

ZIKV: updates on epidemiology, viral dynamics in blood and transfusion infectivity

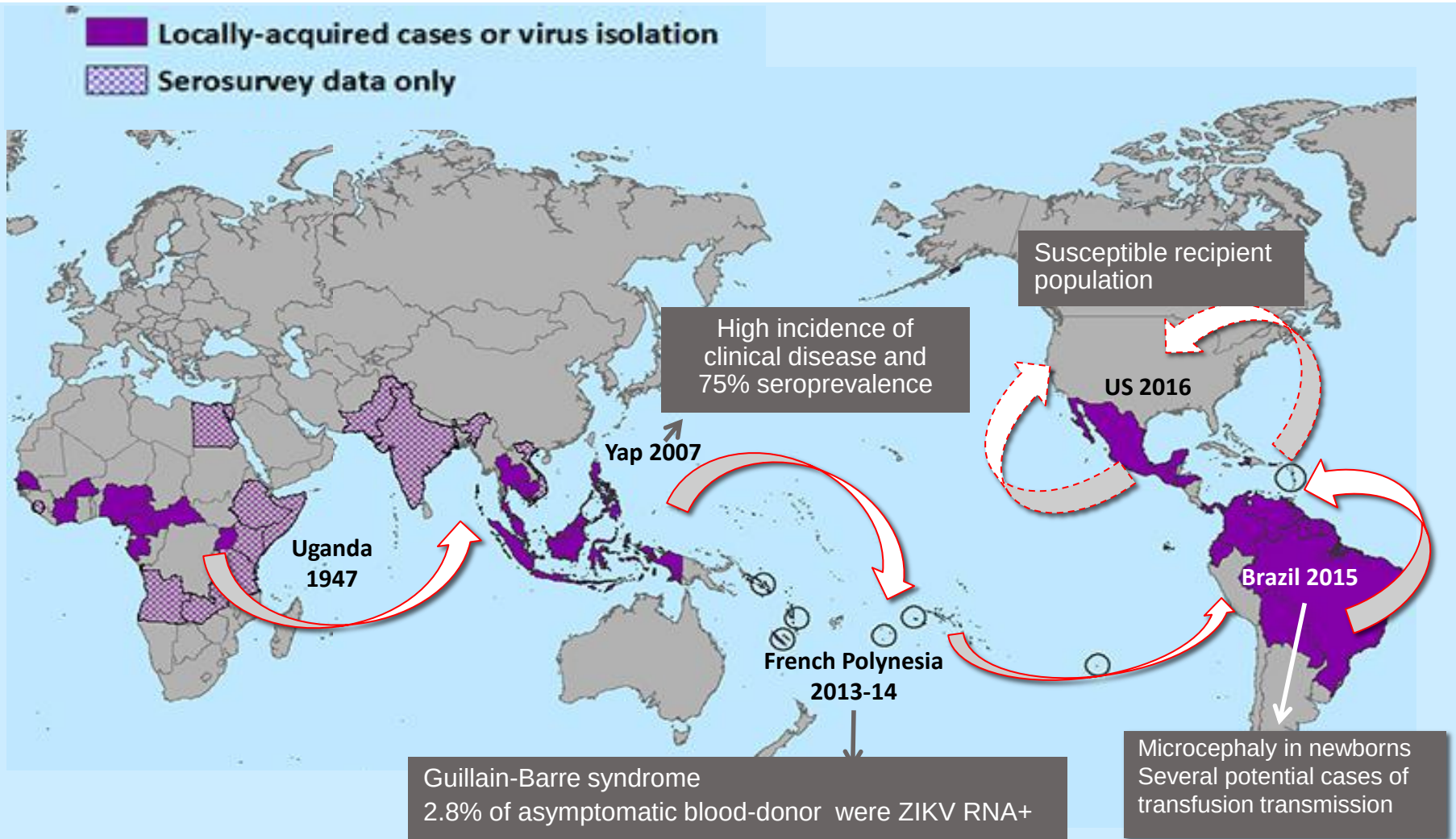
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Blood Systems Research Institute
University of California, San Francisco*



