NHLBI programs to respond to emerging transfusion-transmitted infections

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Uses of donor-recipient repositories

- Proof of transfusion-transmission
  - Sequence identity of isolates
  - Contrast to hospitalized, non-transfused controls
- Establish the rate of tx-transmission at time repository was collected
  - Distinguishes new vs newly discovered agent
  - However, may not be relevant to current risk
- Evidence for lack of tx-transmission

Uses of donor-recipient repositories

- Determine correlates of transmission
  - Level of viremia
- Dynamics of early infection in recipients
  - Window period to antibody detection
  - Kinetics of viremia
  - Cellular immune response
- Defined cohort for long term natural history studies

NHLBI-sponsored donor and recipient repositories

Study	Years	Type	Agents studied
TTVS	1974-79	Linked	HBV,HCV,GBV-C
NIH CC	1973-80	Linked	HCV,GBV,TTV,SEN
TSS	1984-85	Donor	HIV, HTLV
FACTS	1985-91	Recp.	HIV, HTLV, HCV
VATS	1995-98	Linked	HIV, CMV, HCV, HBV
REDS	1991-95	Donor	HBV, CMV, HHV-8, T. cruzi, TTV, PTLV

Retrovirus Epidemiology Donor Study (REDS): donor repositories

Period:	1990-1995 and ongoing
Regions:	LA, SF, Det, Balt/DC, Okl C.
Repository:	508,151 donor sera (GSR)
	147,915 donor plasma and cells (GLPR)
	Special Repositories:

- HTLV
- HIV

Key findings from REDS general repositories

- Rate of HBV NAT pos in HBsAg neg/anti-HBc pos donors
- Prevalence of T. cruzi in donors and risk of transmission through lookback studies
- HHV-8 seroprevalence/viremia in donors
- CMV viremia in seropos and seroneg donors
- TTV seroprevalence in donors

REDS RADAR Repository

- Established from 2000-2003 in seven geographic regions (Balt-DC, Detroit, LA, SF, Oklahoma City, Pitt, Tampa)
- NHLBI and CDC funded
- Full enrollment for recipients included a pre (or peri) tx specimen and a follow-up specimen at 6-12 months
- RADAR units (mostly RBC) were targeted to surgical patients on specific clinical services at specific hospitals
- Enrolled recipients got both RADAR and non-RADAR units

Design goals for RADAR

- Primary aim: To show that a given agent is not transmissible by transfusion (or has a transmission rate less than a given low number: i.e., 25%) with a reasonable level (i.e., 95%) of certainty
- Secondary aim: If transmission occurs, to provide a measure of the transmissibility rate

Prevalence and transmissibility assumptions

- Assumption that donor prevalence assay will correlate with infectivity (i.e. HIV, HCV, HBV)
  - 0.05% -1% based upon prior agents at the time of initial discovery
- Transmissibility assumed to be in the range of HIV, HCV, HBV (>75%) or HTLV (~25%)
- Might not be powered for lower rates

Specimen categories

- Fully enrolled recipients (n=3575)
- Initially enrolled recipients without f/u (n=1402)
- Donation specimens (n=127,864)
  - Linked to fully enrolled recipients
  - Linked to initially enrolled recipients
  - Specimens from donors who had a different repository donation txd to a RADAR recipient
  - Unlinked

Fully enrolled recipients

- RADAR components txd: mean:3.9; median 2.0
- RADAR component types:

  Non LR RBCs: 35% LR RBCs : 42%

  WB platelets: 13% FFP: 10%
- RADAR RBC recipients: 98% (n= 3515)

  LR only: 55% (n=1961)

  Non-LR only: 40% (n=1433)

  Both: 3% (n=121)

REDS-I Mission

- initiate and facilitate investigations of transfusion-transmitted infections in blood donors
- provide NHLBI with the scientific resources to rapidly address critical issues affecting blood safety and availability
- provide scientific data for policy decision-making
- provide data on relevant blood safety and availability issues and identify areas requiring additional research

Research Areas

- Epidemiological Studies
- Donor Research
- Laboratory Studies

REDS-I Laboratory Studies - HIV

- evaluated HIV WB-Indeterminates
- characterized and estimated prevalence of HIV WB-FPs
- evaluated alternative HIV serological confirmatory assays (RIBA or IFA)
- evaluated p24 Ag positive and indeterm results
- helped develop a less sensitive HIV-antibody EIA
- studied early viral dynamics and estimated WPs

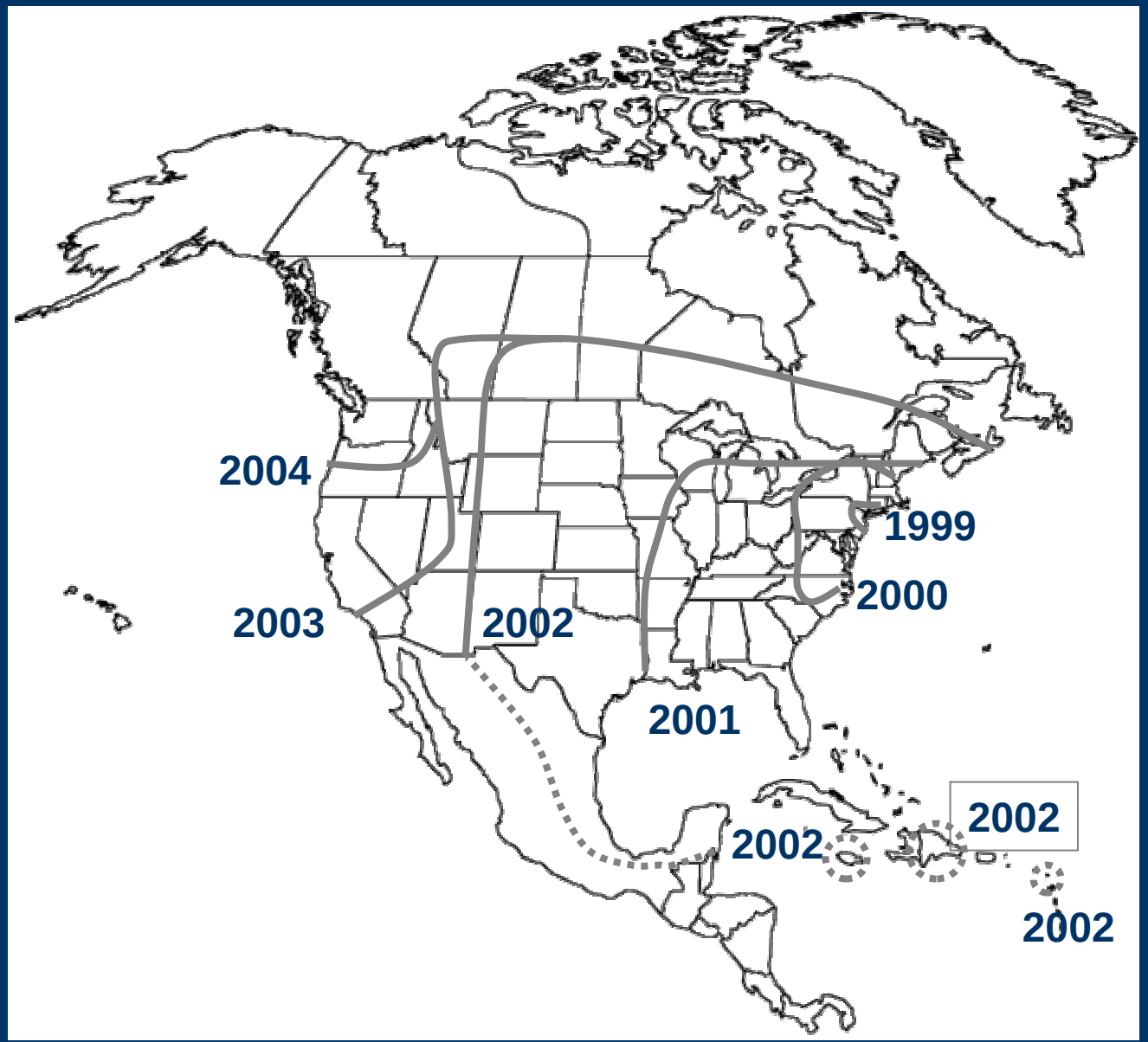
Laboratory Studies - HCV, HBV, HTLV

- HBsAg test algorithm to identify potential false-positives
- effectiveness of anti-HBc screening
- impact of enhanced HCV screening and confirmatory tests (EIA/RIBA 2.0 vs. 3.0)
- HCV transmission by Rh immune globulin
- HCV lookback policy
- HTLV serological tests
- early viral dynamics and estimated WPs

