 8-10 replicate dHIV TMAs ----

  7 (87%) had detectable RNA

- 24 WB+ donations that met criteria of probable FP WBs (low s/c; weak band patterns w/o p31; neg NGI PCR) tested by 10 rep dHIV TMA and all 24 tested neg x 10, on corroborating FP classification and specificity of replicate TMA

Blood Systems

Elite Controllers by Gender

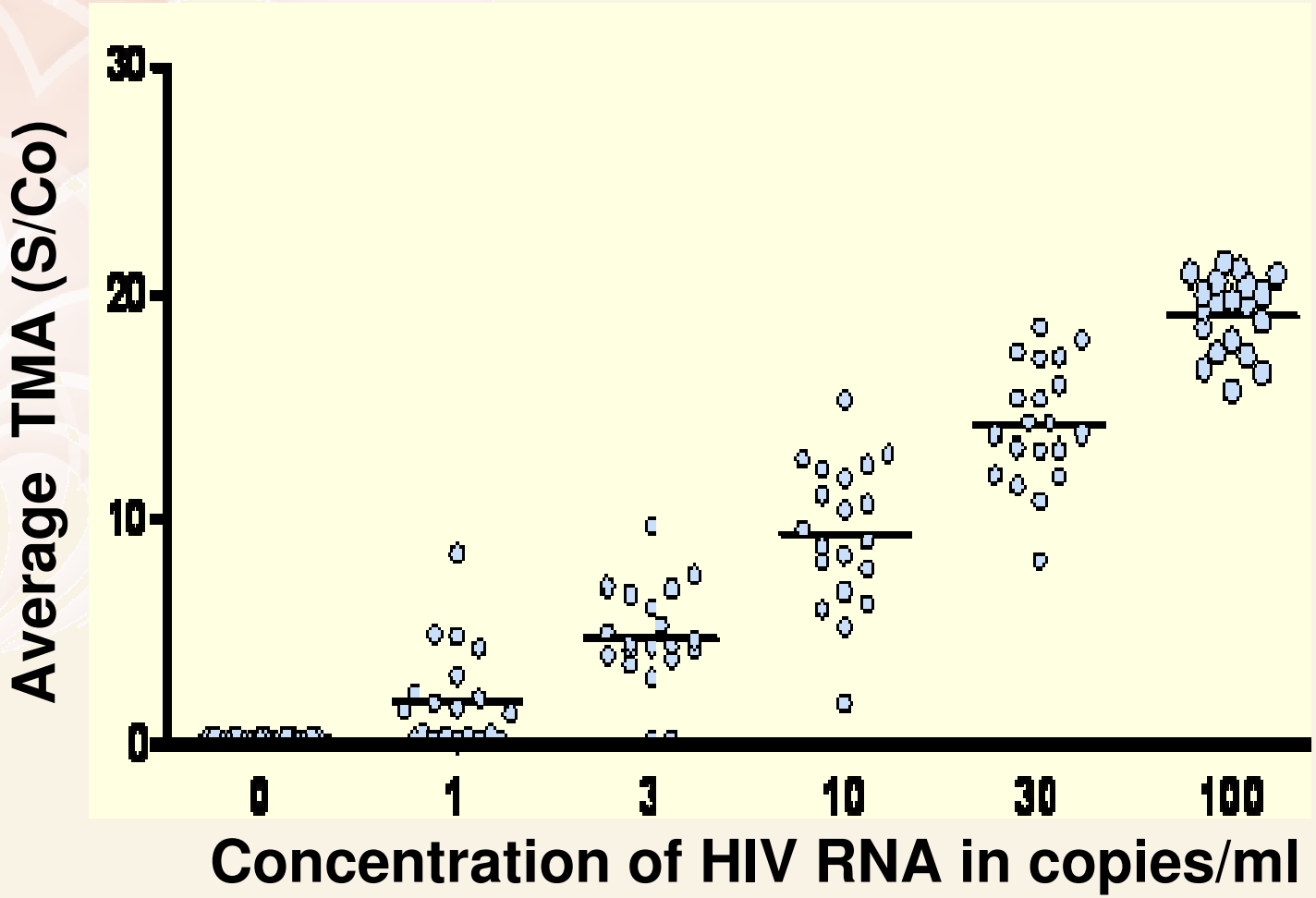
US (Clade B)	Female	Male	Total
No Ab+ Donors	412	1091	1503
No Elite controllers	25	28	53
%	**5.5%**	**2.5%**	**3.3%**