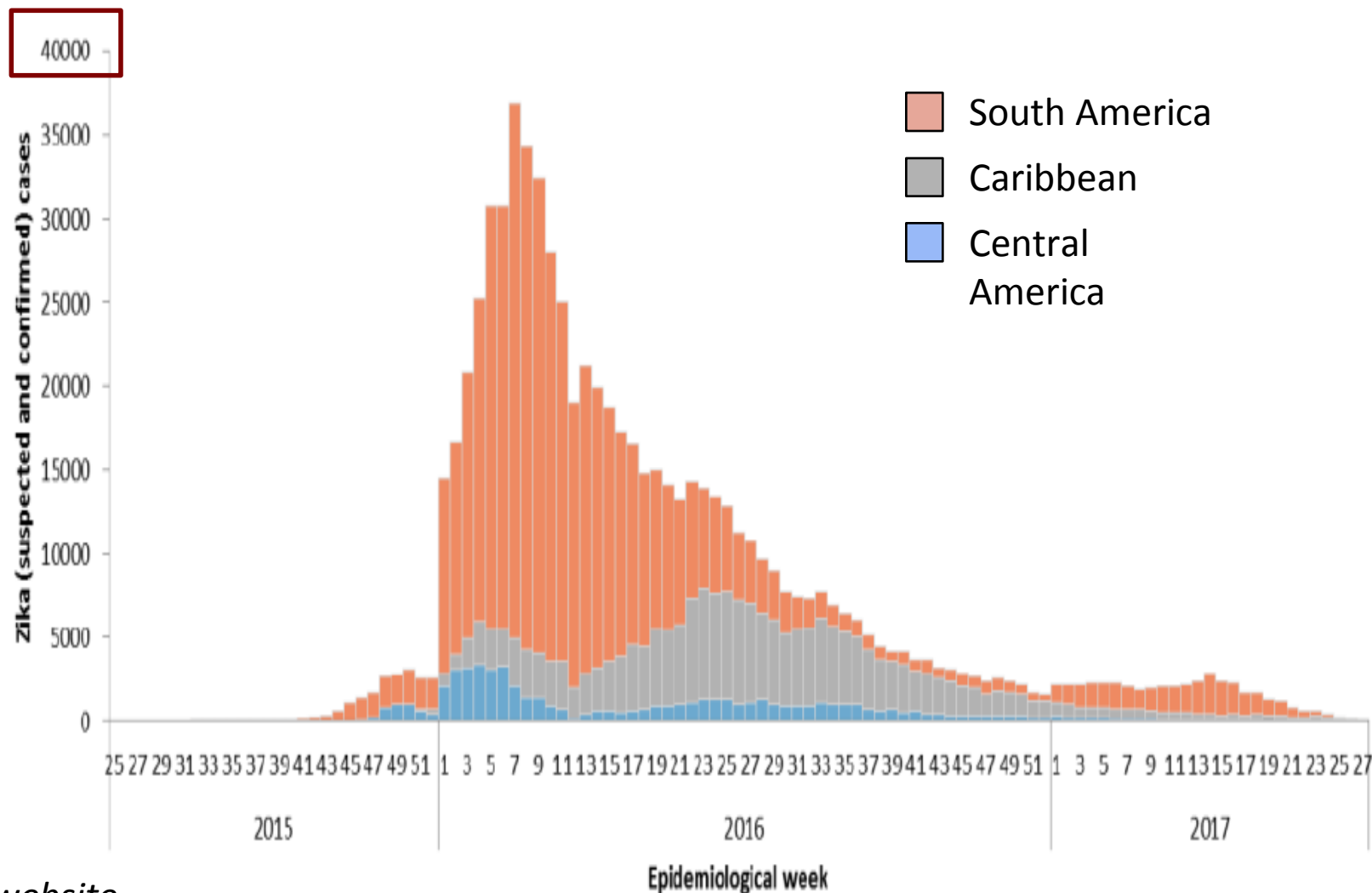
Mission: BSRI is dedicated to advancing blood safety world-wide through scientific research, education and the promotion of evidence-based policies.

