Viremia and antibody studies in WNV infected blood donors

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Introduction

- Screening donors for WNV RNA using NAT detects humans in the viremic, pre-seroconversion phase of WNV infection

- An understanding of the time course and dynamics of WNV RNA and serological markers following acute infection has important implications:
  - donor screening and deferral policies
  - diagnosing WNV infection in clinical settings
  - pathogenesis research

Blood Systems studies to define WNV window period dynamics

- Characterize WNV RNA+ index donations – viral load and serological profile
- Correlate MP-NAT yield with cumulative incidence based on IgM/IgG screening to derive length of MP-NAT+ WP
- Analyze relative yield of MP-NAT and ID-NAT in BSL retrospective ID-NAT study sites to derive lengths of pre- and post MP-NAT WPs that are detected by ID-NAT
- Analyze serial f/u samples from NAT yield donors to derive estimates for IgM and IgG SC and ID-NAT+ WPs

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Index donation RNA/antibody profiles

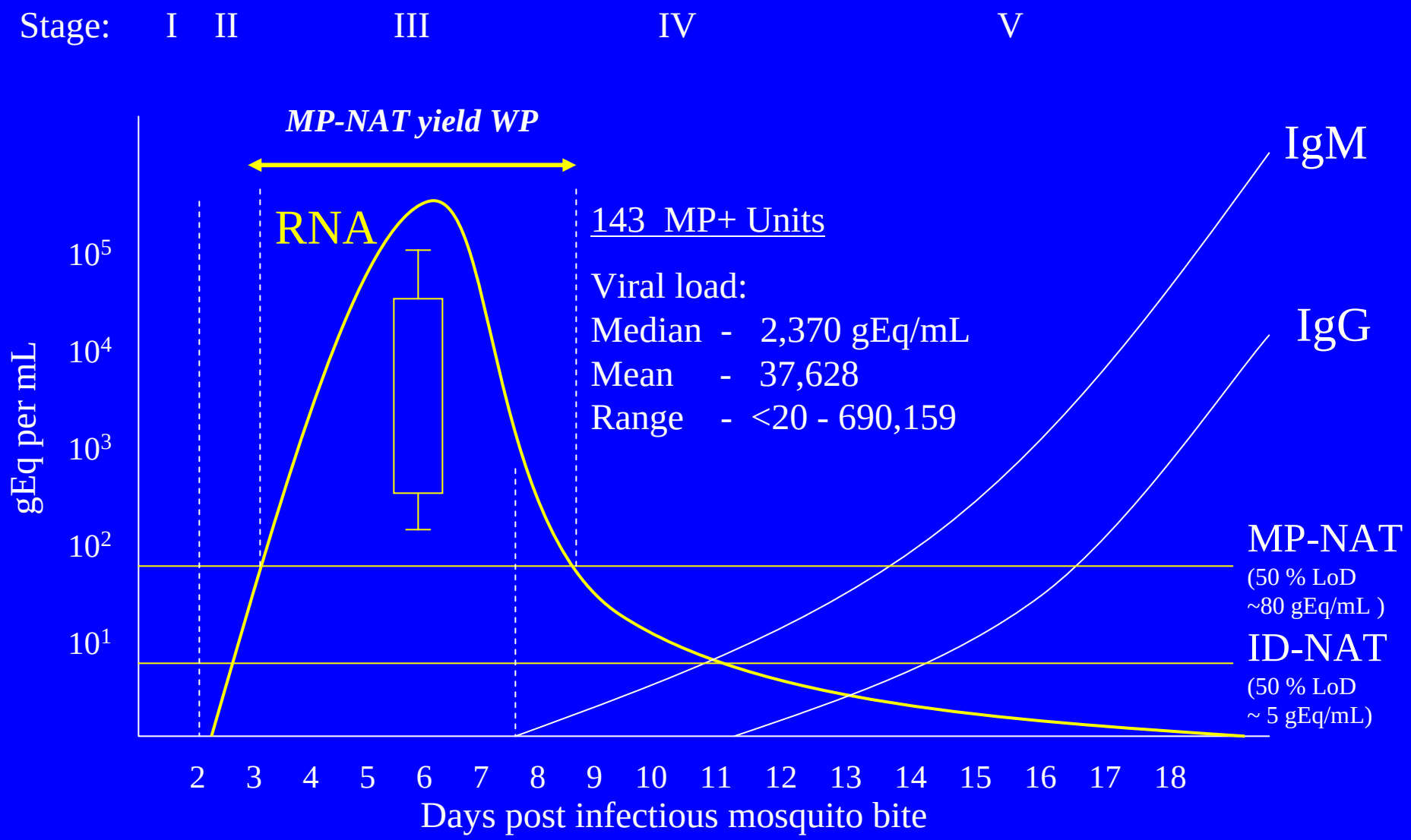
- Donations screened for WNV RNA by either Mini-Pool NAT (MP-NAT) with 16 units/pool, or targeted Individual Donation NAT (ID-NAT), using investigational WNV TMA assay (Gen-Probe/Chiron)
- Index unit viral loads determined using a target-capture/real-time PCR assay (Chiron Corporation)
- IgM and IgG status determined using EIAs (Focus Technologies)

Characteristics of viremic donations

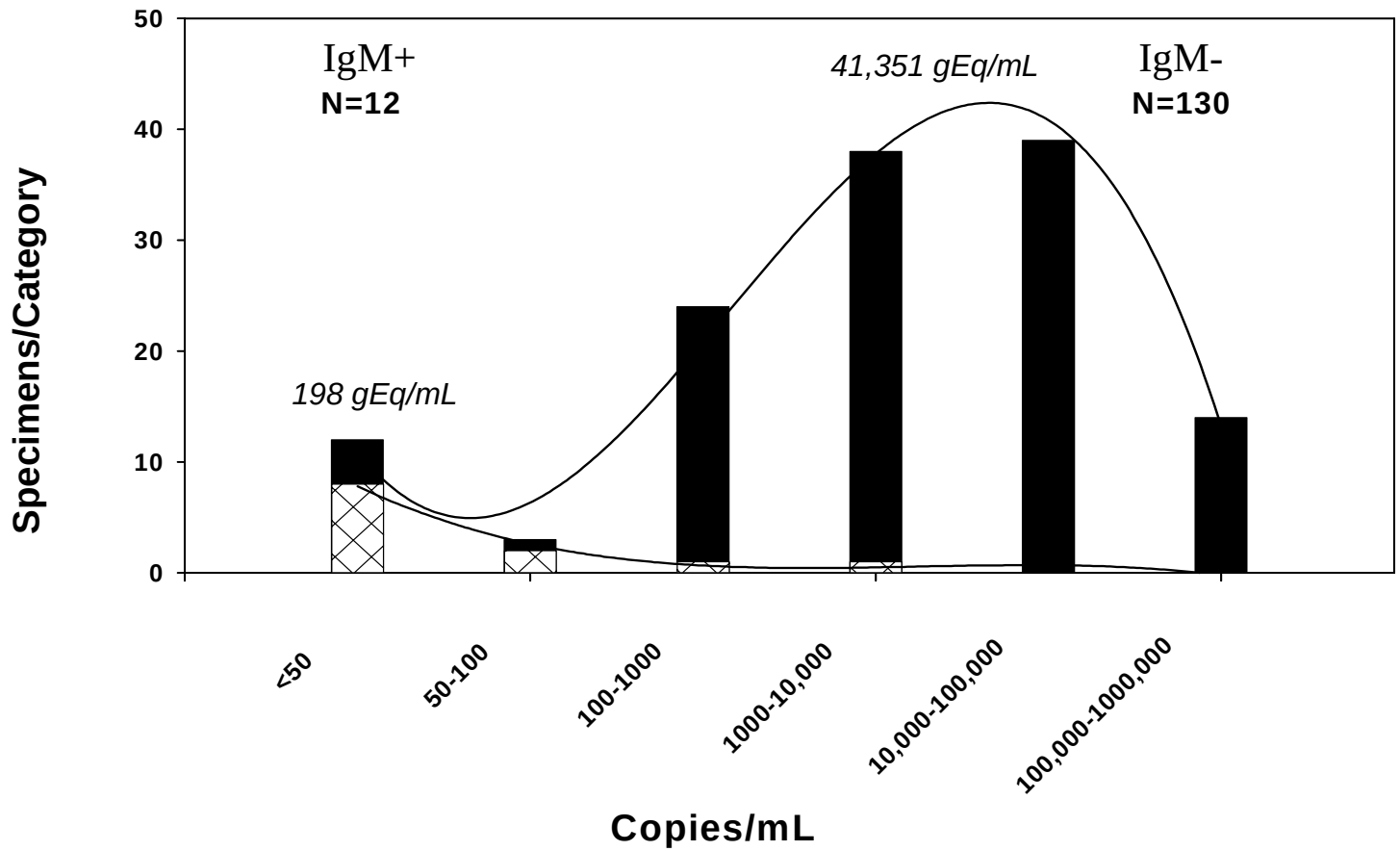
- **681,567 donations screened from 7/1-10/31, 2003**
  - **230 confirmed viremic donors identified (1 in 3000)**
    - **186 (82%) identified by MP-NAT (1 in 3700)**
    - **44 (18%) identified by targeted retrospective or prospective ID-NAT (1 in 15,000)**

- **20% (47/224) confirmed viremic index donations tested IgM-reactive and 18% (39) IgG-reactive**
  - **IgM detected in 16/183 (8%) MP-yield cases**
  - **IgM detected in 31/41 (75%) ID-only case**

Characteristics of MP-NAT yield donations



Viral load distribution of viremic units detected by WNV MP-NAT



Correlate MP-NAT yield with cumulative incidence from IgM/IgG screening to derive MP-NAT+ WP

- Focused on collection sites based in Bismarck and Minot, North Dakota
  - epidemic region in 2003 and no prior WNV activity
  - archived donation plasma samples for retrospective testing for IgM

- MP-NAT rates obtained for each calendar week between July 1-Sept 27, 2003
  - 28 WNV-NAT confirmed positive donations identified by MP-NAT screening of 7073 donations