SA (Clade B)	Female	Male	Total
No Ab+ Donors	908	832	1740
No Elite controllers	8	3	11
%	**0.88%**	**0.36%**	**0.63%**

France	Female	Male	Total
No Ab+ donors	61	162	223
No Elite controllers	3	3	6
%	**4.9%**	**1.8%**	**2.7%**

Blood Systems

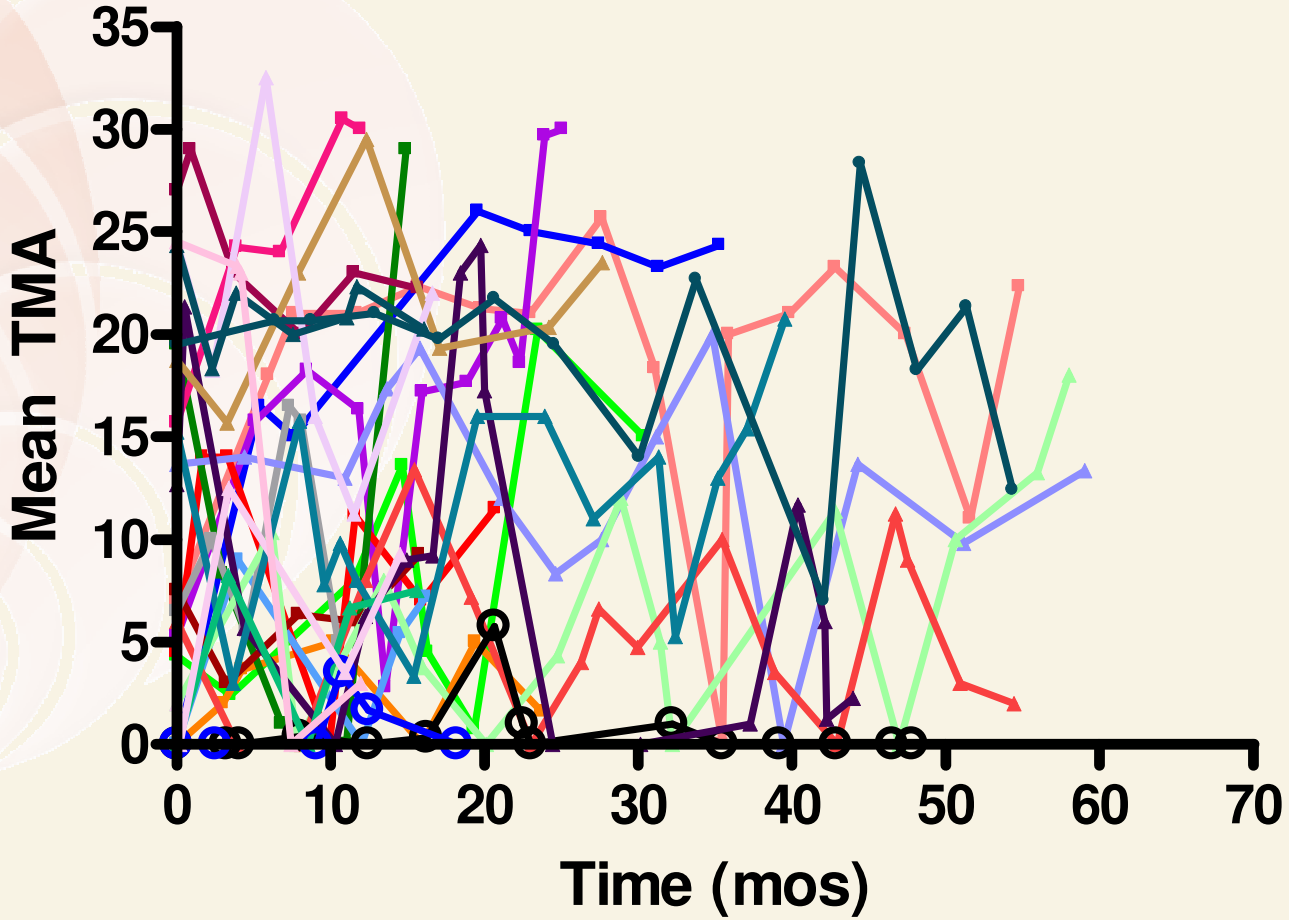
Validation of TMA Assay for Measurement of Low-level Viremia