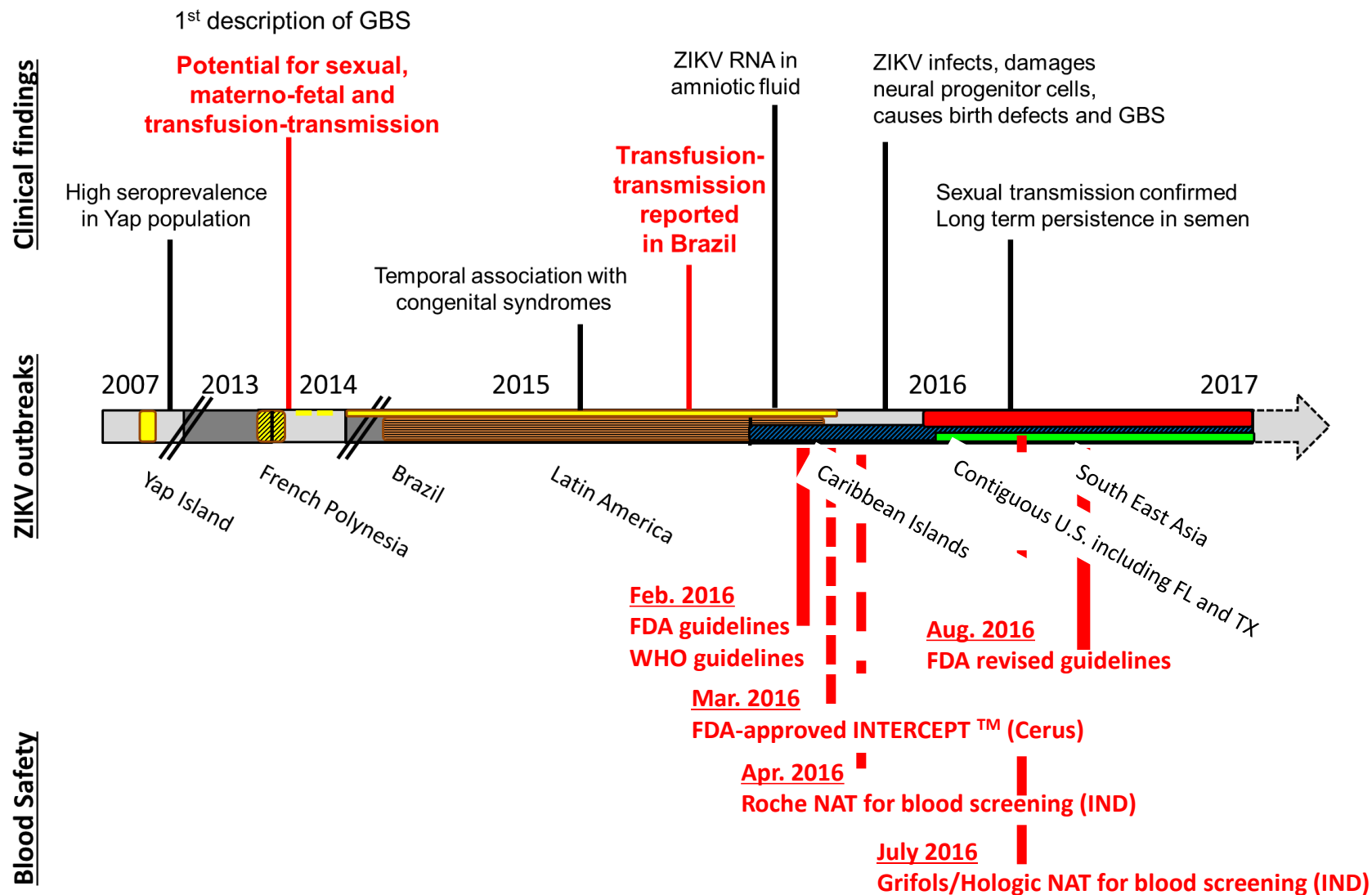
Timing of outbreaks and worldwide concerns