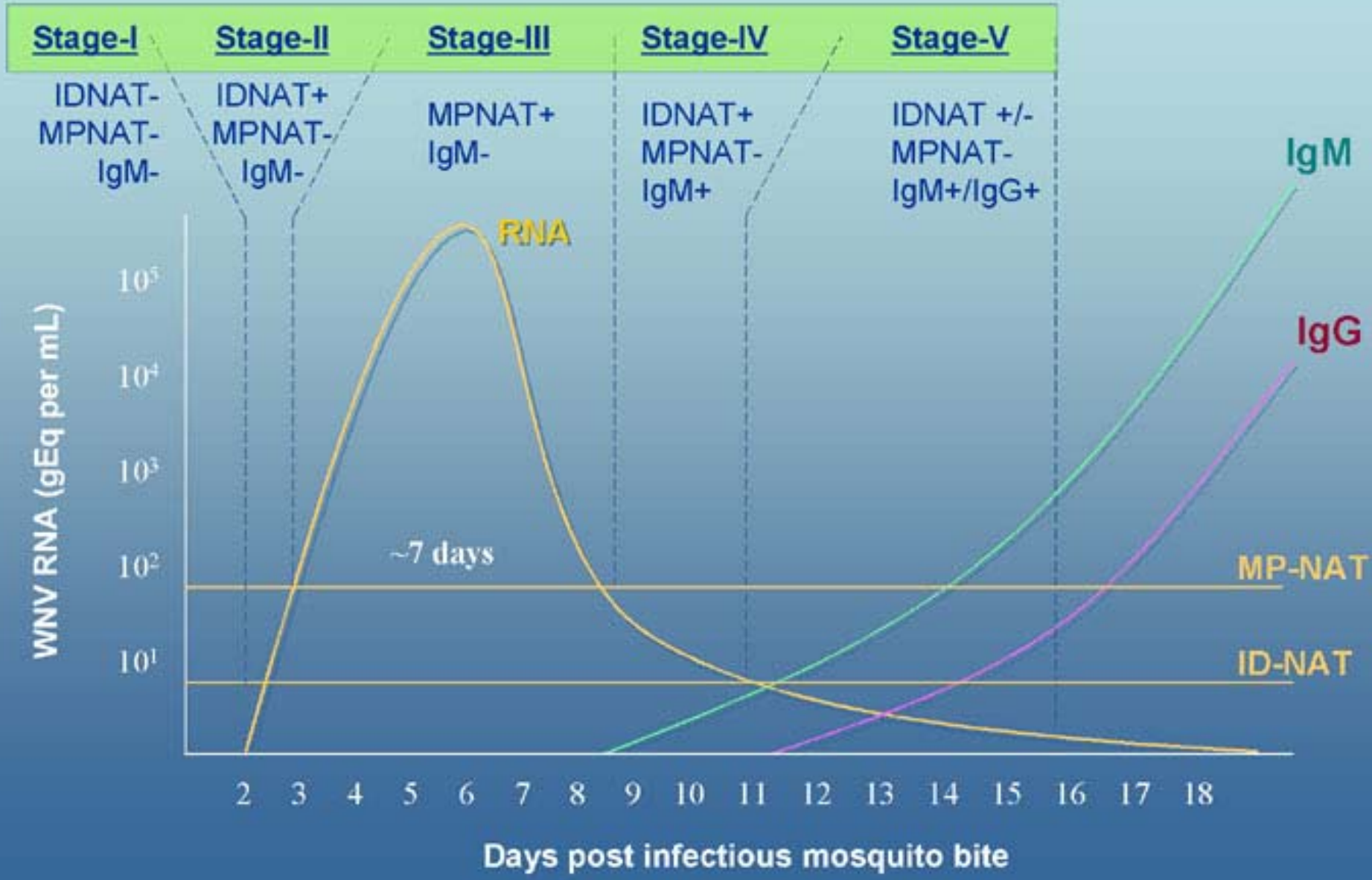
Laboratory Studies - Emerging Infections

- idiopathic CD4 lympocytopenia (ICL) studies
- prevalence of *T. cruzi* and conducted lookback investigation
- presence of Primate T-Lymphotropic viruses in blood donors with HTLV sero-indeterminate results
- evaluated HHV-8 serological assays and estimated HHV-8 prevalence in US blood donors
- WNV studies

Distribution of West Nile Virus in N America

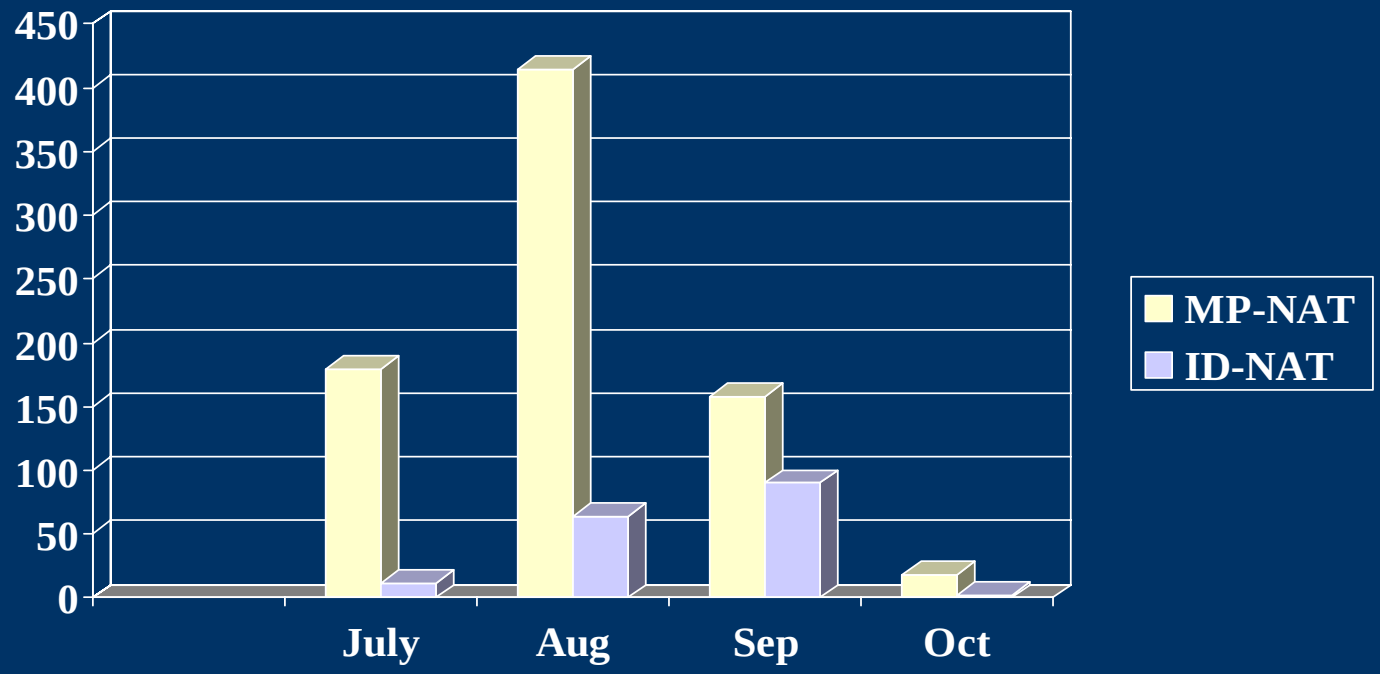


Stages of viremia during WNV infection



Yield of WNV NAT screening of 4,585,573 donations from July-October, 2003

ARC & ABC (~95% of U.S. collections)