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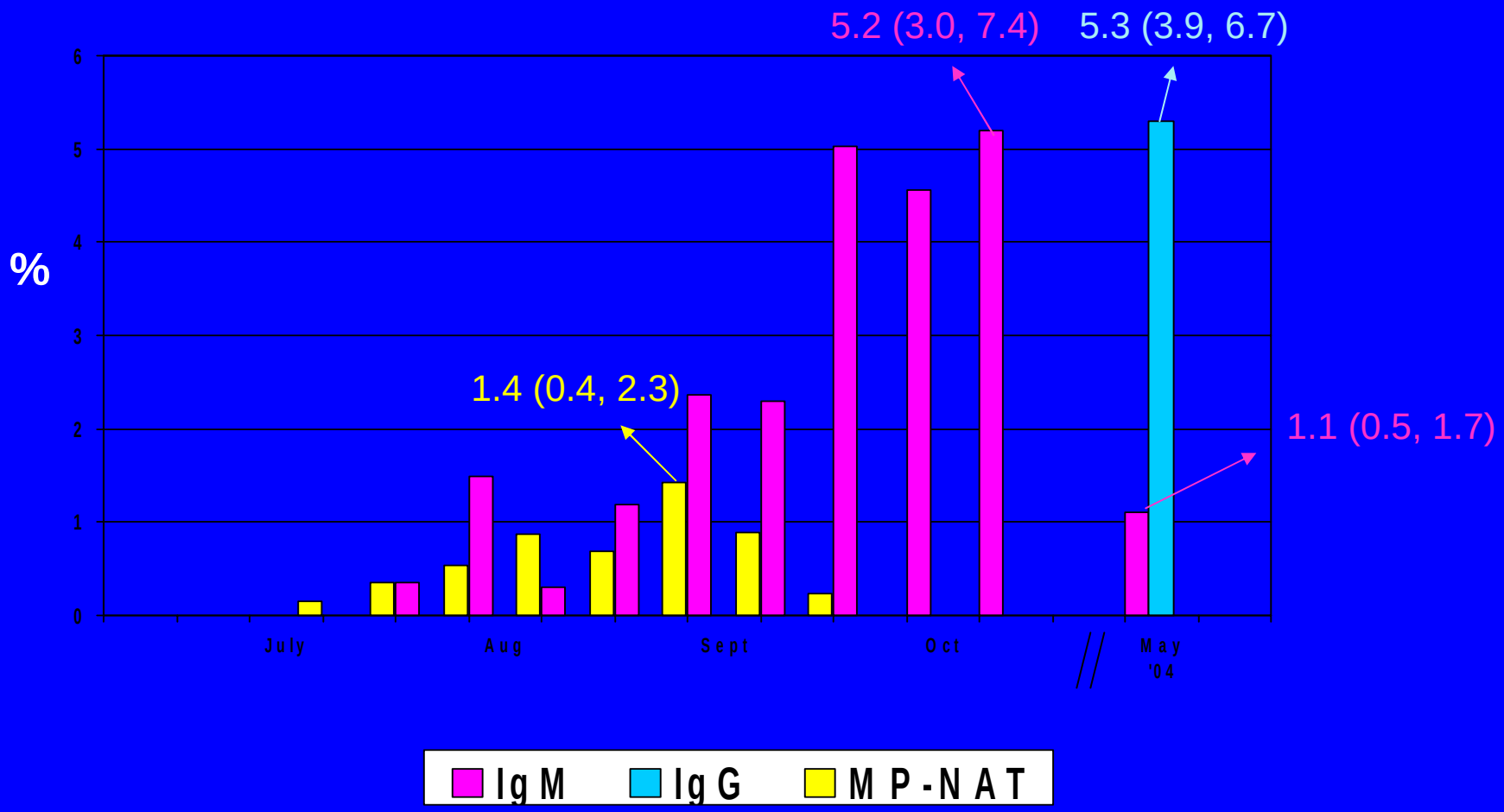
- Measure serial IgM prevalence for same period (7/1-9/27, 2003)
  - Obtained results of Focus IgM EIA performed on NAT-reactive donations as part of routine confirmatory evaluation
  - Evaluated IgM status by performing IgM EIA (Focus) on 3922 (56%) of 7012 MP-NAT screened non-reactive donations
- Prevalence of IgM and IgG 9-11 months after 2003 epidemic (but prior to a possible 2004 epidemic)
  - Prospectively obtained 1000 specimens collected from Bismarck from June 7-24, 2004
  - Tested for IgM and IgG using Focus EIAs

WNV MP-NAT yield relative to IgM and IgG seroprevalence rates North Dakota, 2003 epidemic



Key observations from correlations of MP-NAT, IgM and IgG

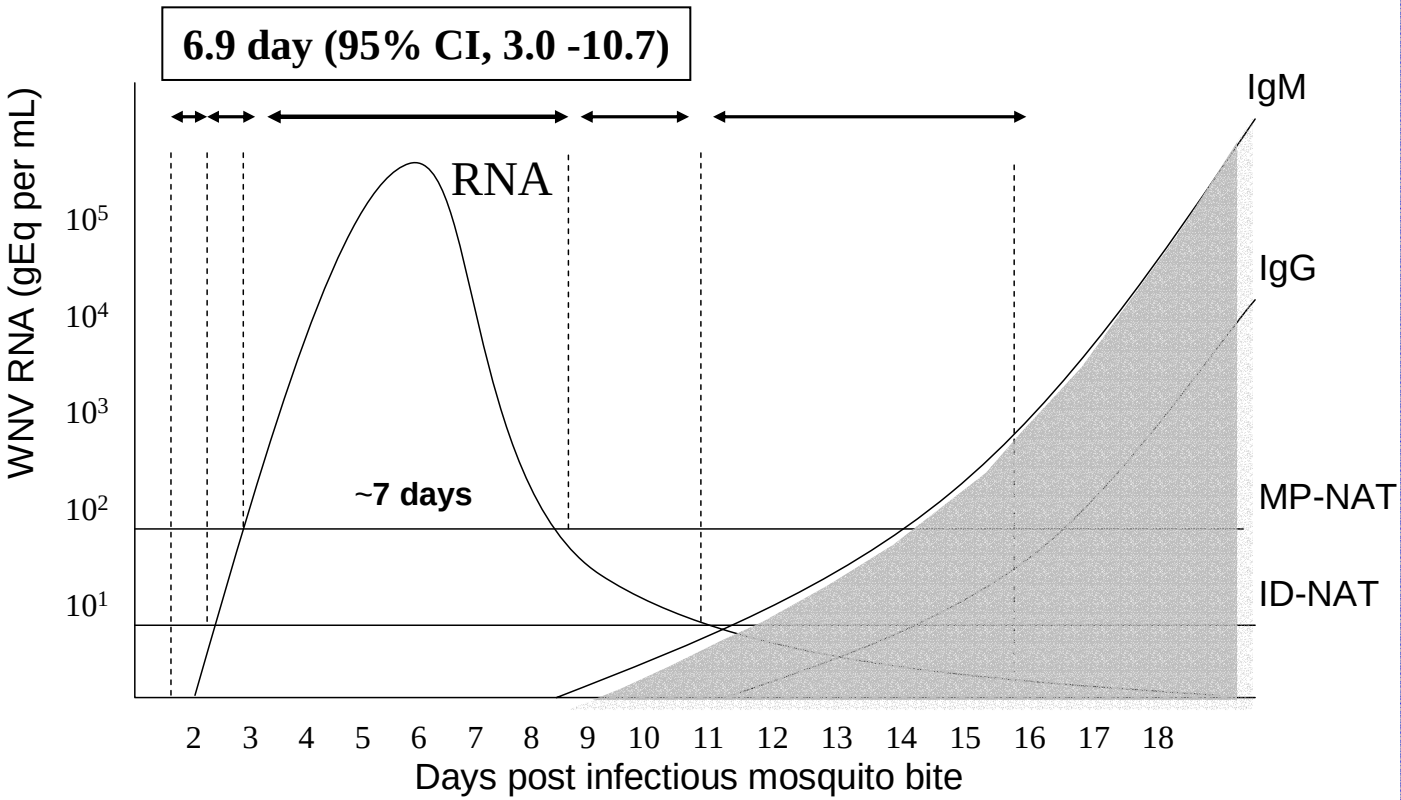
- IgM not detected early in epidemic, and therefore not useful as a screening test prior to peak of MP-NAT
- IgM rates peak 3-4 weeks after detection of peak viremia rates, with IgM prevalence ~4 times peak MP-NAT rate
- IgM screening may have value if persistent low-level viremia observed during IgM+ convalescent phase is proven to be infectious

Key observations from correlations of MP-NAT, IgM and IgG (cont)

- Late in an epidemic, IgM testing would lead to significant rates of unit loss and donor deferral, with low risk of infectivity of IgM+/NAT- units
- After 6 months, IgM wanes to 20% of peak rate, while IgG rate is c/w IgM peak rate (assumes no prior WNV or cross-reactive flavivirus epidemics)
- Even in a highly affected region, most donors show no evidence of exposure and would be susceptible to infection in future years, indicating need for ongoing donor screening (vaccine?)

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

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



Blood Systems 2003 Retrospective WNV ID-NAT Study

- Screen and release products based on MP-NAT
- Save samples from centers with high MP-NAT yield
- Retrospectively test samples by ID-TMA
- If reactive: retrieve products; retest by WNV TMA, IgM & IgG and Alt-TMA; trigger donor follow-up
- CDC assists with recipient notification/follow-up

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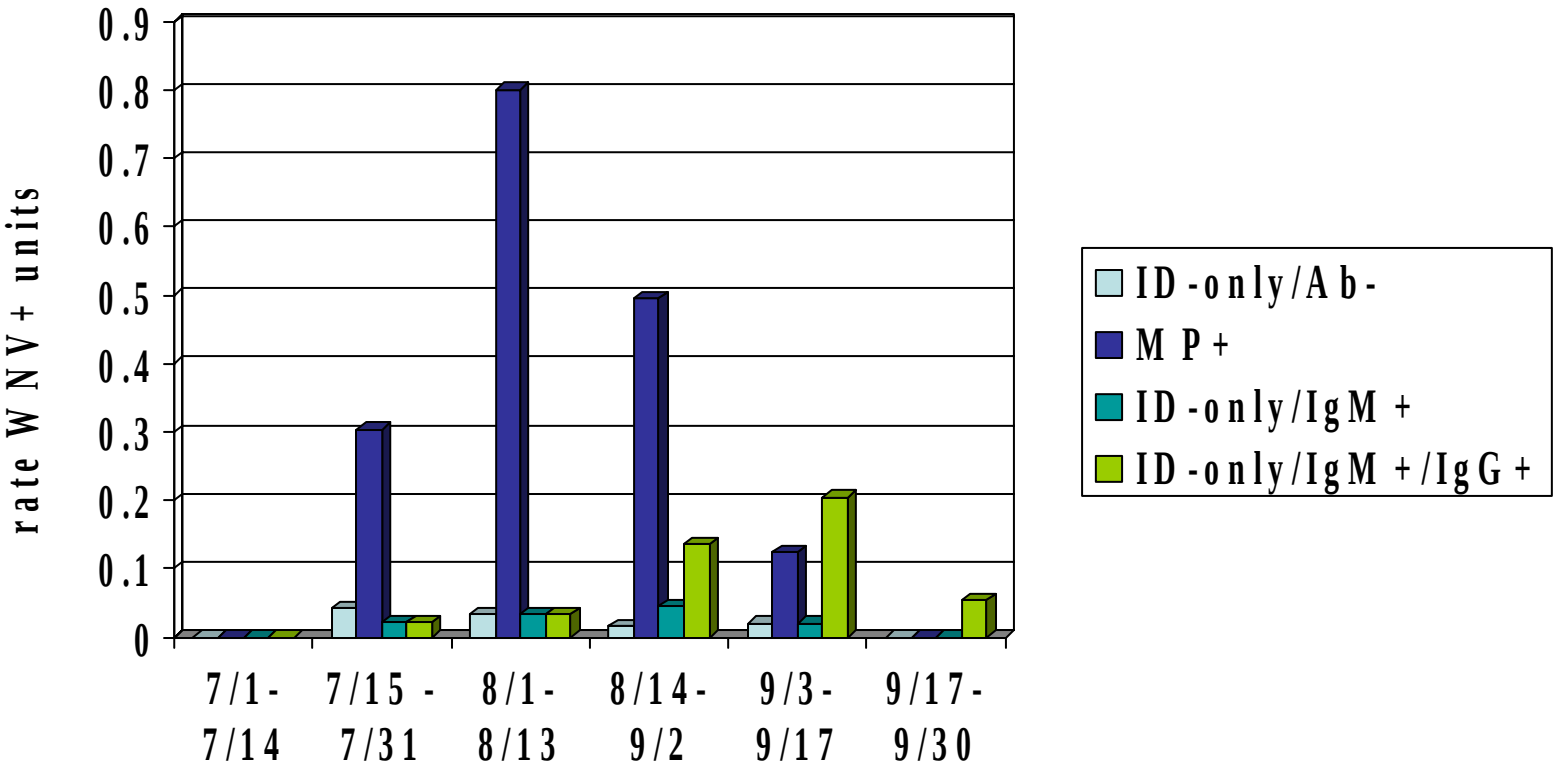
BSL 2003 ID-NAT Study Summary

- **Retrospective ID-NAT on 23,088 MP-NAT negative donations from Texas, North and South Dakota yielded 30 confirmed positives**

- **Prospective ID-NAT on 3,964 donations from Dakotas during September yielded 17 confirmed positives (3 reactive at 1:16 dilution)**

MP-NAT-detectable vs. ID-NAT-only donations in Blood Systems retrospective/prospective ID-NAT studies, N. and S. Dakota, 2003