ZIKV spread, expanded clinical associations, and evidence for potential transfusion-transmissions

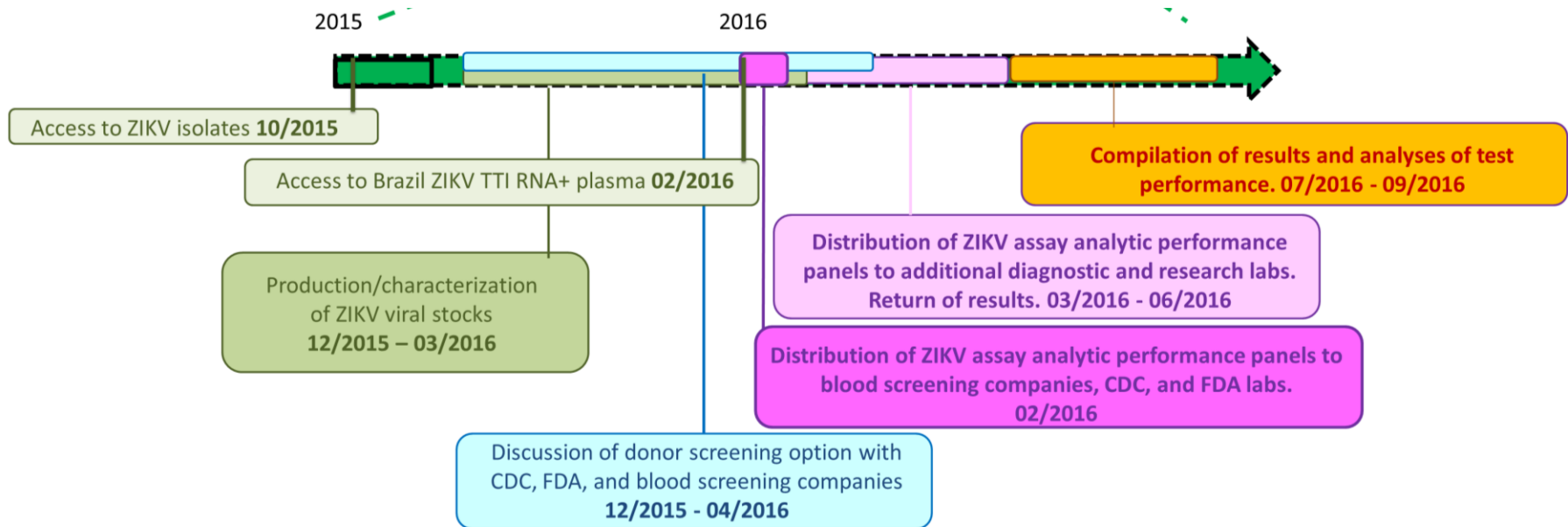


Distribution of suspected and confirmed ZIKV cases by epidemiological week and sub-region Americas, 2016 – 2017





BSRI study to support evaluation and implementation of ZIKV screening in US toward



Analytical sensitivities of potential blood screening and diagnostic ZIKV RNA assays available in early 2016