Each data point represents average of 4 replicates

Blood Systems

Mean TMA in Elite Controllers



*Includes only subjects (n=26) with >=5 observations

Blood Systems

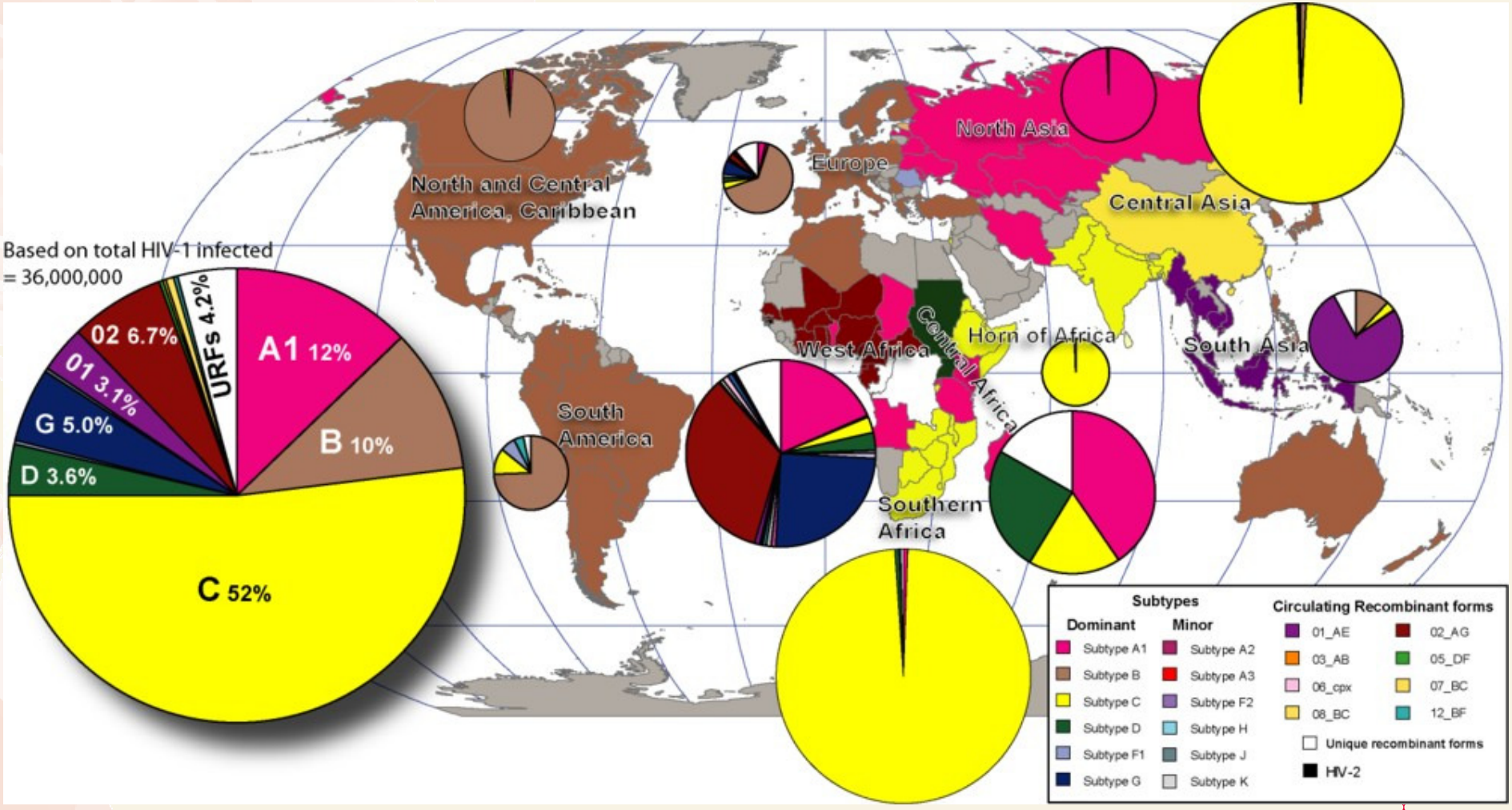
Conclusions

- Parallel screening of blood donors using HIV NAT and antibody assays provides the first systematic estimate for the frequency of ECs among newly diagnosed, asymptomatic HIV-infected persons (0.7-3.2%)
- The higher rate of ECs among HIV-1 infected donors in the US, France and Germany relative to South Africa probably reflects use of MP-NAT in those countries and ID NAT in South Africa
- Additional ID-NAT testing of EC donors detected very low-level plasma viremia in the large majority of cases evaluated
- The rates of EC are similar among demographic subgroups, except for 2-fold higher rates in females in three countries, indicating similar immunopathogenesis of ECs in these divergent clade settings and a possible role of gender on control of viremia
- Detection of very low level viremia in EC donors, and published studies documenting viral isolation from ECs, indicates that NAT screening cannot replace HIV Ab screening, even when using ID-NAT

Blood Systems

HIV-1 subtype prevalence in the world

Subtype C is dominating the epidemic



Why Study HIV Variation in Blood Donors?

- Assure that screening, diagnostic and confirmatory assays detect circulating strains
  - Assays presently are based on prototype HIV strains
  - Numerous studies have demonstrated failure of assays to sensitively detect and accurately quantify divergent subtypes
  - Documentation of viral divergence in the donor pool will lead to accelerated development and licensure of robust serological and NAT assays for donor, diagnostic and clinical management

Blood Systems

Why Study HIV Variation in Blood Donors?

- Blood donors are a “convenience sample” likely to represent the larger population