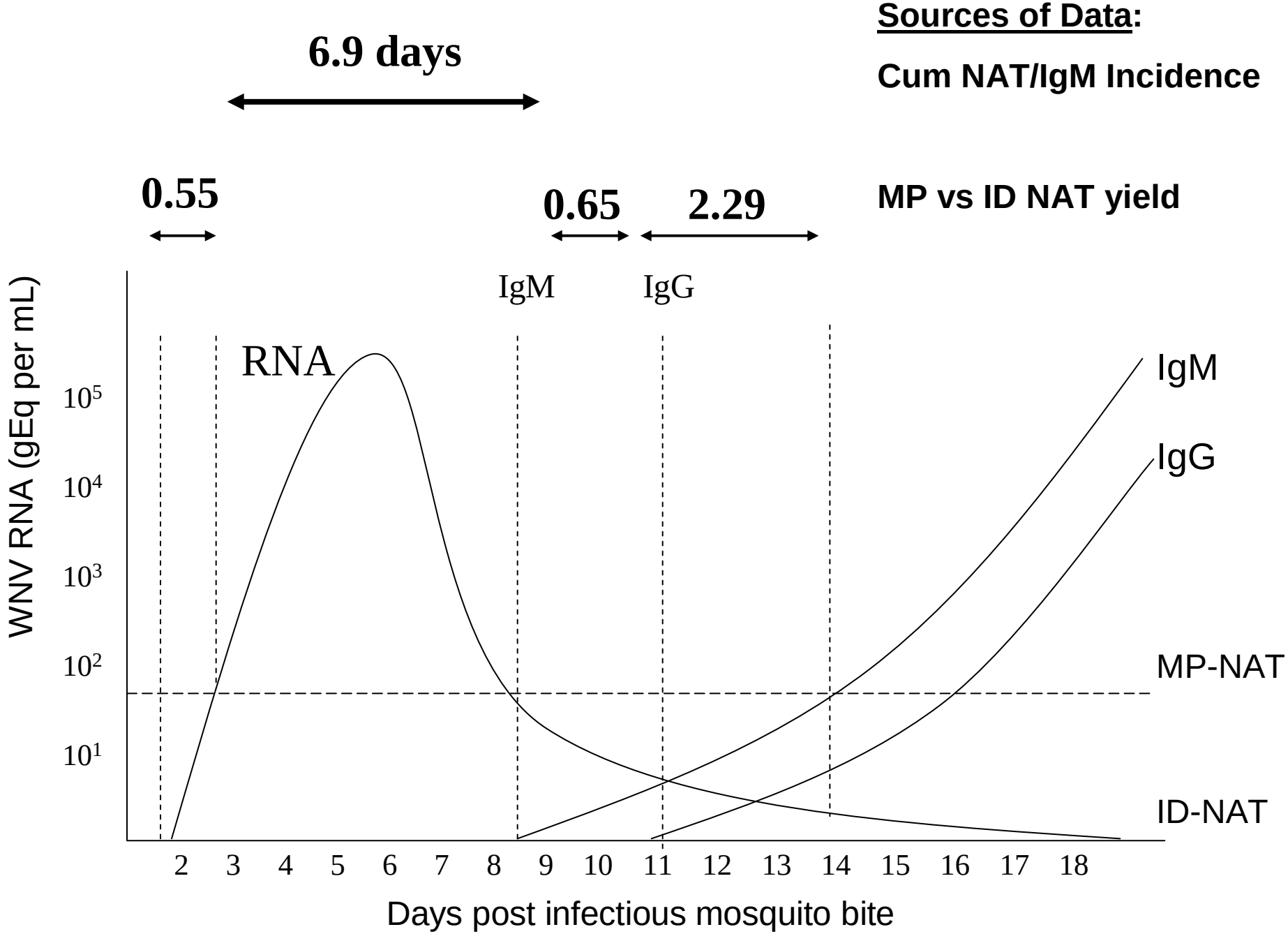
MP-NAT vs. ID-NAT yield: estimated WP lengths and proportion of viremic donations

	MP-NAT yield ***infectious***	**ID NAT+, IgM-** ***probably infectious***	**ID- NAT+ IgM+** ***possibly infectious***	**ID- NAT+ IgM/IgG+** ***possibly infectious***
# cases	79	6	8	26
WP length	**6.9 d**	**0.55 d**	**0.65 d**	**2.3 d**
Proportional yield	~66%	~ 5%	~ 6%	23%

Sources of Data:

Cum NAT/IgM Incidence

MP vs ID NAT yield



Viremic donor follow-up study

- **NAT-reactive donors offered enrollment into follow-up study that included a symptom questionnaire at enrollment and sampling at weekly intervals**
- **Follow-up ended when donors tested negative by ID-TMA and developed WNV IgM**
- **All follow-up samples tested for RNA by TMA and RT-PCR, and for IgM and IgG antibodies**
- **Subset of panels further tested by 5 replicate TMA, IgA and Plaque Reduction Neutralization Titration (PRNT) assays (CDC)**

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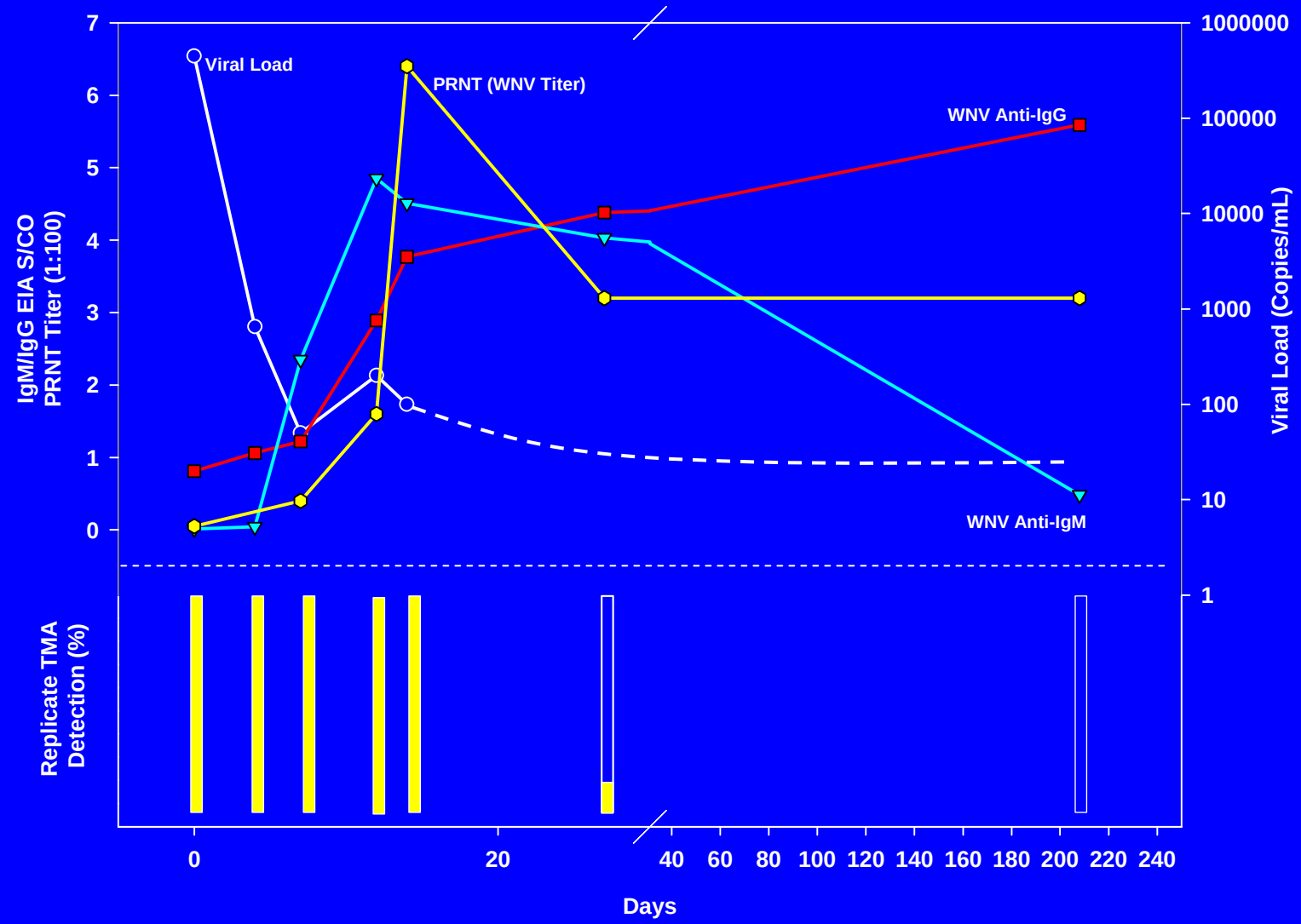
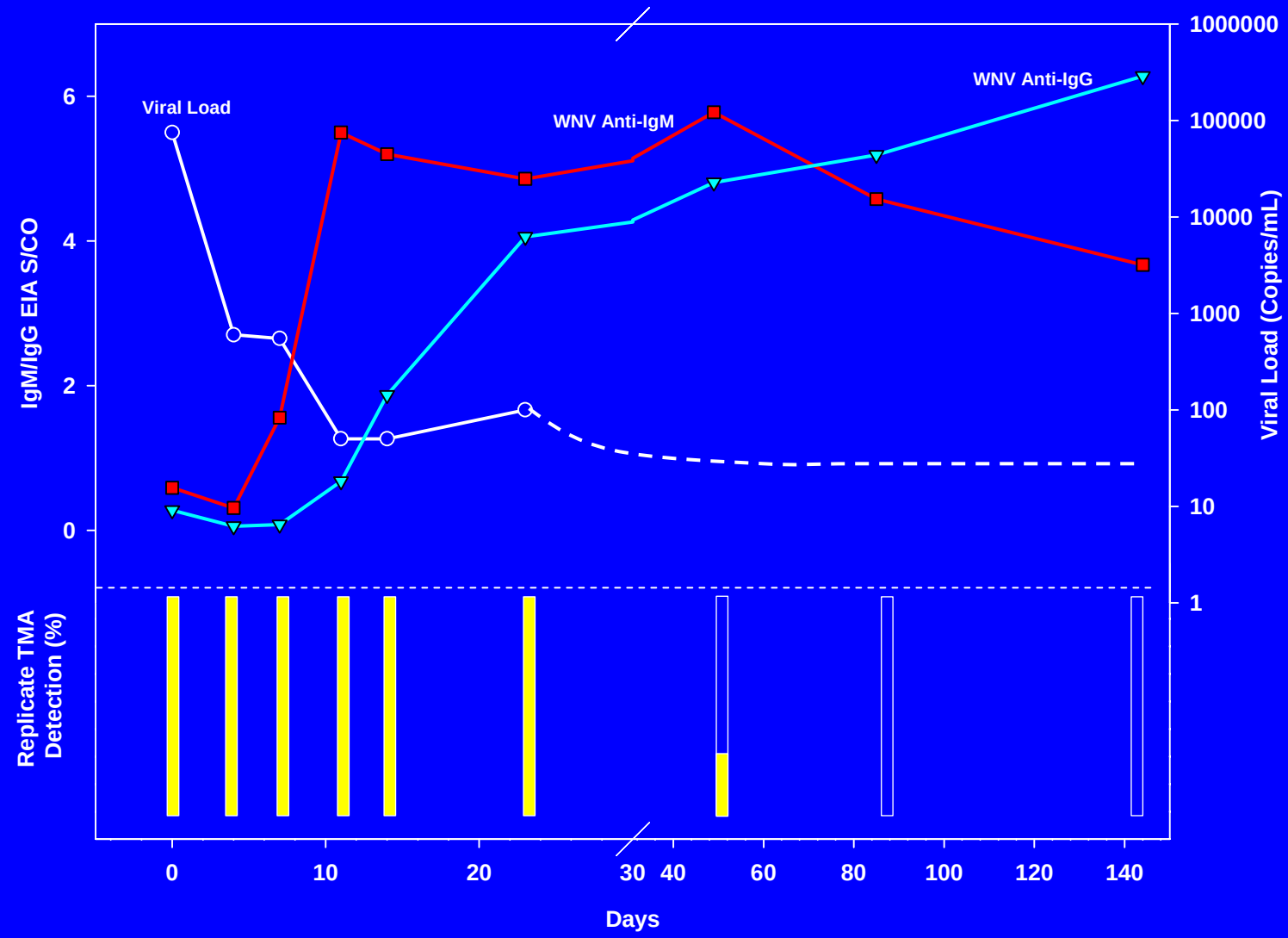
Results of follow-up study