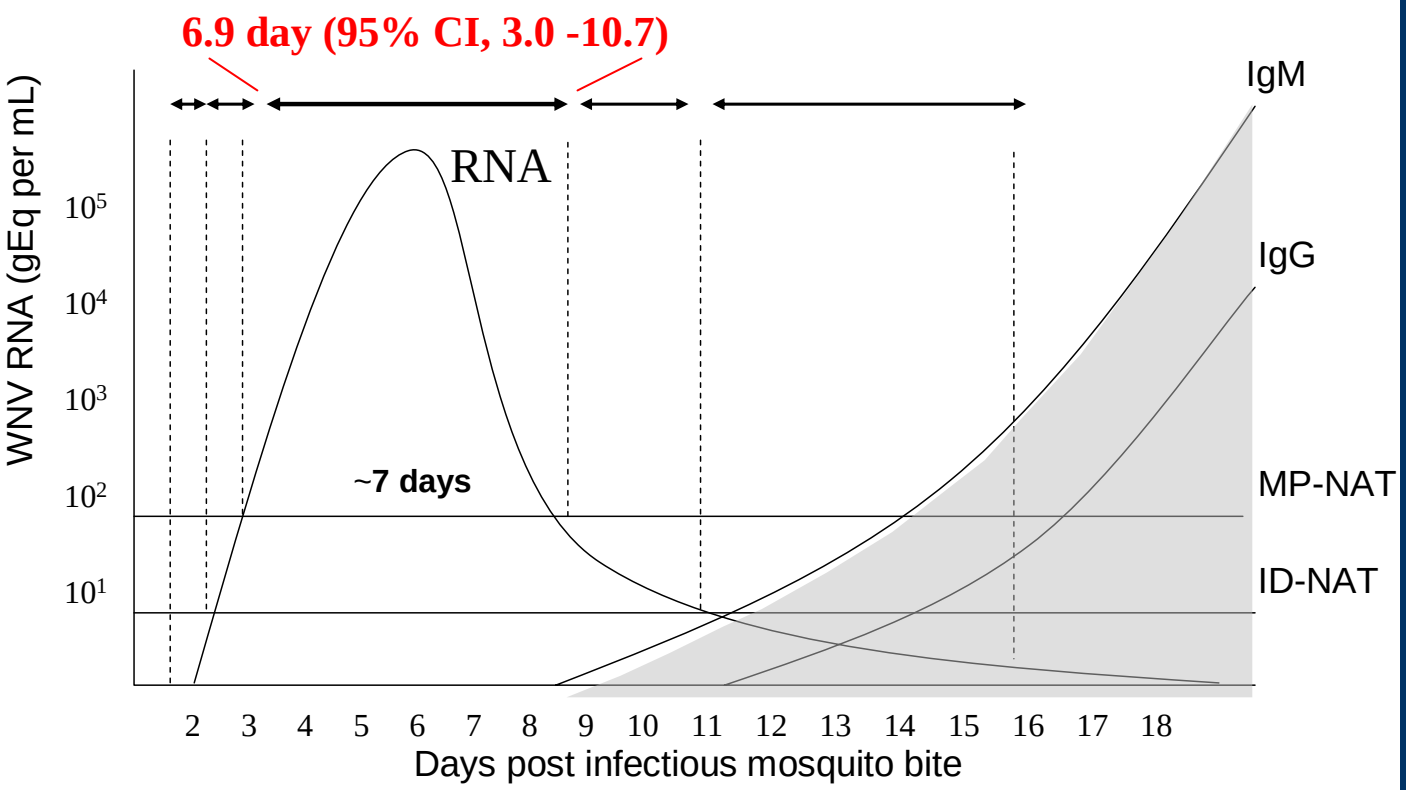


944 confirmed viremic donors.:

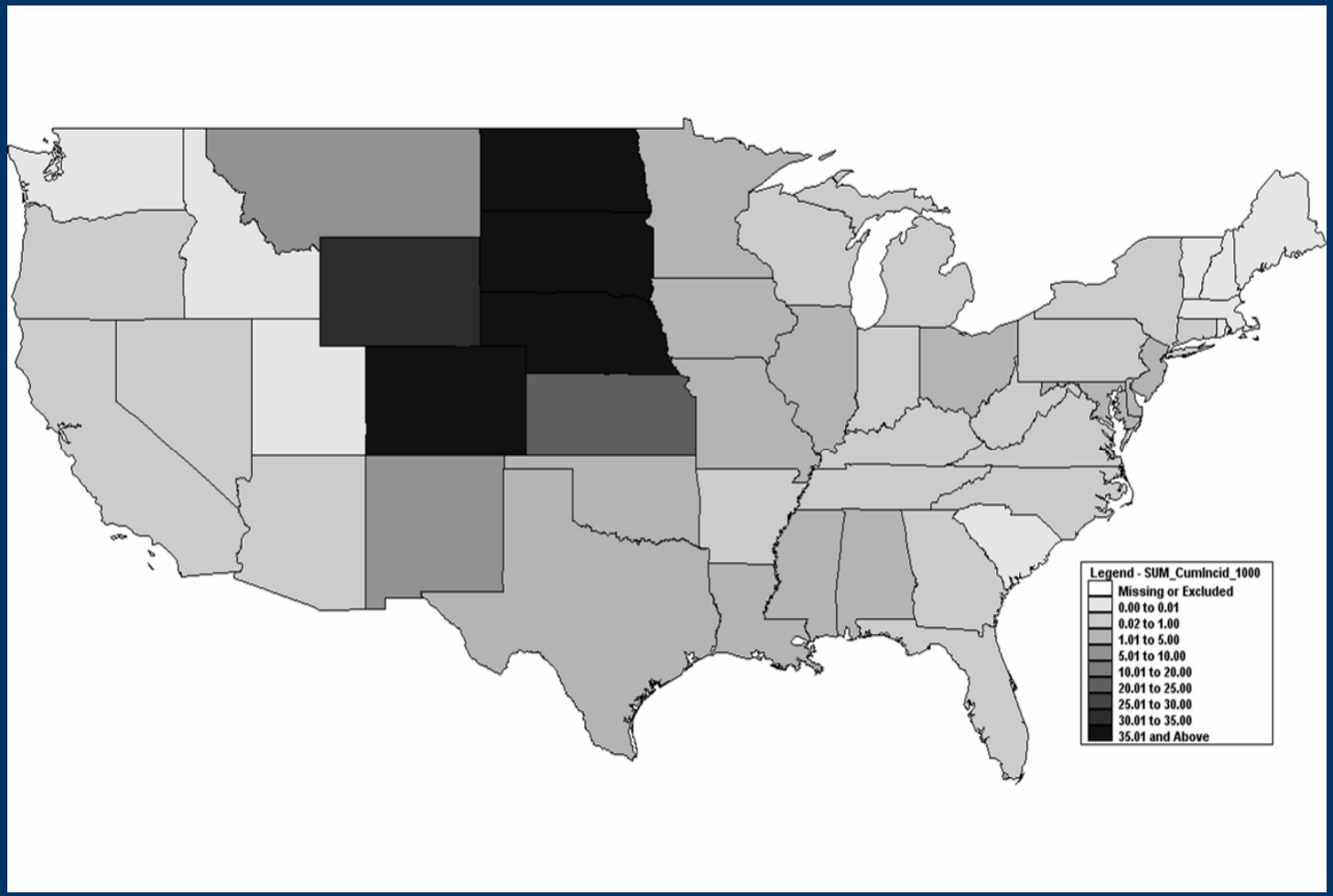
- **770 detectable by MP-NAT**
- **174 ID-NAT-only (BSI and ARC screened 36,269 donations)**

Derivation of $T_{MP\text{-}NAT}$ from period-specific MP-NAT yield and peak IgM prevalence rates

$T_{MP\text{-}NAT}$ (expressed in weeks) derived by dividing sum of weekly MP-NAT estimates by the peak IgM prevalence



State-specific WNV infection rates (per 1000) in 2003, projected from MP-NAT yield and T_{MP-NAT}