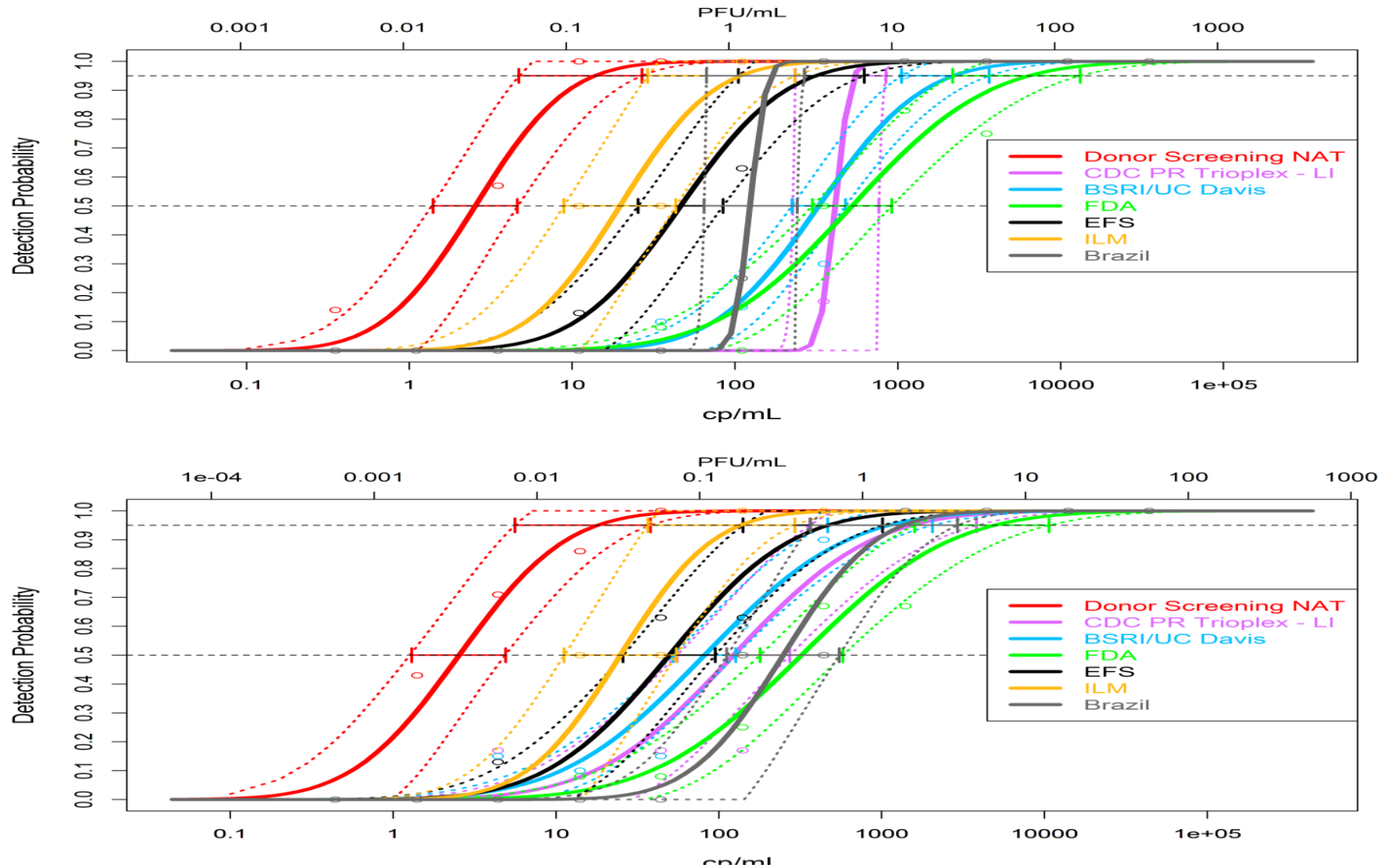