  - Studies in donors permit population based monitoring of recently transmitted viruses, including drug resistant phenotypes.
  - Knowledge of virus variation is critical to public health strategies for AIDS prevention
- Detection of variants in blood donors allows access to large volume plasma components for test development, evaluation and Quality Control

Blood Systems

HIV Genetic Subtypes in U.S. Donors

Period	Source	Tested	Non-B	Clades
’84-’85	TSS donors & hemophilliacs	143	0	
’93-’96	Donors in CDC study	383	2 (0.8%)	1 C, 1 CRF A/G
’97-’98	Donors in CDC study	163	3 (1.8%)	3 Cs, 1 HIV-2
’99-’00	Donors in CDC study	130	4 (3.1%)	1 C, 1 A 1 CRF A/E
’00-05	HIV NAT yield/ /Ab+ donors	26/46	3 (4.7%)	1 CRF_AG; 2 CRF_AE 4 drug res

De Oliveira et al. Transfusion,, 2000
Delwart et al. ARHR 2004
Brennan et al. Transfusion 2008

Blood Systems

HIV-1 Incidence Among Blood Donors in France, 1992-2006

(TRANSFUSION 2008; 48:1567-1575)

Percent recent HIV-1 Infections (<180d) by virus subtype

	No.+ donors	% Recent[a]	95%CI
Clade B	327	15.6	11.9-20.1
Non-B	93	25.8	17.5-36.1
Non-typable	39	76.9	60.3-88.3

[a] $p<10^{-4}$

Blood Systems

HIV Viral Panels Project: Purpose

In cooperation with other HIV surveillance efforts, to establish a set of fully characterized viruses from early acute HIV infections that are **consistent with the degree of viral evolution present globally**, for

- -Developing new assays
- -Validating assay platforms
- -Assisting regulators to evaluate test kits
- -Monitoring HIV drug resistance
- -Informing vaccine development

HIV Viral Panels Project

Mission Statement:

To establish a set of fully characterized viruses from early HIV infections that are **consistent with the degree of viral evolution** present globally for developing new assays, validating platforms, assisting regulatory bodies in evaluating assay performance, and collaborating with the scientific community.