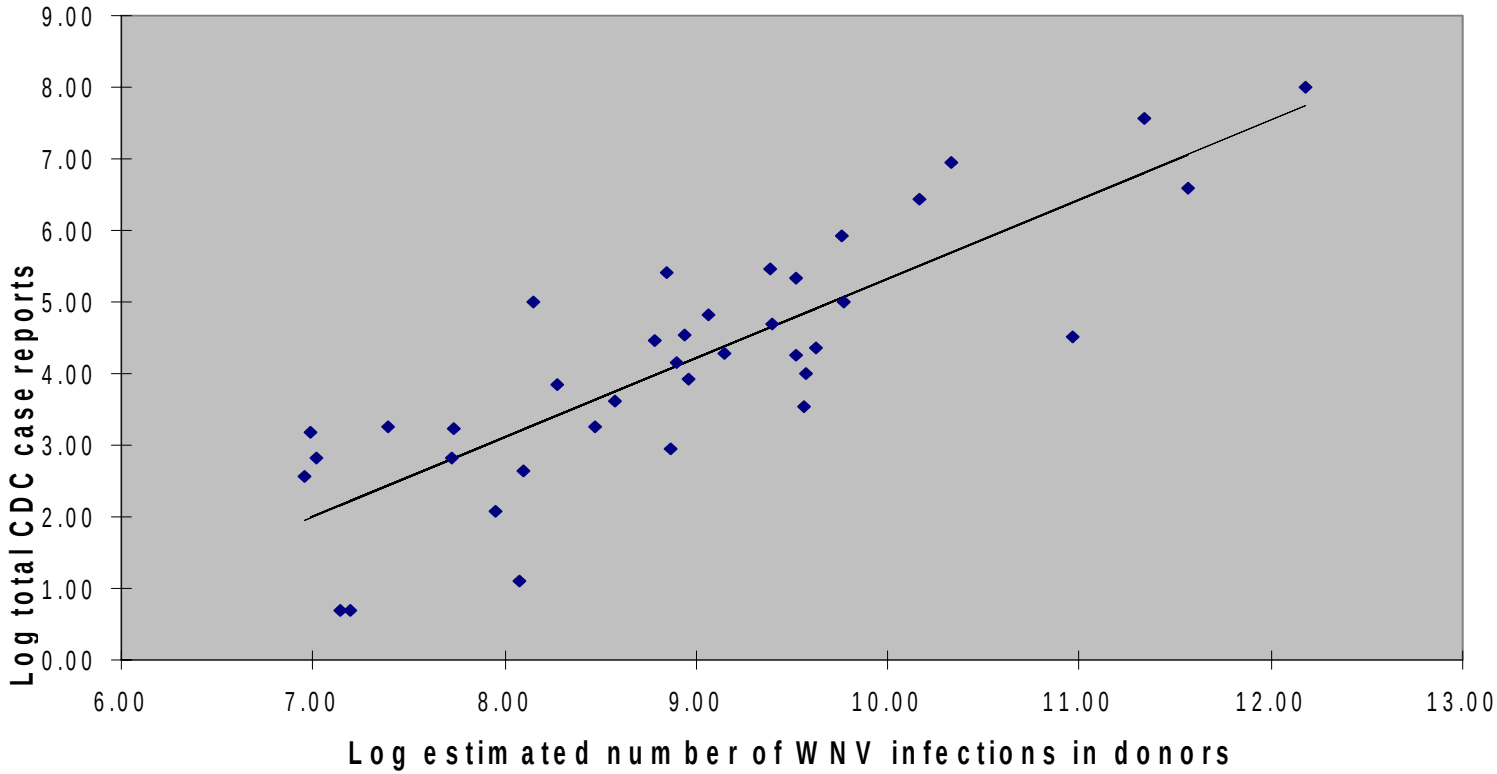


Highest infection rates in Nebraska (4.9%), Colorado (4.3%), North Dakota (4.1%), South Dakota (4.0%), Wyoming (3.5%) and Kansas (2.1%)

Nationally, 735,000 persons (95% CI 583,000-887,000) infected with WNV in 2003.

Correlation of MP-NAT yield-based population infection rates with WNV neuroinvasive cases

1 neuroinvasive case per 256 WNV infections



REDS II

- Funding – NHLBI (10/04-9/09)
- Coordinating Center –Westat
- Central Laboratory – BSRI/BSL
- Participating US Blood Centers
  - Blood Center of Southeastern Wisconsin
  - ARC New England Region
  - Emory University/ARC Southeast Region
  - University Cincinnati/Hoxworth
  - Institute For Transfusion Medicine
  - UCSF/Blood Centers of the Pacific/BSRI
- International Blood Centers
  - 2-3 developing countries

REDS II

Objectives

*To conduct epidemiologic, laboratory and survey research on volunteer blood donors to ensure the **safety** and **availability** of the US blood supply*

REDS II

- Two proposals were submitted by each site, the coordinating center, and the central laboratory
- The proposals fell into 6 groups:
  - Infectious disease
  - TRALI
  - Iron/hemoglobin in donors
  - Donor recruitment/retention
  - Donor deferral
  - Donor database and surveys

REDS-II International Component

- RFP issued by Westat April 22, 2005 (available at www.westat.com/reds2rfp.html)
- Proposals due June 14, 2005
- Anticipate up to three awards made to U.S. blood centers/blood banks/academic institutions with established collaborative programs with foreign blood centers/blood banks (3 or more) in developing countries where HIV/AIDS is highly prevalent.
- 4 year program with anticipated award date of September 1, 2005

REDS-II International - Objectives

Conduct epidemiologic, laboratory, and survey research on blood donors in selected developing countries in regions seriously affected by the HIV/AIDS epidemic such as Africa, Asia, and South America to help increase the safety and availability of blood for transfusion.

- Assess and monitor the prevalence and incidence of HIV-1, HIV-2 and other existing as well as newly discovered infectious agents that pose a threat to blood safety
- Assess risks of transfusion-transmitted infections
- Assess the impact of existing and new blood donor screening methodologies on blood safety and availability
- Evaluate characteristics and behaviors of blood donors including risk factors for acquiring HIV and other blood-borne agents
- Evaluate the donation process for ways to improve the safety and adequacy of the blood supply, and reduce infectious disease burden.

REDS-II International Component

To address important blood safety issues, the REDS-II International Component will

- develop a comprehensive longitudinal donor and donation database for each foreign program containing information on all donations collected during the period of the contract (≥25,000 annual donations/site; total of ≥ 250,000 annual donations/country); and

- conduct a research program that includes epidemiologic, survey, and /or laboratory studies.

REDS-II Infectious Disease Projects

Proposed

- RADAR prevalence/transmission studies
  - HHV-8
  - Parvo B19
- Molecular surveillance of incident case of HIV, HCV, HBV and WNV

In development

- Assessing risk of TT of tick-borne pathogens
- Effect of LR on EBV and CMV viral load
- Emerging infectious agents in US (global) donor pool

Early discussion

- Simian Foamy Virus prevalence/transmission
- Role of HBsAg post-NAT
- Impact of vaccines on blood screening

HHV-8 and transfusion

Pro

- Recent study suggesting transmission to two cardiac surgery patients (out of 284) in Baltimore at end of 1980s
  - However, no donor linkage established
- Cross-sectional studies of SS anemia pts in Uganda

Con

- No transmission in 32 recipients in two previous studies
- KS not reported in chronic transfusion recipients

Parvovirus B19 and transfusion

- Known to be transmitted by blood components, SD plasma, and factor concentrate
  - Resistant to inactivation
- Can cause disease in specific patient populations
  - Fetus, hemolytic anemia and cellular immunodeficiency pts.
- Significance of clinical disease from transfusion transmission is unknown
  - Anecdotal reports of mild disease

Parvovirus B19 and transfusion

- Transmission rate is unknown
  - No prospective studies
- Many recipients (50-75%) may be protected due to pre-existing antibody
- Relationship to viral titre in single donor blood components is unknown
  - This may have important implications regarding the need for more sensitive NAT applied to transfusable components