- 182 (83%) confirmed positive donors enrolled.
- First follow-up specimen median of 9 days (mean 15 days) following index donations
- Enrolled donors gave average of 2.5 follow-up specimens (range 1–8)
- Of 140 donors w/ IgM negative index donations, IgM detected in first f/u bleed in 113 (81%) and second f/u bleed in remaining 27 (19%) cases

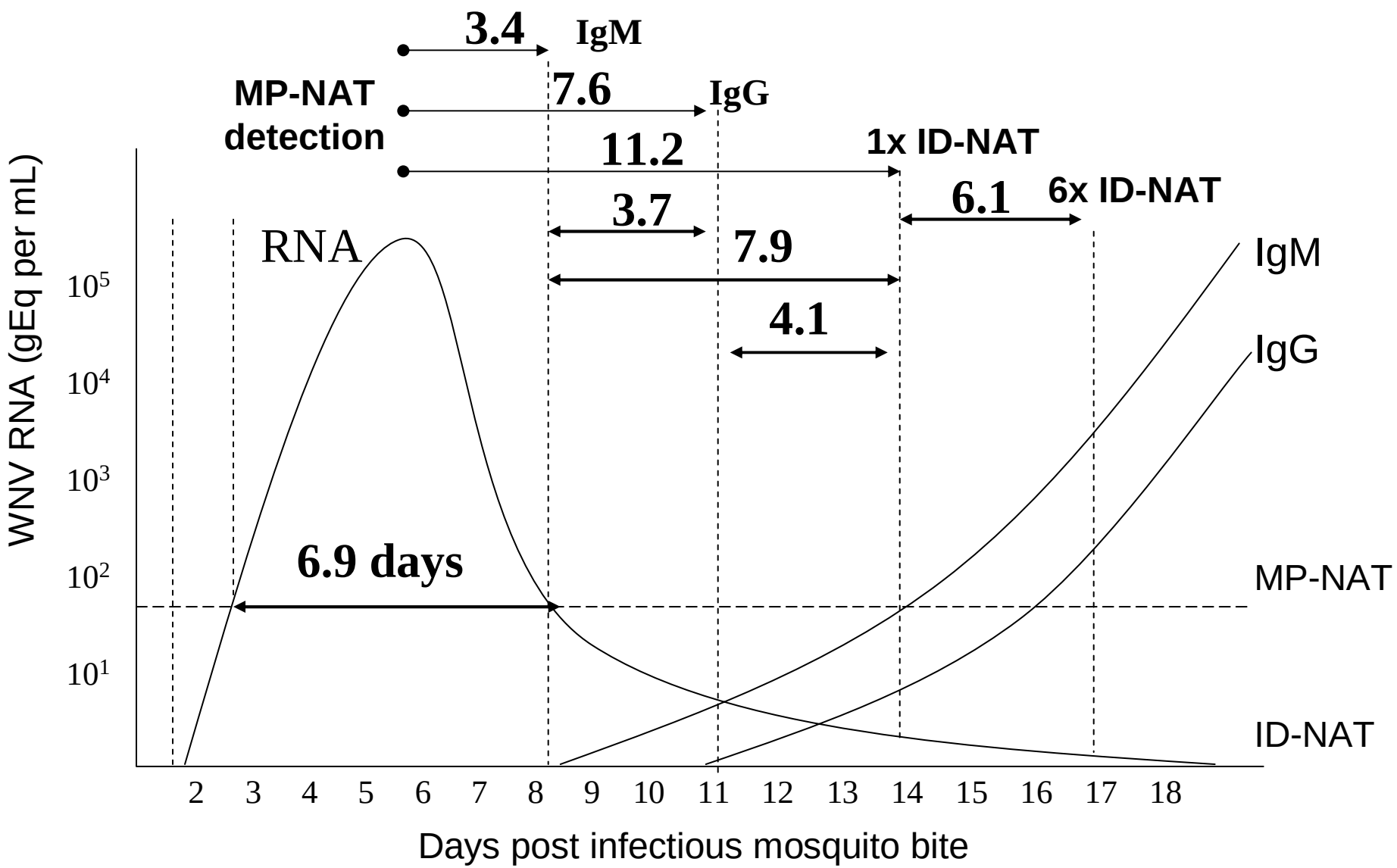

IgM/IgG EIA S/CO
PRNT Titer (1:100)
Viral Load (Copies/mL)
Viral Load
PRNT (WNV Titer)
WNV Anti-IgG
WNV Anti-IgM
Replicate TMA Detection (%)
Days
0
1
2
3
4
5
6
7
1
10
100
1000
10000
100000
1000000
0
20
40
60
80
100
120
140
160
180
200
220
240


Viral Load
WNV Anti-IgM
WNV Anti-IgG
IgM/IgG EIA S/CO
Viral Load (Copies/mL)
Replicate TMA Detection (%)
Days
0
2
4
6
1
10
100
1000
10000
100000
1000000
0
10
20
30
40
60
80
100
120
140

Statistical analysis of f/u data

- Interval censored longitudinal regression analysis
- 182 MP yield donors w/ follow-up
  - Time from index unit to:
    - IgM SC
    - IgG SC
    - Loss of RNA by single TMA assay
  - Time from IgM to IgG SC
  - Time from IgM and IgG SC to loss of RNA
- 56 cases studied for low-level viremia by performing 5 TMA replicates (Tigris) on 180 f/u samples
  - Time from index unit to no detectable RNA by TMA (1x & 6x)
  - Time from loss detectable RNA by 6x TMA relative to 1 x TMA

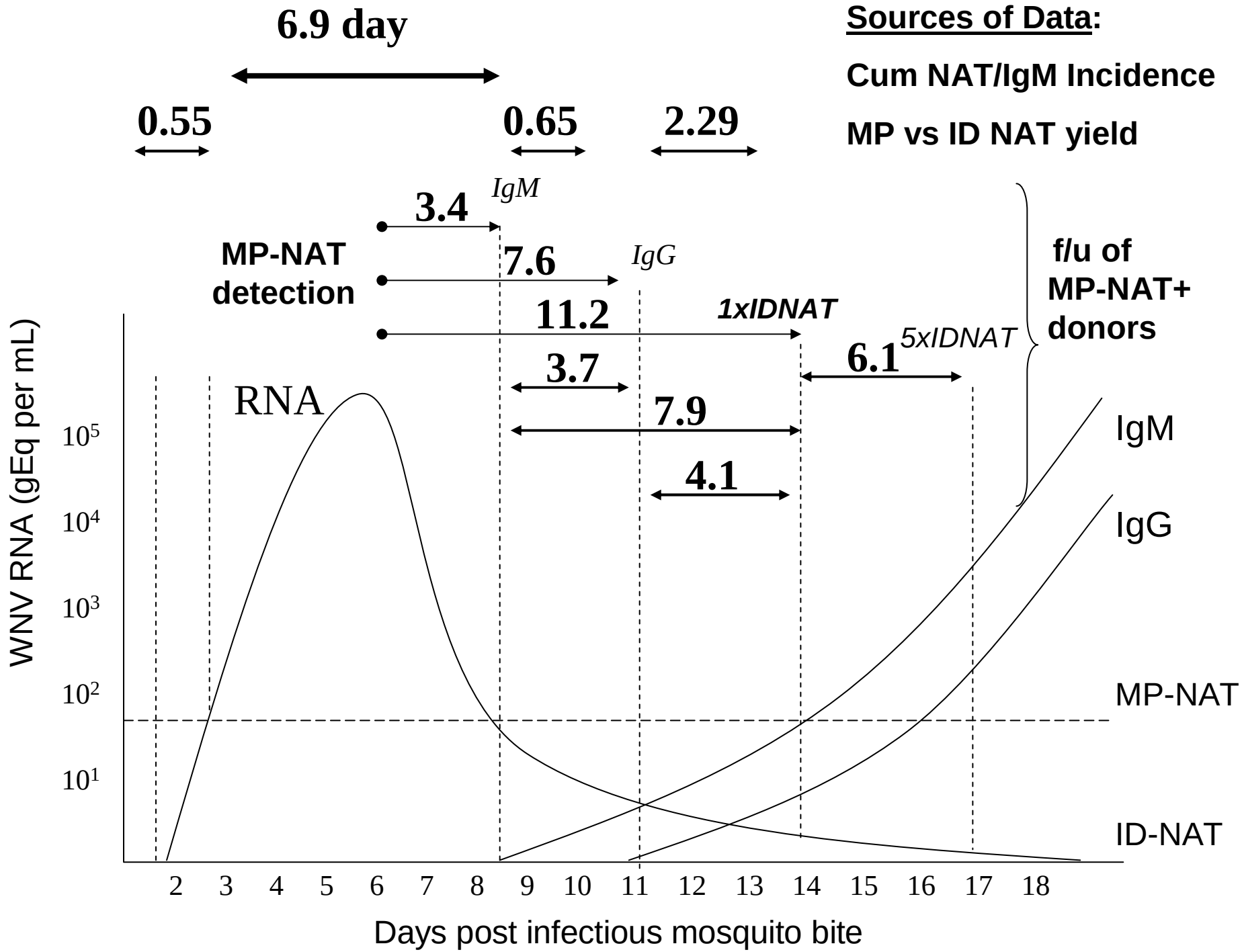
Point estimates for mean WPs for acute WNV infection parameters



WNV window period estimates

Window Period	Mean (days)	95% CI for mean WP
MP+ to IgM SC	3.4	2.9 - 4.0
MP+ to IgG SC	7.6	6.6 - 8.6
IgM SC to IgG SC	3.7	2.6 - 4.8
MP+ to TMA_{neg} (1x)	11.2	9.8 - 12.3
TMA_{neg} (1x) to (6x)	6.1	4.2 - 8.0

Assuming a normal distribution, 99% of NAT yield donors clear viremia by mean + 2.33 times SE (8.6 d) = 31.2 days


6.9 day
0.55
0.65
2.29
Sources of Data:
Cum NAT/IgM Incidence
MP vs ID NAT yield
IgM
3.4
MP-NAT detection
7.6
IgG
11.2
1xIDNAT
5xIDNAT
6.1
f/u of MP-NAT+ donors
3.7
7.9
4.1
RNA
IgM
IgG
MP-NAT
ID-NAT
WNV RNA (gEq per mL)
10^5
10^4
10^3
10^2
10^1
2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
Days post infectious mosquito bite

ARC Viremia and SC Studies

- **415 positive donors from 2003 prospective screening**
  - 335/350 (96%) in follow up seroconverted
  - **186/335 studied in detail**
    - Multiple follow up samples
    - First follow up ≤ 35 days (selected to include the donor with the longest viremic period at the first follow up)
  - 76/186 (41%) **TMA "observed" reactivity 2-39 days** post index
    - 12/76 had fluctuating viremia