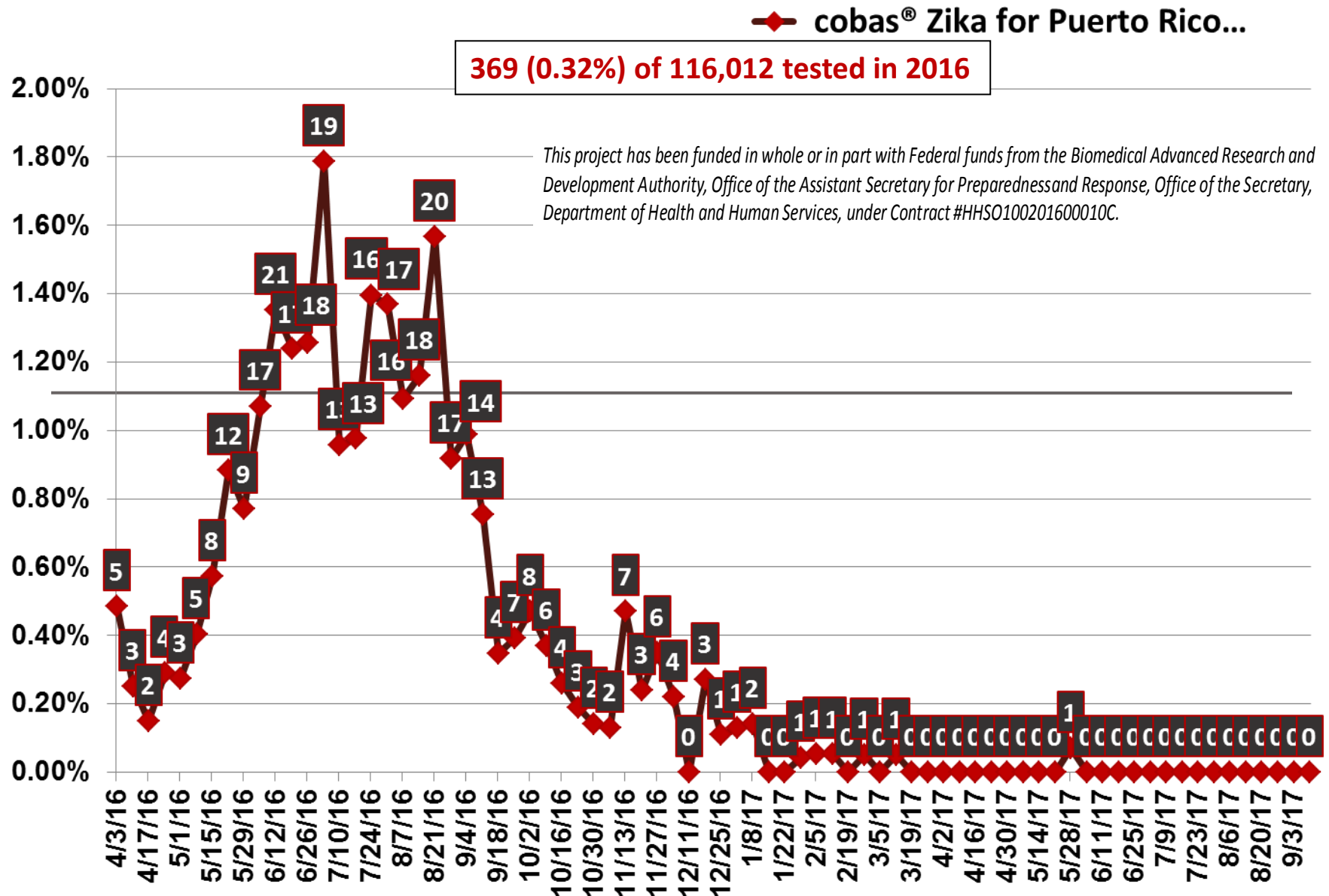