Parvovirus B19 and transfusion

- In SD plasma setting, transmissions did not occur in units with viral titres below 10^4 copies/ml
  - Unknown whether this was due to protective antibody in pool or due a higher required infectious dose of virus or both
- Currently US source and recovered plasma manufacturers are performing desensitized NAT (~10^6 copies/ml) but not linking these results to transfusable components
  - Decreases yield but provides safety
  - Ethical/safety concerns over “in process” testing that does not interdict B19 DNA+ transfused components

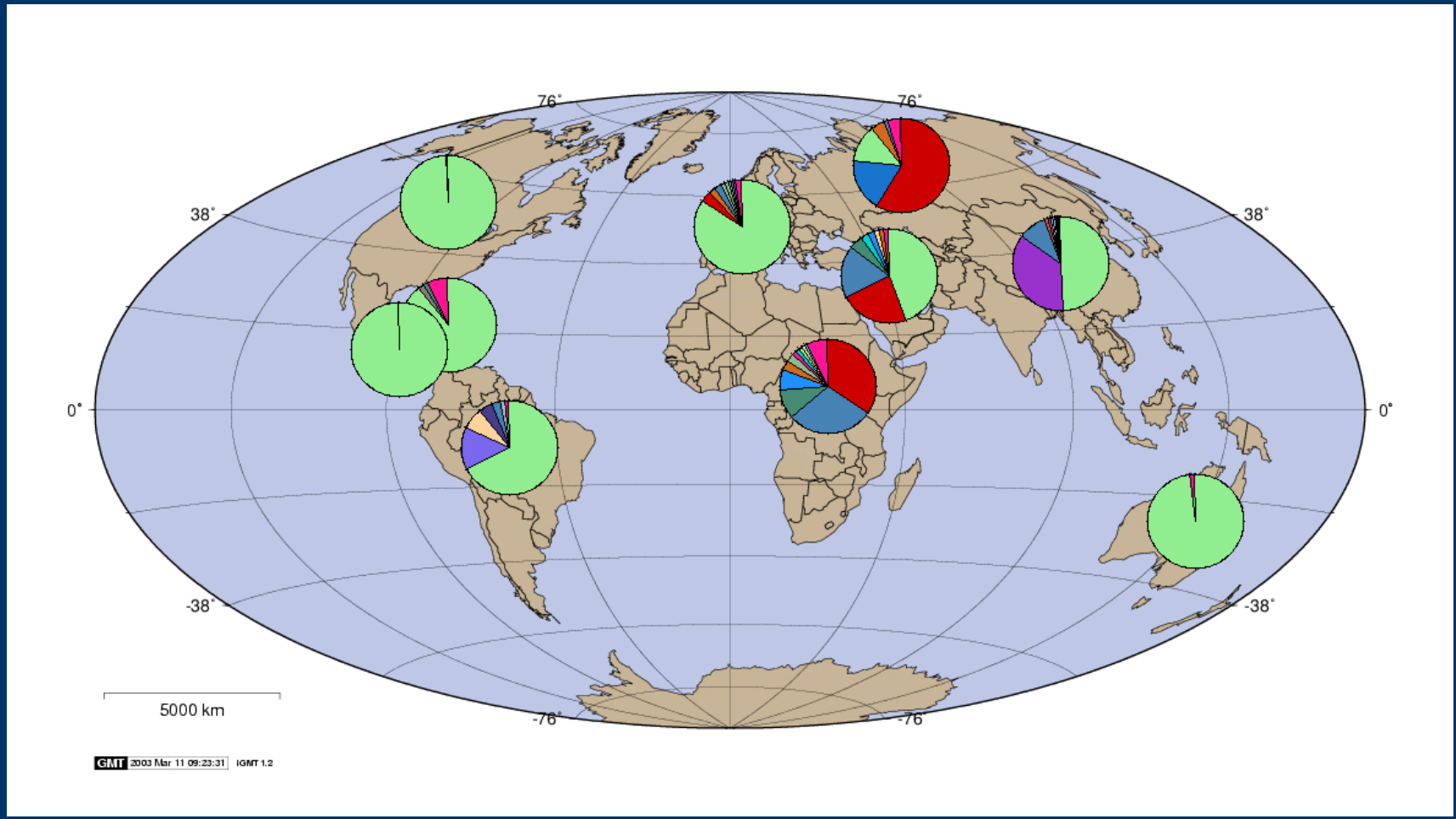
Why surveillance for molecular variants?

- Assure screening, diagnostic and supplemental assays are sensitive to circulating strains
  - U.S. assays still based on prototype strains
  - Numerous studies have demonstrated failure of these assays to sensitively detect and accurately quantify divergent subtypes
  - Documentation of divergence in donor pool will lead to accelerated development and licensure of robust serological and NAT assays
- Pathogenesis implications (e.g., HCV-1a non-responsive to Rx)
- Vaccine implications (HBV escape mutants; HIV/HCV vaccines)

Molecular surveillance of incident cases of HIV, HCV, HBV and WNV

- Major risk of TTV is from donations during acute infection WP (incident infections); testing errors, viral variants and immunosilent infections are minor contributors
- Combined NAT and serological screening, supplemented by novel serological test strategies (e.g., detuned EIAs), identifies incident cases (and test errors)
- Systematic program proposed for genetic characterization of viral genomes in donors with incident infections
  - monitor circulating strains of viruses transmitted to donor population, and this within “low risk” general population
  - detect rare variants, including vaccine and drug escape mutants, that may be increasing in U.S. population

Global distribution of HIV genotypes



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
’99-2000	Donors in CDC study	130	4 (3.1%)	1 C 1 CRF A/E 1 A variant

p=0.06

Surveillance of HIV-1 Genetic Subtypes and Diversity in the U.S. Blood Supply
de Oliveira CF, Diaz RS, Machado DM, Sullivan MT, Jacobs T, Gwinn M, Lackritz EM, Williams AE, Kessler D, Operskalski EA, Mosley JW, **Busch MP.** Transfusion, 40:1399-1406, 2000

Two percent of HIV-positive US Blood donors are infected with non-subtype B strains
Delwart E, Orton S, Parekh B, Dobbs T, Clark M, Busch MP. ARHR 2004

Use of the sensitive/less-sensitive (detuned) EIA strategy for targeting genetic analysis of HIV-1 to recently infected blood donors

DaisyM. Machado[a,b], Eric L. Delwart[a,c], Ricardo S. Diaz[a,b], Carlos F. de Oliveira[a,b], Katia Alves[a,b], Bhupat D. Rawal[a], Marian Sullivan[d], Marta Gwinn[e], Kenneth A. Clark[e] and Michael P. Busch[a,f]

AIDS 2002, **16**:1–7

Keywords: Acute infection, epidemiology, HIV diagnostic tests, HIV sequence variability, seroprevalence, surveillance, detuned EIA