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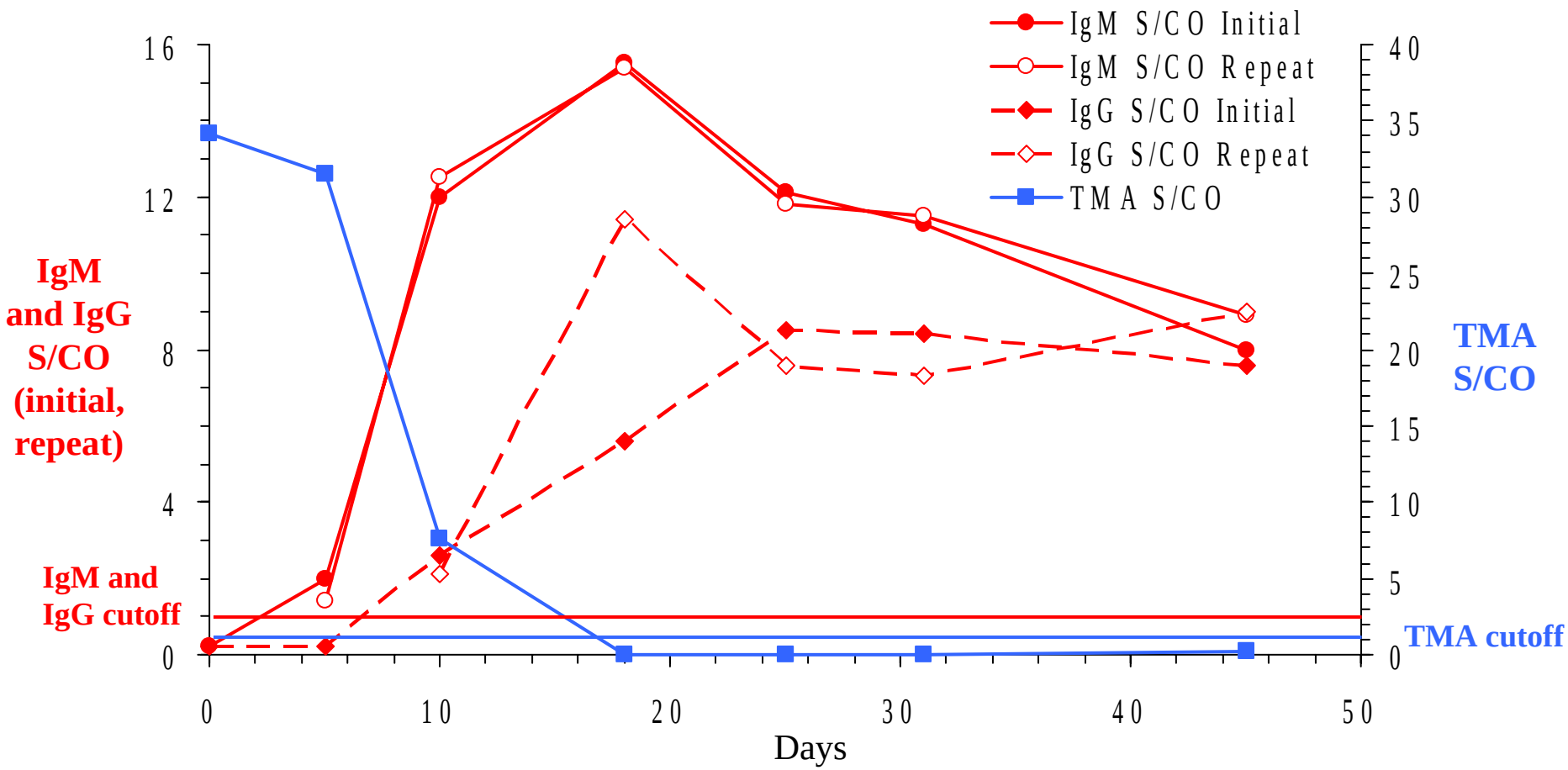
WNV Confirmed Positive Donor Profile

WR0620

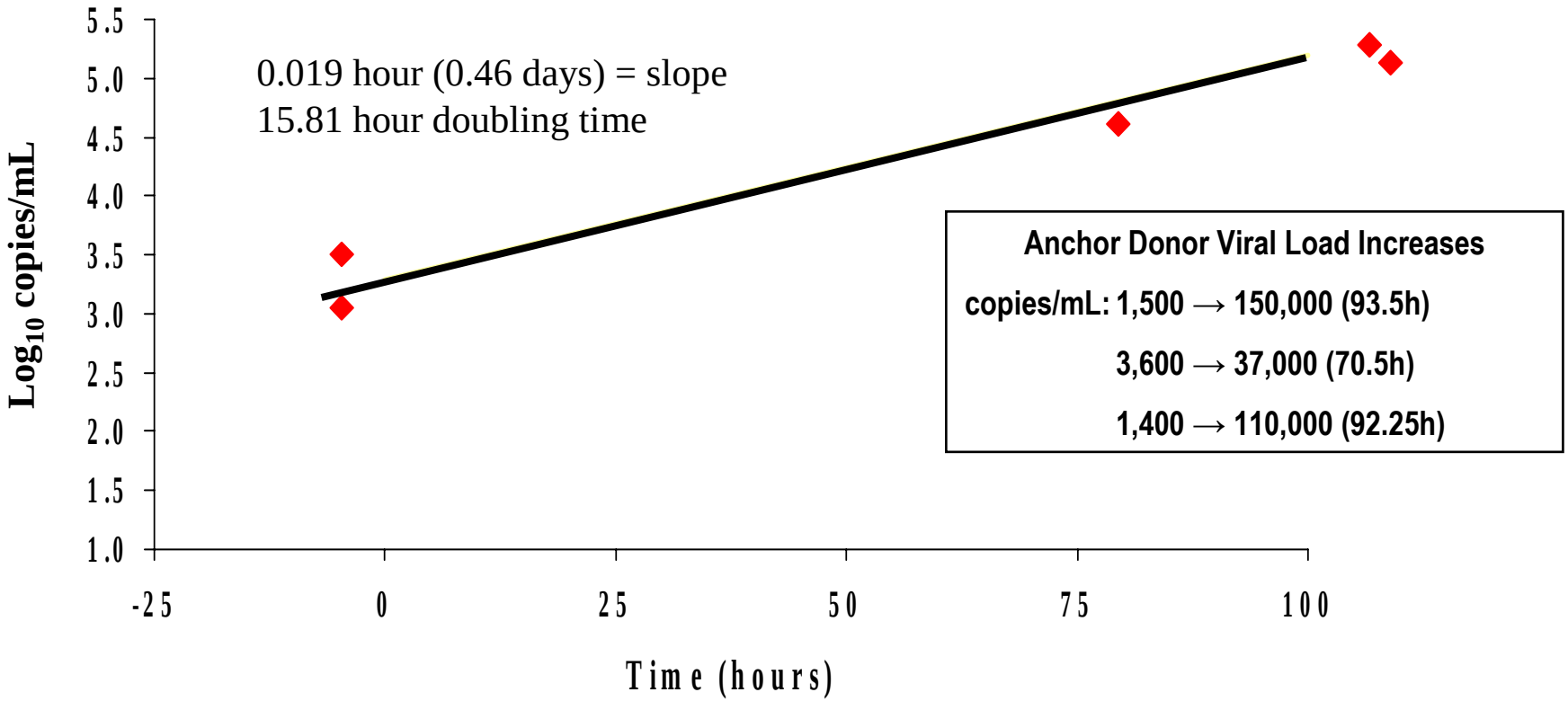


Days	0	5	10	18	25	31	45
PCR copies/mL	45,000	100	50	50	<5	<5	<5

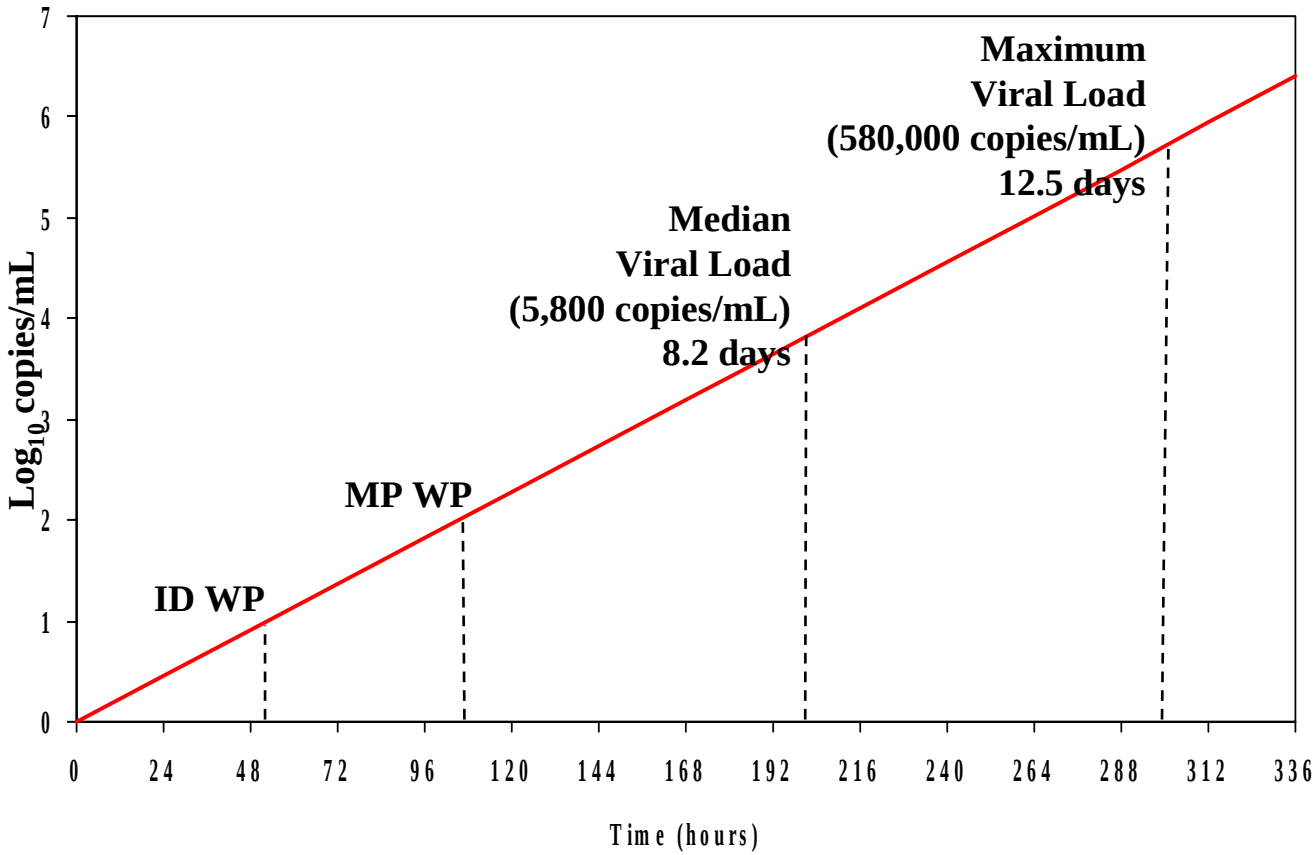
Window Period Determinations

- **0.46 log increase/day (0.019 log inc/hr) calculated based on 3 "anchor donors"**
  - Donors with ramp-up viremia between index and follow up
- **Doubling time = 15.81 hours** (mean of 3 donors)
  - Back calculating to 1 copy/mL= time zero (t_0) and using TMA 95% LoD (10 copies/mL), window period from t_0 to NAT reactivity:
    - ID NAT = **2.2** days
    - MP NAT = **4.8** days