Panel Criteria and Challenges

1. Obtain plasma viruses from acute or early seroconversion infections
   - Early viruses closer to transmitted virus (vaccine interest)
   - Use blood donor populations and partner with clinical protocols
2. Obtain representative emerging viruses from distinct clades (common & rare)
   - Evaluate vaccine efficacy (antibody, T cell epitopes, unique signatures…)
3. Full virus characterization
   - FGS, coreceptor usage, serological reactivity
   - Identify recombinants for diagnostic evaluation

Blood Systems

Identification and characterization of transmitted and early founder virus envelopes in primary HIV-1 infection

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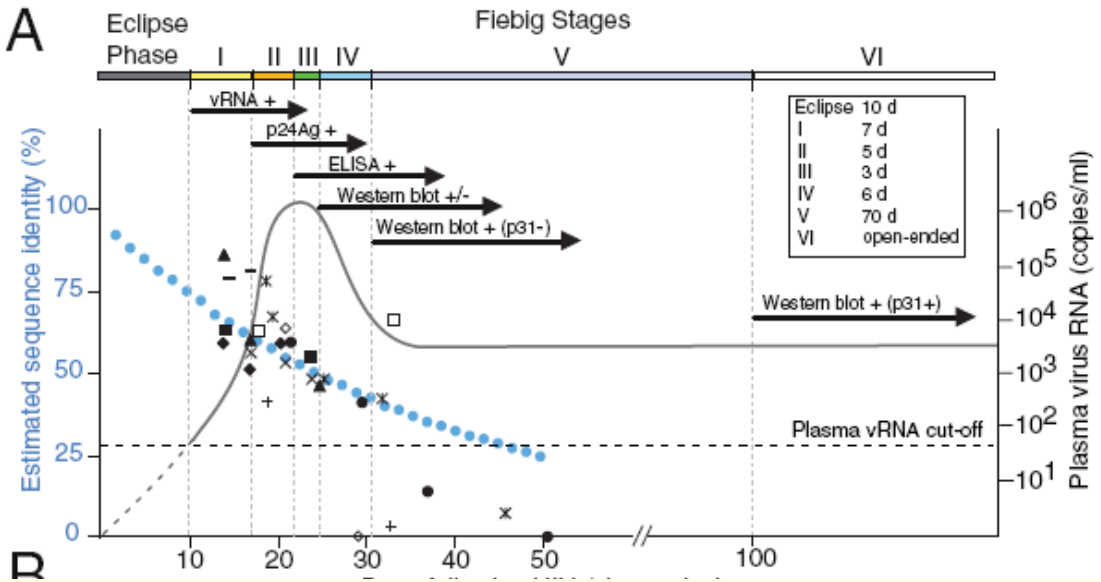


102 acutely infected plasma donor panels

3476 complete *env* sequences from single genome amplifications

Inferred consensus sequence at estimated time of virus transmission

78 donors infected by single virion; 24 by 2-5 virions

Scope of HIV Panel

- 50-60 Member Panel
- Dynamic panel: updated and rebalanced as epidemic evolves
  - Focus on isolates from acute infections
- Specimen Source:
  - Aliquots from plasma components from acutely infected donors
  - Plasma viral isolation/propagation for rare Groups, Subtypes, CRF