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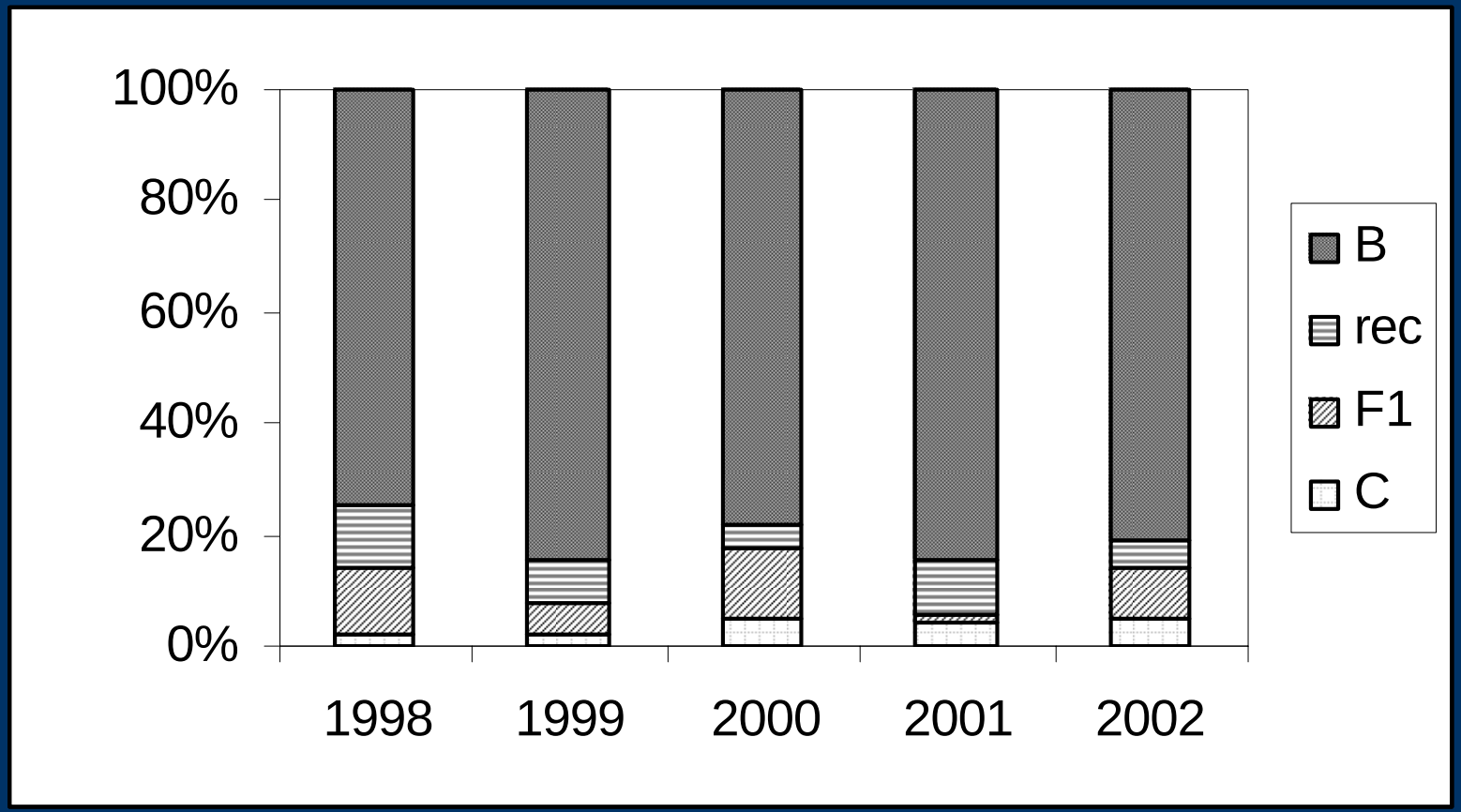
HIV Subtypes in Sao Paolo blood donors, according to STAHRS Results

STAHRS results	Pure subtypes			Recombinants			
	B N(%)	C N(%)	F1 N(%)	BF N(%)	BC N(%)	CRF02_AG N(%)	Total
recent infected	49 (89.1)	1 (1.8)	2 (3.6)	2 (3.6)	0	1 (1.8)	55
long standing	222 (79.2)	12 (4.3)	23 (8.2)	21 (7.5)	2 (0.7)	0	280
Total	271 (80.9)	13 (3.9)	25 (6.7)	23 (7.5)	2 (0.6)	1 (0.3)	335

Detuned results were not obtained for 6 subtype B samples

Proportion of non-B subtypes according to the year of sample collection.



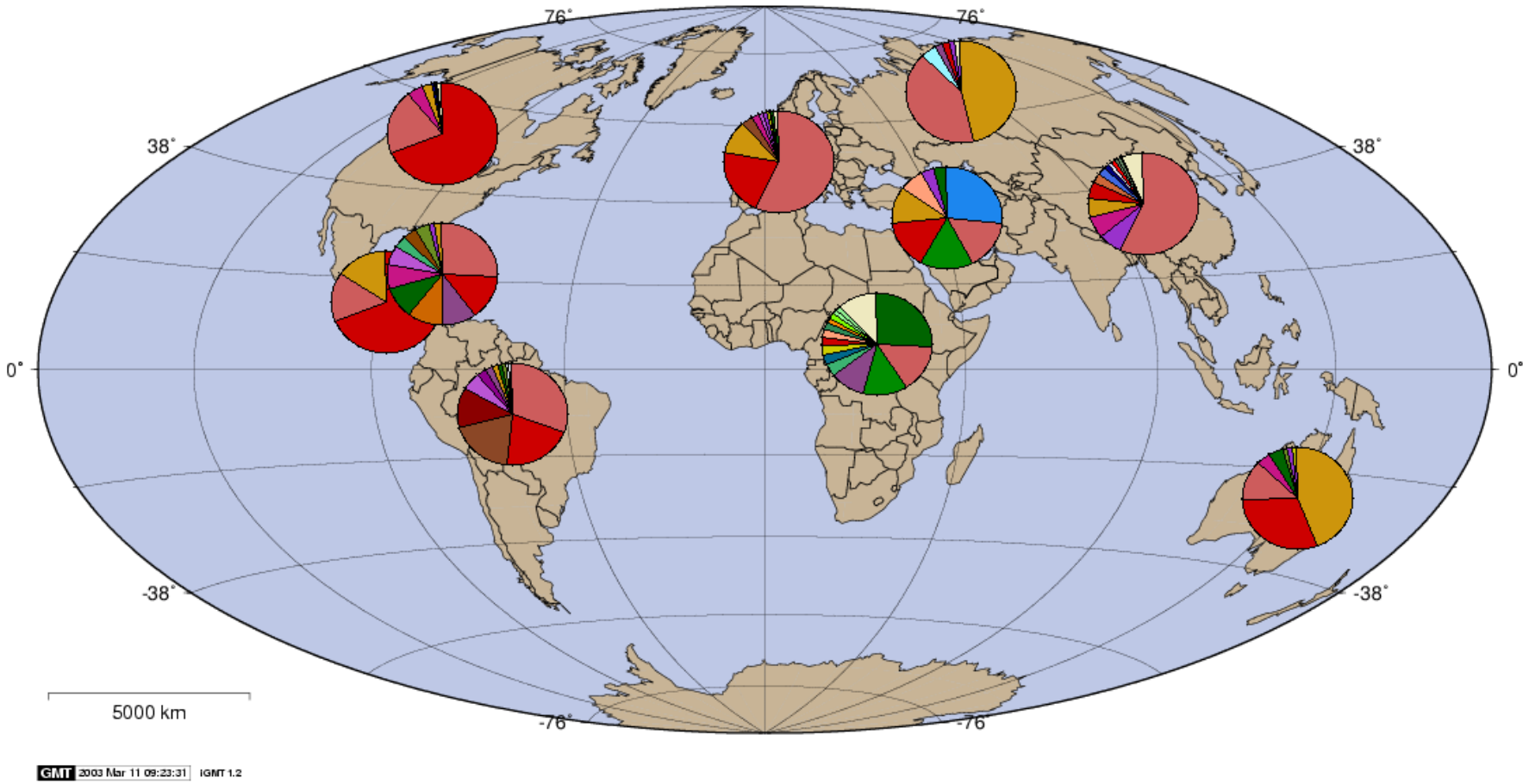
Number and percentage of drug resistant strains in HIV-infected Sao Paulo blood donors

STAHRS results	N of resistance strains	Prevalence (95% CI)	Total
Recent Infection	7	12.7 (5.2 - 245)	55
Long Standing	16	5.7 (3.3 - 9.1)	280
Total	23	6.8 (4.4 - 10.1)	335 *