WNV Viral Replication
Slope Based on 3 Anchor Donors



Predicted Viral Load Based on 3 Anchor Donors



Assume: 1 copy/mL WNV at time zero

Assume: IDT 95% LoD = 10 copies/mL; **IDT WP = $Log_{10}(10)/0.46 = 2.2$ days**

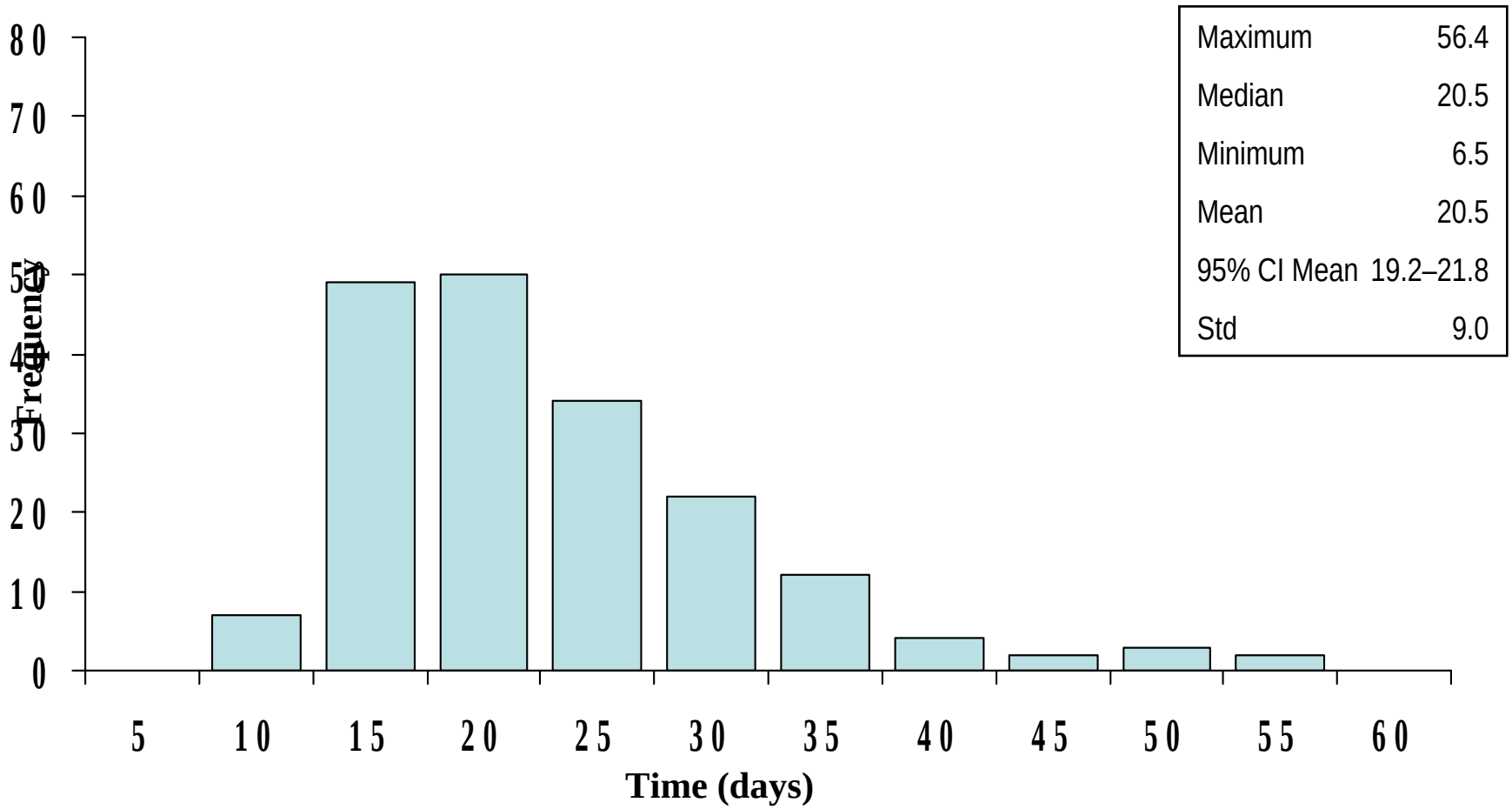
Assume: MP 95% LoD = 160 copies/mL; **MP WP = $Log_{10}(160)/0.46 = 4.8$ days**

*** Median observed viral load at index = 5,800 copies/mL**

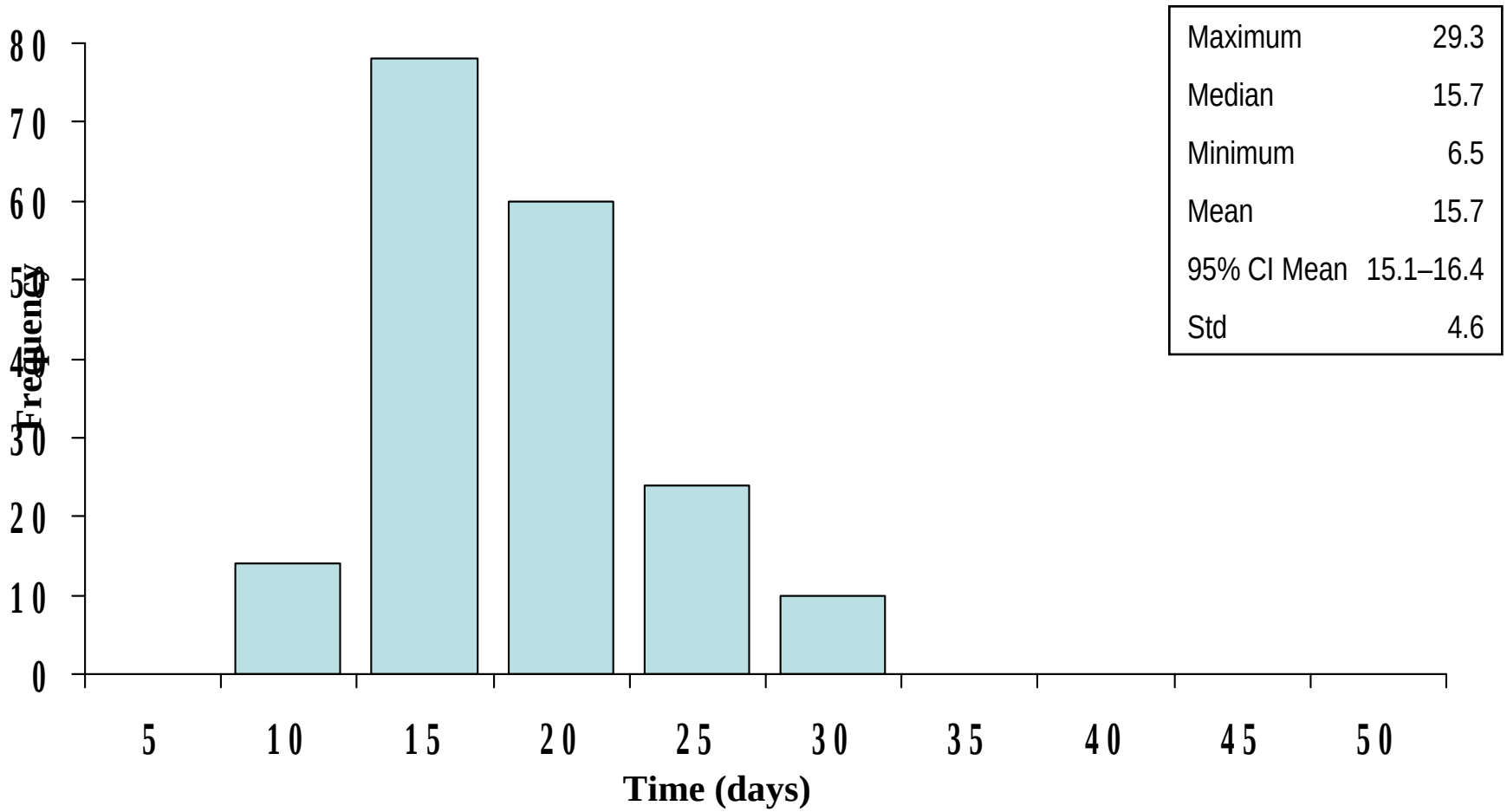
**** Maximum observed viral load at index = 580,000 copies/mL**

N= 241 IgM NR donors

Duration of Viremia Based on Last TMA Reactive Bleed Post Index (N=186)