Blood Systems

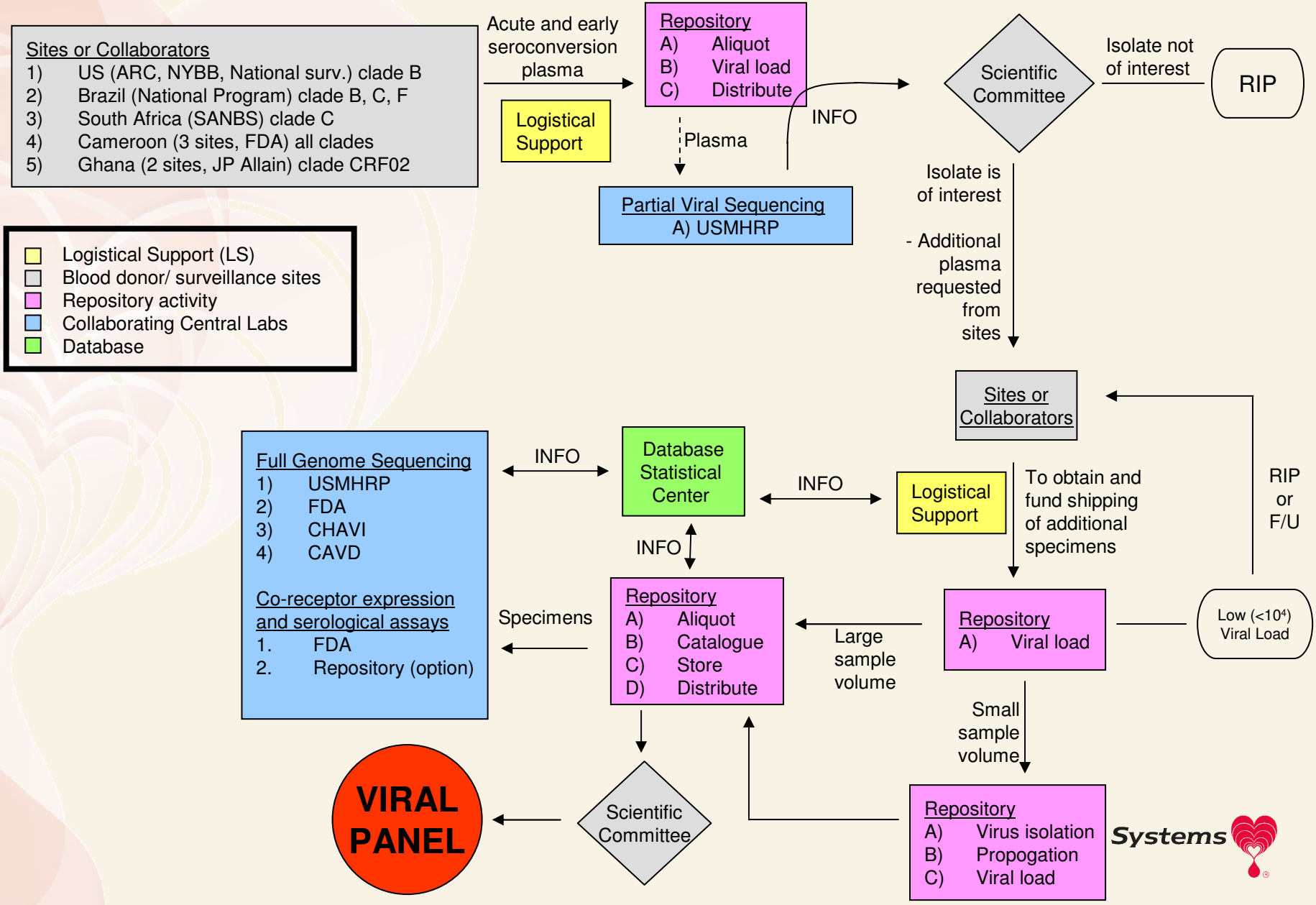
Tier 1 Isolates

Subtype	Region of Interest
A1	Uganda, Rwanda, former Soviet Republics
B	North America, Western Europe, Australia, Western South America
C	South Africa, Botswana, Zambia, Malawi, Tanzania, Ethiopia, India, Southern Brazil
D	Uganda
G	Nigeria, Spain (IDU), Portugal (IDU)
CRF01_AE	Thailand, Vietnam, Cambodia
CRF02_AG	Senegal, Nigeria, Ghana, Cote d'Ivoire, Cameroon