Detuned results were not obtained for 6 samples with no resistance mutation

P = 0.06

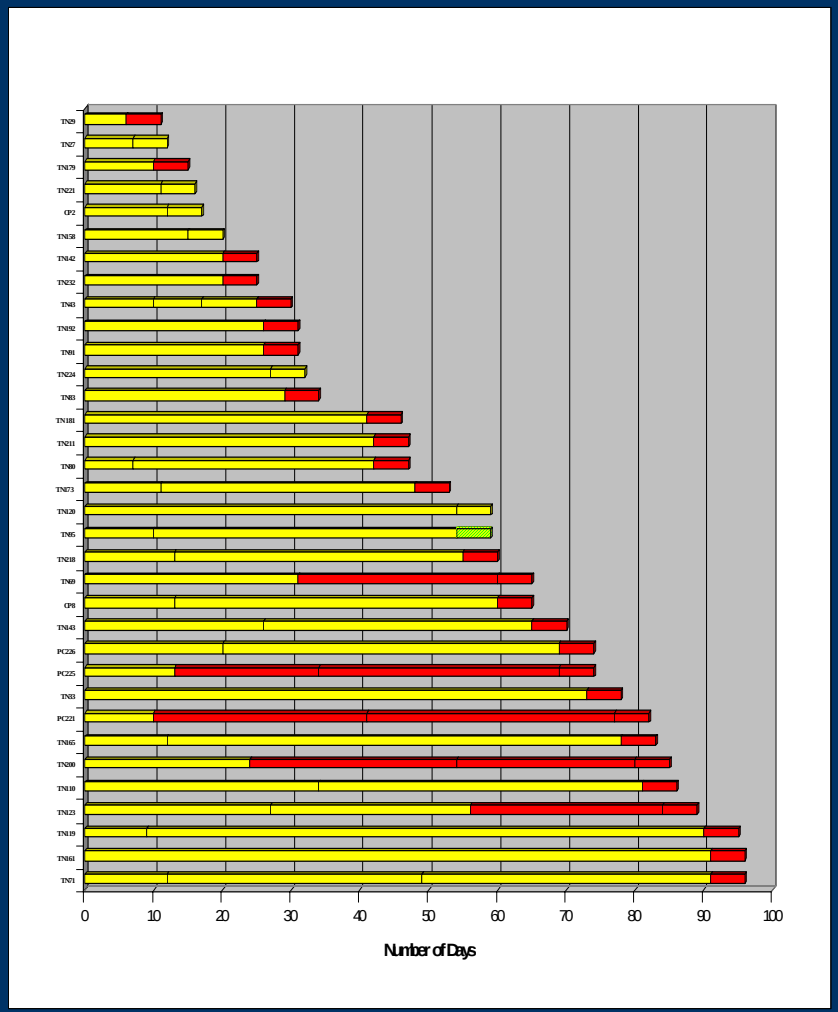
Global distribution of HCV genotypes

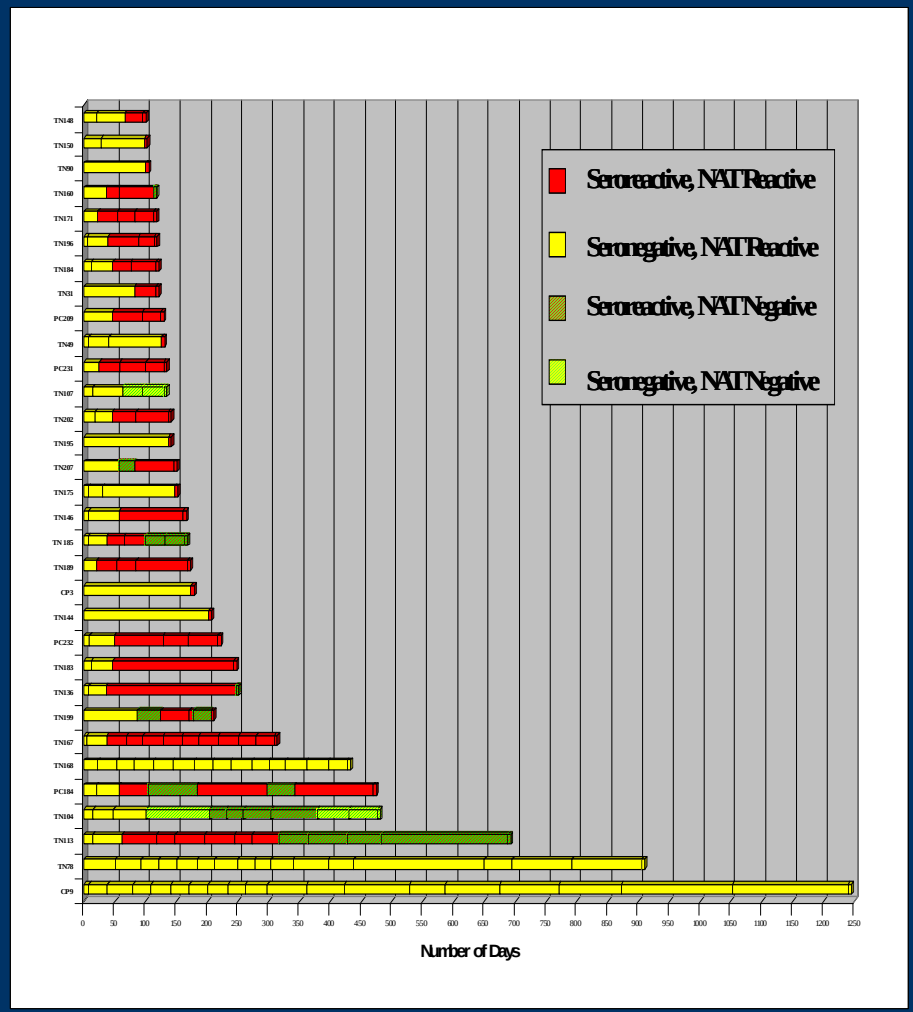


Changing distribution of HCV genotypes in US donor setting

TTVS 1970s:	**90% genotype 1b**
Plasma donors 1990s:	**80% genotype 1a**
NAT+ blood donors:	**50% genotype 1 (a>b)**
	20% genotype 2
	20% genotype 3

Evolution of HCV infection in NAT-yield donors





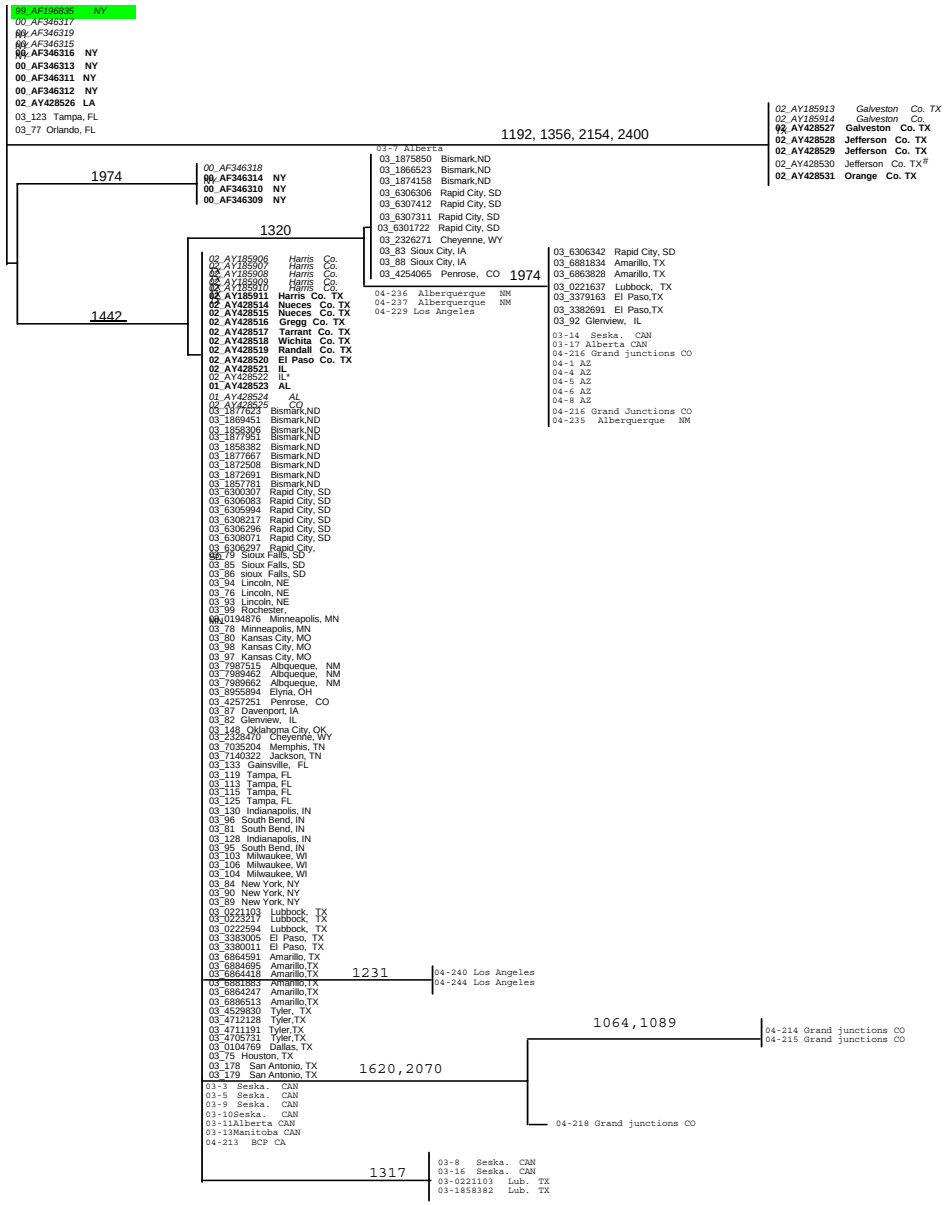
HCV Genotypes in NAT yield donors, 1999-2002