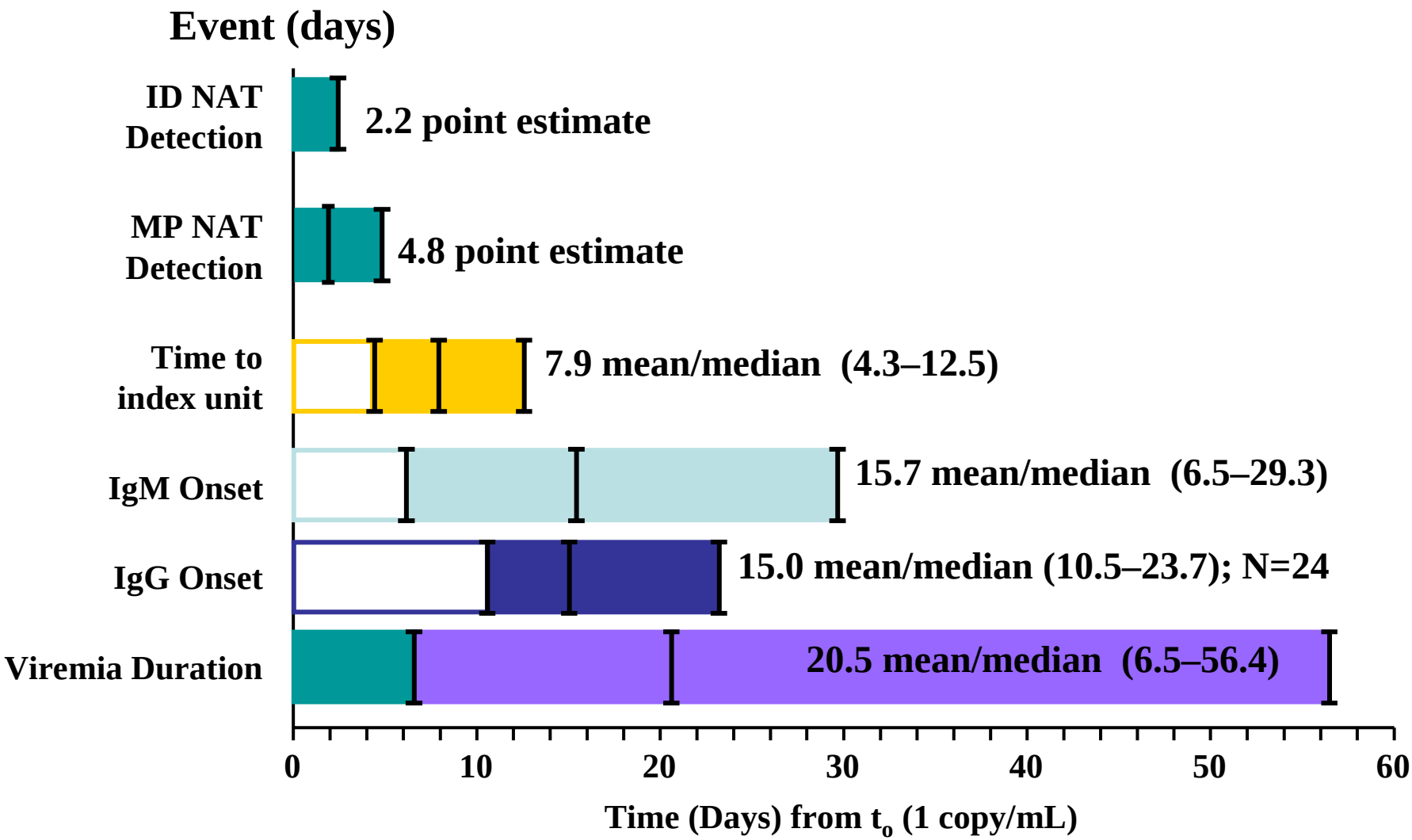


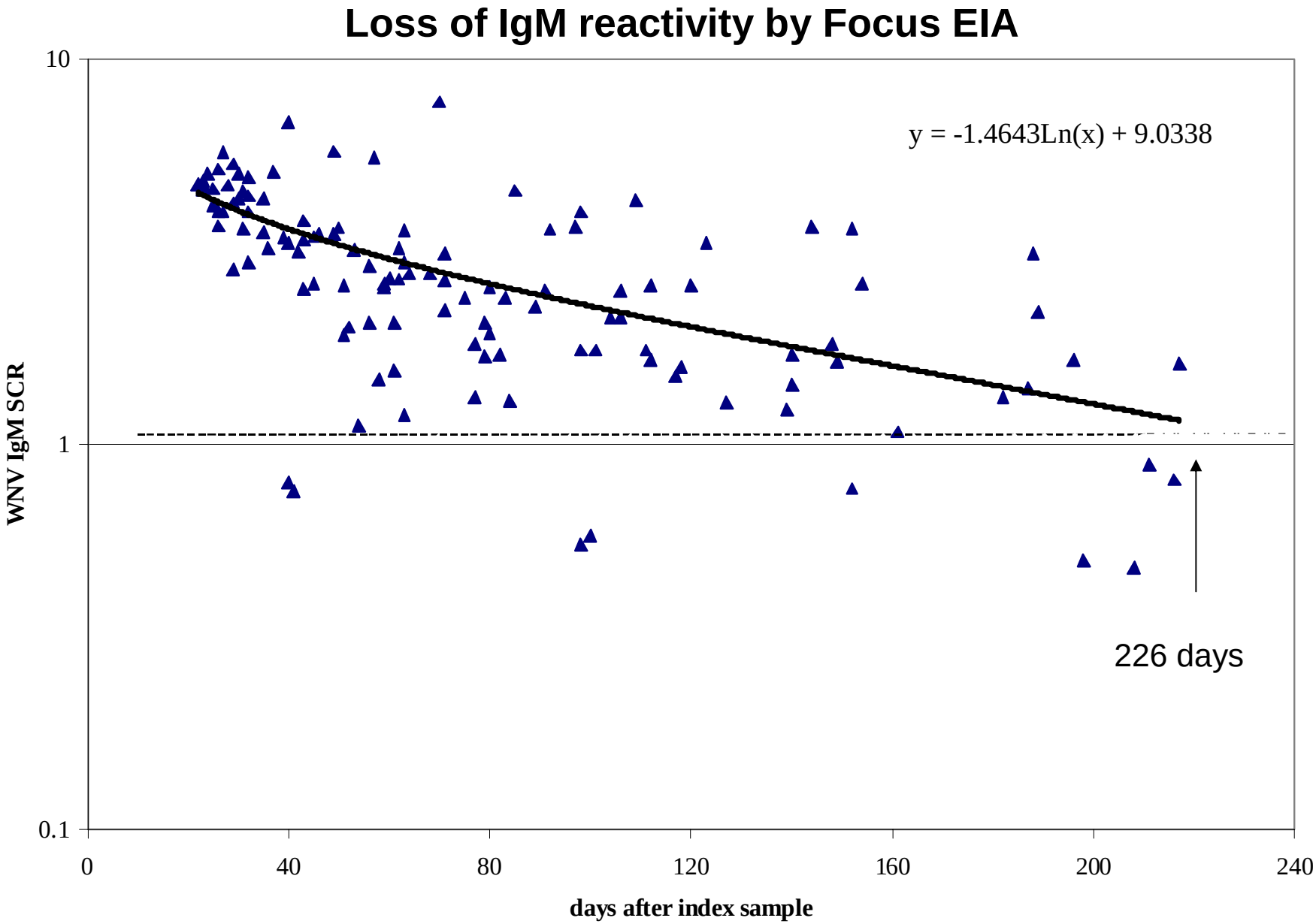
Time Zero to IgM Seroconversion (N=186)



Modeled WNV Dynamics of Infection from Estimated Time Zero (1 copy/mL)

N=186 Donors with Multiple Follow-up Samples





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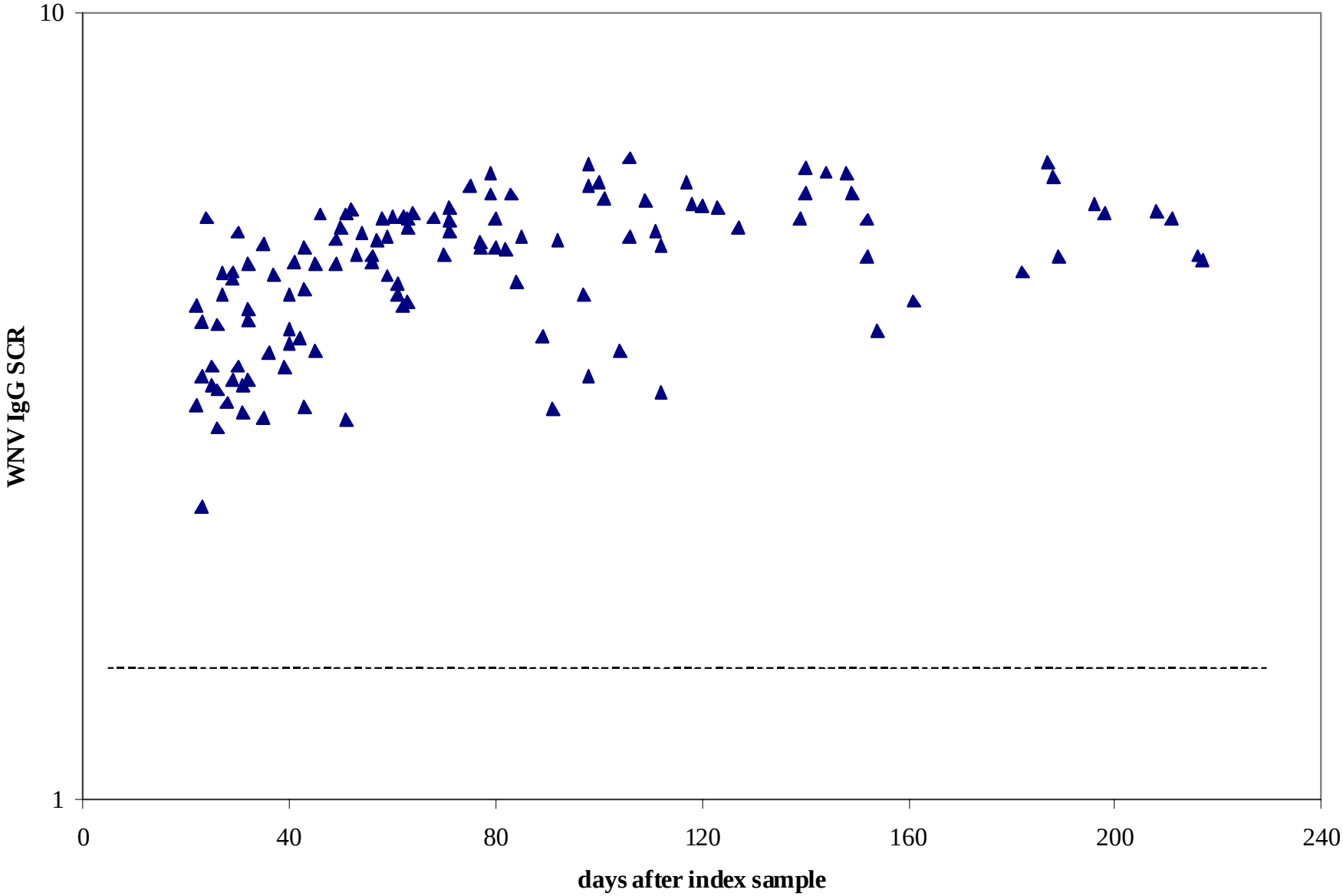
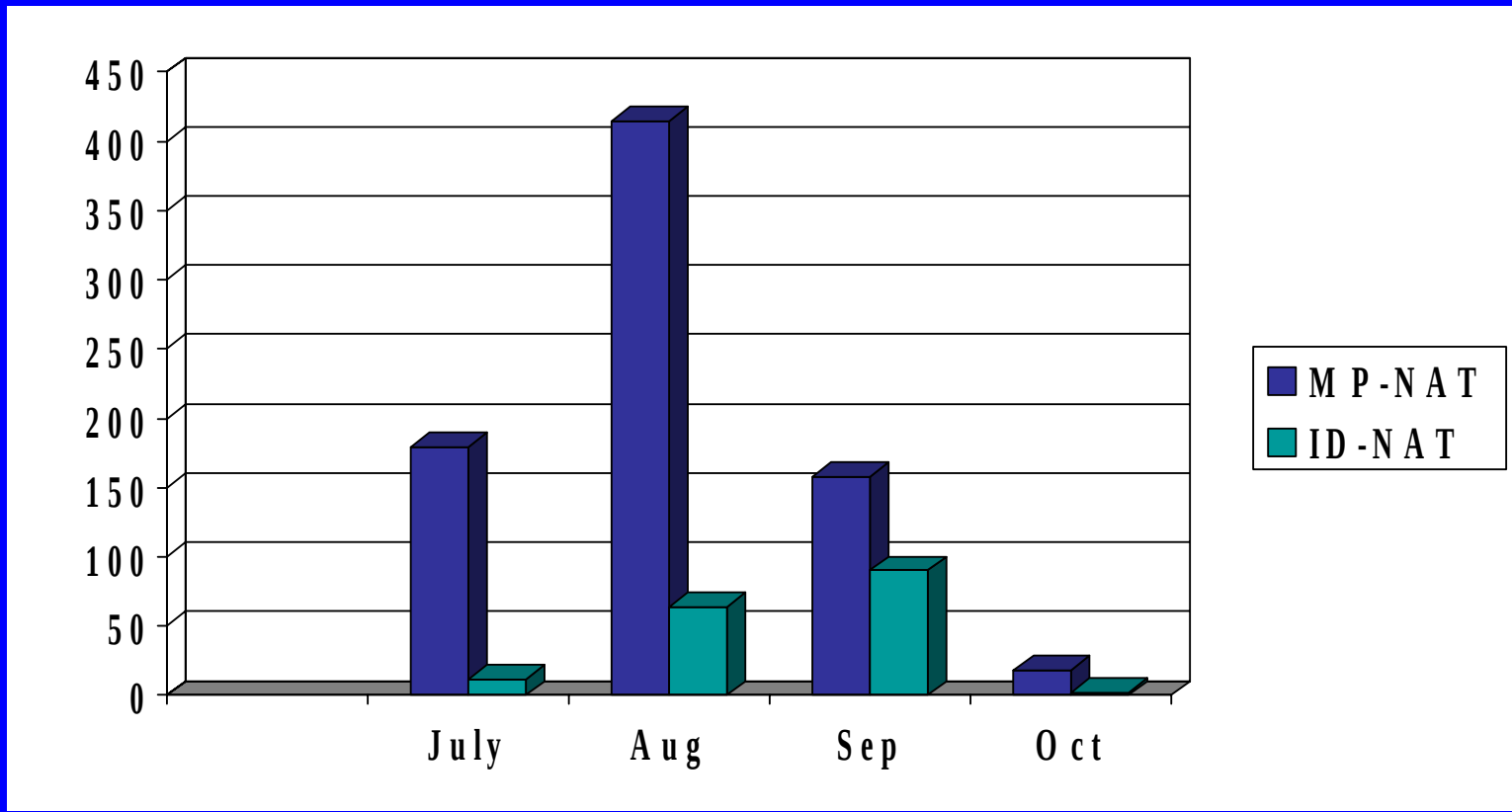
WNV IgG reactivity stable over time



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)