Tier 2 Isolates

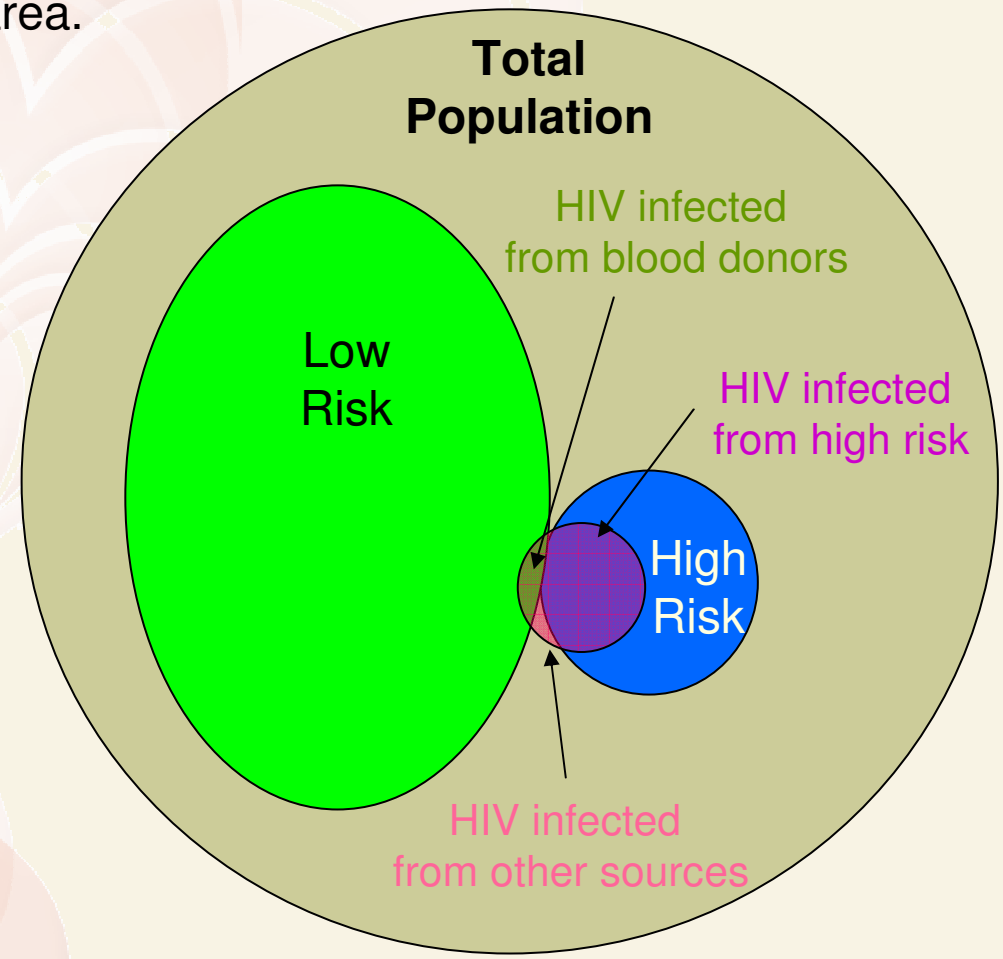
Subtype	Region of Interest
F1	Brazil, Romania, Spain
F2	Cameroon
H, J, K	DRC, Cameroon, Congo
CRF04_cpx (A,G,H,K,U)	Cyprus, Greece
CRF05_DF	DRC, Belgium
CRF06_cpx (A,G,J,K)	Niger, Mali, Cote d'Ivoire
CRF07_BC	China
CRF08_BC	China
CRF09_cpx (02,A,U)	Cote d'Ivoire, Mali
CRF11_cpx (A,G,01,J)	Cameroon, DRC, CAR
CRF12_BF	Argentina
CRF13_cpx (A,01,11,G,J,U)	Cameroon, CAR
CRF14_BG	Spain, Portugal
CRF18_cpx (A,F,G,H,K,U)	Cuba
CRF20_BG, CRF23_BG, CRF24_BG	Cuba
CRF31	Brazil

Sample Flow


Sites or Collaborators
1) US (ARC, NYBB, National surv.) clade B
2) Brazil (National Program) clade B, C, F
3) South Africa (SANBS) clade C
4) Cameroon (3 sites, FDA) all clades
5) Ghana (2 sites, JP Allain) clade CRF02
Logistical Support (LS)
Blood donor/ surveillance sites
Repository activity
Collaborating Central Labs
Database
Acute and early seroconversion plasma
Logistical Support
Repository
A) Aliquot
B) Viral load
C) Distribute
Plasma
INFO
Partial Viral Sequencing
A) USMHRP
Scientific Committee
Isolate not of interest
RIP
Isolate is of interest
- Additional plasma requested from sites
Sites or Collaborators
RIP or F/U
To obtain and fund shipping of additional specimens
Logistical Support
INFO
Database Statistical Center
INFO
INFO
Full Genome Sequencing
1) USMHRP
2) FDA
3) CHAVI
4) CAVD
Co-receptor expression and serological assays
1. FDA
2. Repository (option)
Specimens
Repository
A) Aliquot
B) Catalogue
C) Store
D) Distribute
Large sample volume
Repository
A) Viral load
Low (<10⁴) Viral Load
Small sample volume
VIRAL PANEL
Scientific Committee
Repository
A) Virus isolation
B) Propogation
C) Viral load
Systems

Statistical Consideration Regarding Viral Panels: Are the viruses representative?

Hypothesis: The viral sequence diversity of transmitted viruses derived from HIV acutely infected individuals is not statistically significant between low risk (blood donor) versus high risk (VCT, STD clinics) populations within the same geographic area.