Genotype	# Yield Cases
1	2
1B	17
1A	60
1A or1B	6
2	2
2A	5
2B	28
3A	31
3B	1
6A	3
INDETERMINATE POSSIBLE MIXED 1 and 4 INFECTION	1
unable to type	2
pending	0

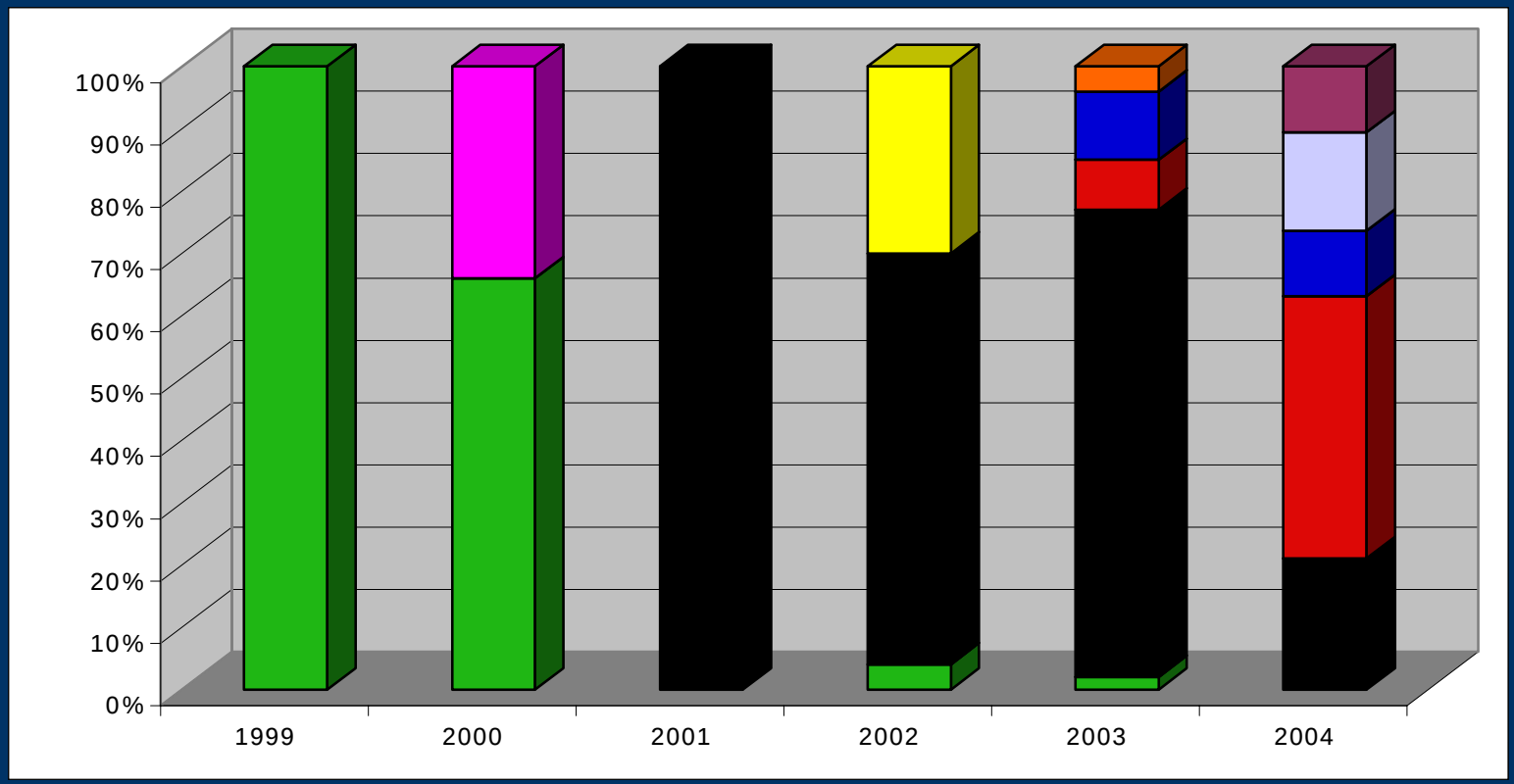
Total	**158**

Data from Susan Stramer, ARC

Phylogenetic tree of WNV sequences in U.S., 1999-2004



Distribution of WNV strains 1999-2004



N=	1	12	2	24	103	20

Objectives of REDS-II Molecular Surveillance Study

- **Primary: Detect genetically divergent HIV, HCV, HBV, WNV (?)**
  - Donations representing incident infections will be identified at REDS-II, ARC and BSI (incl DT testing of HIV seropositive donor specimens).
  - Central Laboratory will sequence the phylogenetically most informative regions for each virus.
  - Strains will be compared to Genbank data to determine whether highly divergent variants are transmitted within the US blood donor population.

- **Secondary: Monitor for testing errors and immunosilent infections**
  - Donations that were originally classified as NAT (or HBsAg) yield cases but which on repeat serological testing are found to be seroreactive will be classified as test errors.
  - NAT-positive donors who fail to seroconvert on follow-up will be classified as immunosilent carriers.
  - The rates of test error and immunosilent carriers, relative to window period donations and variant viral strains, will be tracked so that the relative contribution of each source of risk can be monitored.

Proposed Design and Testing

REDS-II Screening Laboratories
(plus ARC and BSL Ref Labs)

- HIV NAT+, Ab-; HIV Ab+
- HCV NAT+, Ab-
- WNV NAT+
- HBsAg+, anti-HBc- (HBV NAT yield)

→ REDS-II Central Laboratory

↓

- DT-EIA of HIV Ab+
  - Identify recent seroconversions
- HIV, HCV, WNV NAT-onlys
- HBsAg+, anti-HBc negative

↙

- Nested PCR using primers targeting
  - HIV: V3-V5 domains of env gene
  - HCV: E1E2 region containing HVR1
  - HBV: S gene
  - WNV: env gene
- Genetic Characterization - PAUP Software

Prevalence of Emerging Infectious Agents in the U.S. (Global) Donor Pool

1. Establish an “emerging infections working group”.
    - Composed of select REDS-II investigators and PHS liasons.
    - Responsible for monitoring pathogen discovery and infectious disease surveillance findings.
    - Oversee BSRI’s program to develop assays for sensitive isolation and amplification of pathogen-specific nucleic acids.
    - Encourage rapid development of antibody assays for use by the REDS-II program.

2. Develop improved methods for enrichment, purification and detection of nucleic acids of infectious agents in the cellular compartments of blood.

3. Establish a network for rapid access to residual volume from pooled plasma samples used for NAT screening.

4. Respond to emerging infectious threats by rapidly establishing NAT & serological assays and applying them to determine prevalence of infection (nucleic acids) and exposure to (antibodies) the agent in donor and recipient populations.