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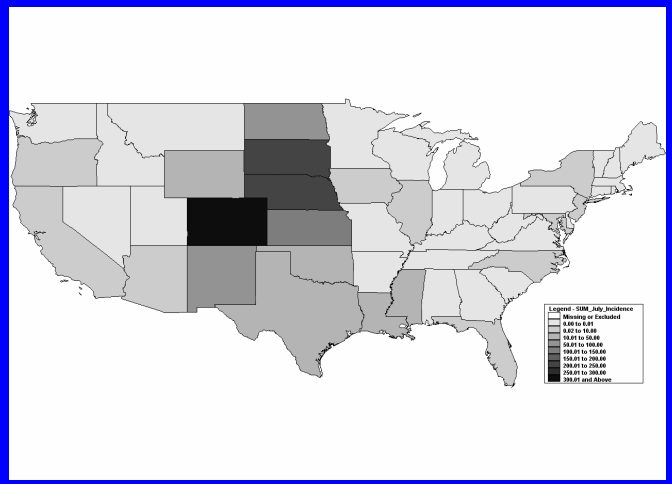
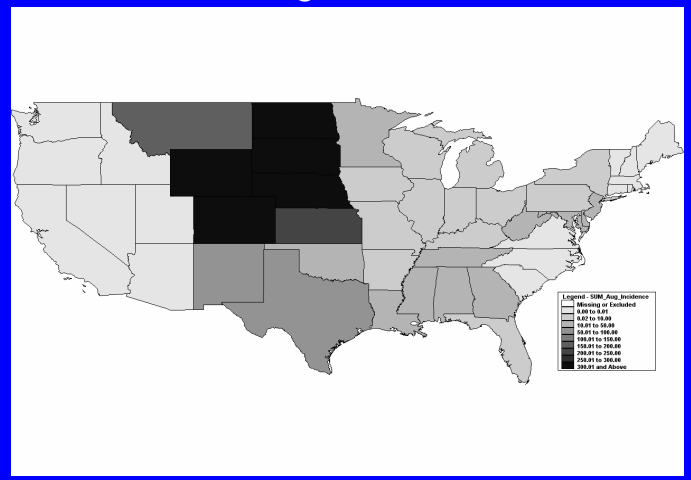
WNV MP-NAT yield by state and month, July – Oct, 2003

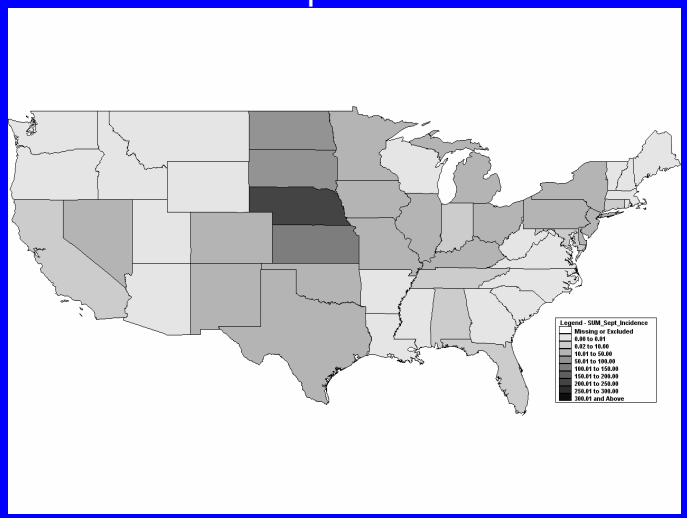
July



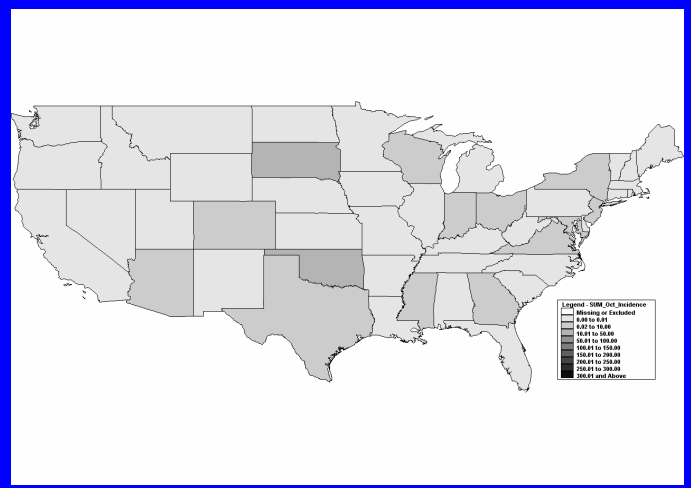
August



September

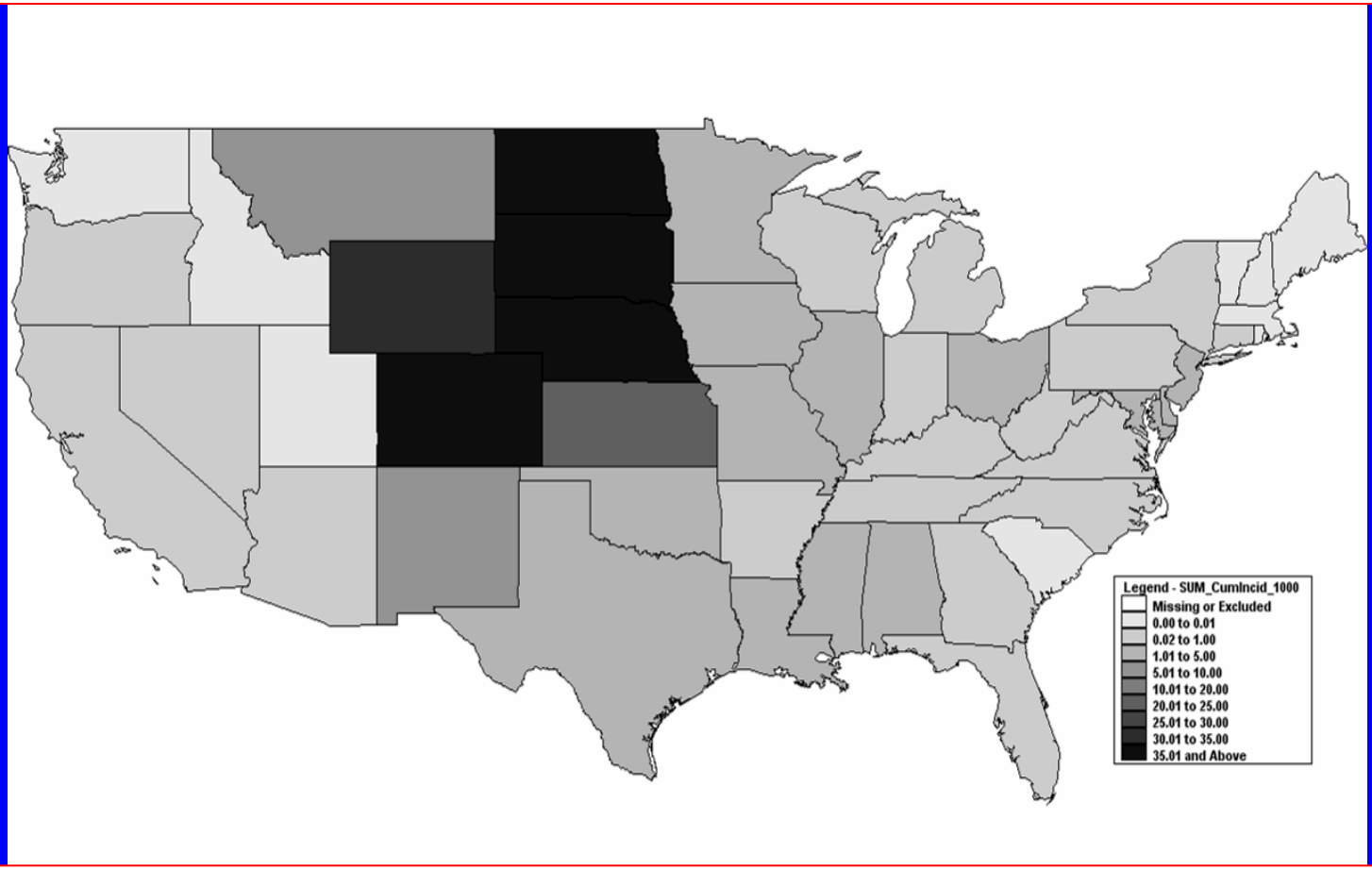


October



peak rates >3 per 1,000 in Colorado in July and 4 other central plain states in Aug

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

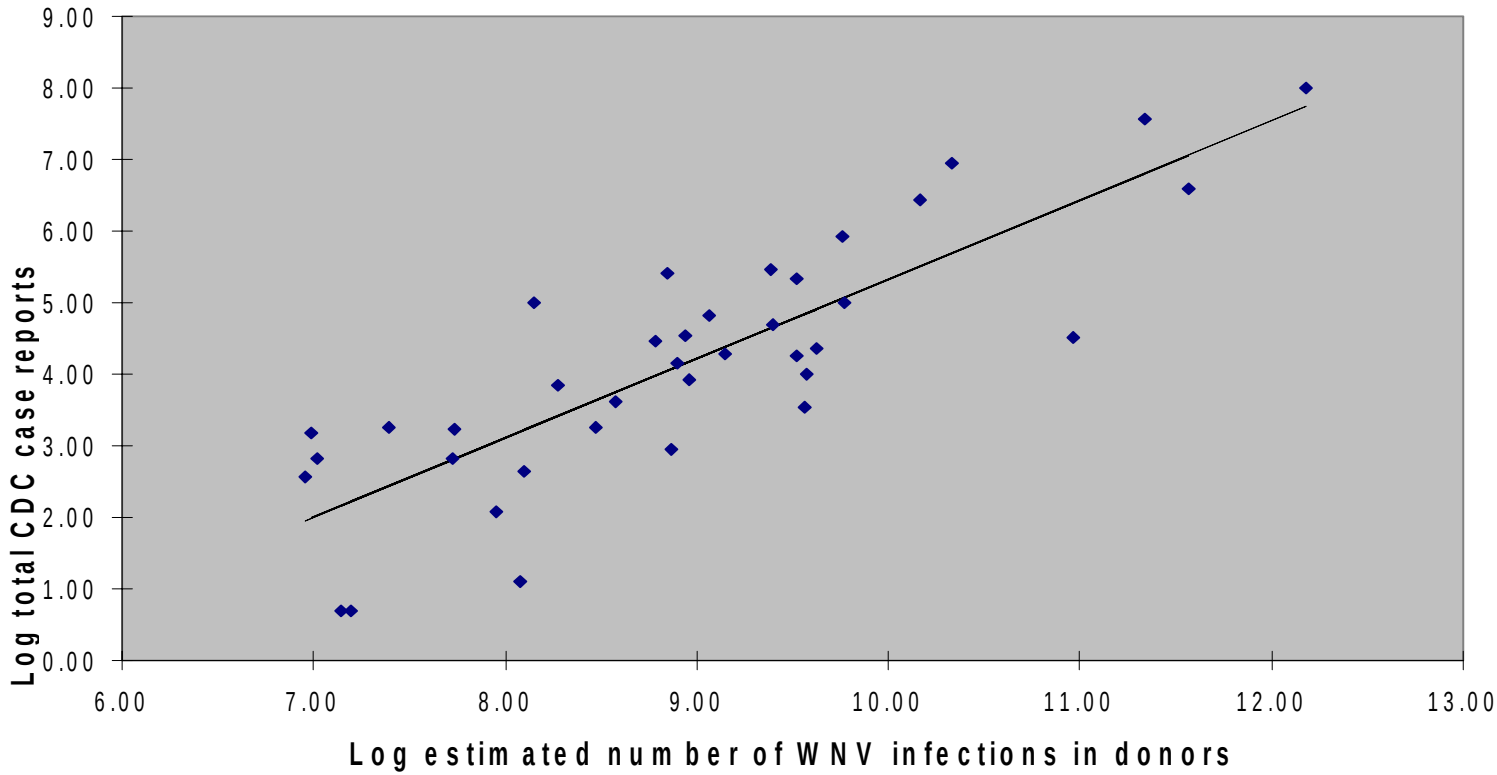


Highest infection rates in Nebraska (4.9%), Colorado (4.3%), North Dakota (4.1%), South Dakota (4.0%), Wyoming (3.5%) and Kansas (2.1%)

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 reported to ArboNET

1 neuroinvasive case per 256 WNV infections



Acknowledgements

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- Gen-Probe: Jeff Linnen, Cristina Giachetti
- Roche: Mike Strong, Jim Gallarda
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