DATA:

- Demographics
- Risk factors
- Viral load
- Full viral genome sequence analysis
- Fiebig stage
- Biological analysis
- Serological analysis

Countries:

- USA
- South Africa
- Brazil

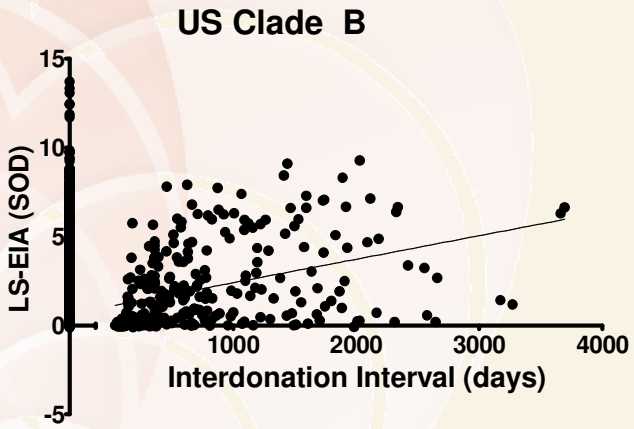
Blood Systems

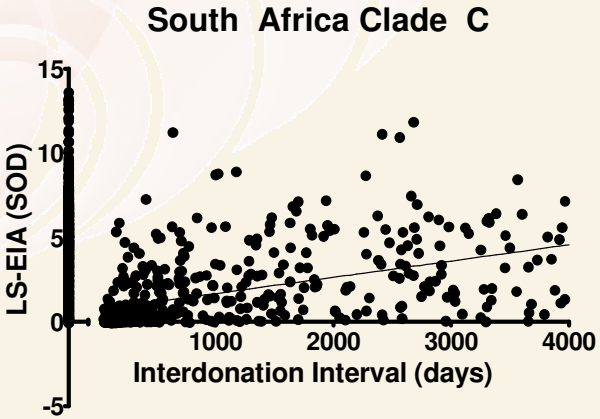
HIV Viral Panels: Early Goals and Accomplishments

1. Goal to complete a pilot study 6 months
   - 20 pre or very early post-SC plasma units from 5 countries (US, SA, Brazil, Cameroon, Ghana)
   - Obtain country support and resolve IRB issues and logistical challenges; standardize procedures
2. Accomplishments
   - Identified needed strains and geographic locations
   - Partnering with different groups to collaborate and pool resources
   - Identified initial start-up funds for FY'09-10
   - Continued support anticipated from NIH and Gates foundation

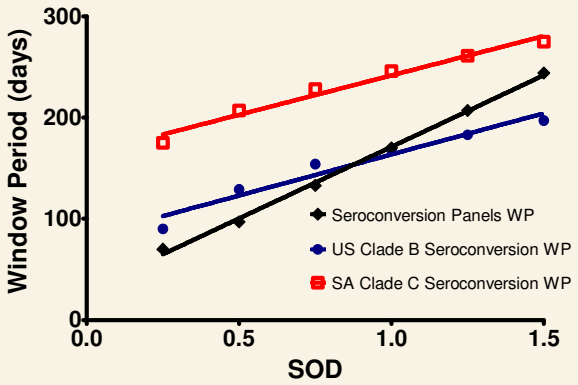
Blood Systems
National Institute of Allergy and Infectious Diseases

Derivation of HIV Incidence Assay "Window Periods" from SC Blood Donors in Countries with Diverse HIV Clades





V-LS-EIA cutoff (SOD)	Clade B WP from SC panels (days)	US clade B donors N=307		SA clade C donors N=504	
		# with SODs < cutoff	Calculated WP (days)	# with SODs < cutoff	Calculated WP (days)
0.25	70	119	90	183	175
0.5	97	169	129	223	207
0.75	133	203	154	249	228
1	170	222	168	271	246
1.25	207	241	183	289	261
1.5	244	260	197	306	275



A representative calculation for an LS-EIA window period for an SOD of 1.0 is shown below:

$$\text{WP (days)} = \frac{\text{Adjusted number seroconverters}}{\text{Incidence} \times \text{number tested}} \times 365$$

$$= 232.5/(1.57/100{,}000 \times 32{,}120{,}470) \times 365$$

$$= 168$$

Blood Systems