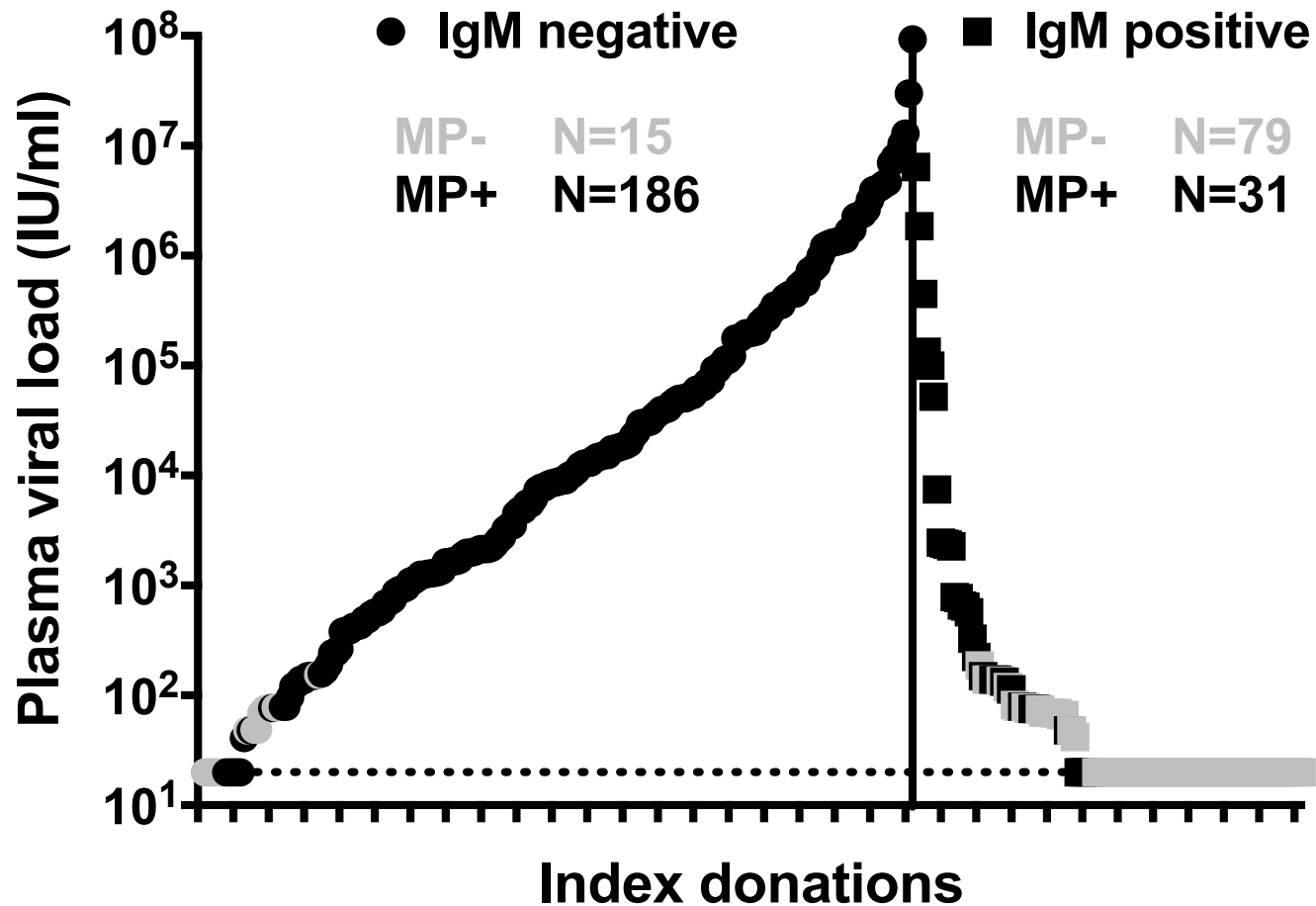
Fig. 2. Probit curves comparing the analytical sensitivity of assays evaluated in this study by replicate testing of (top) the Brazil plasma and (bottom) the French Polynesia isolate supernatant coded panels, grouped by assay category or laboratories (see

ZIKV RNA Reactivity in Blood Donors in Puerto Rico to Nov 4, 2017



Williamson et al., 2017 AABB meeting

IgM status relative to viral load of Puerto Rico index donations



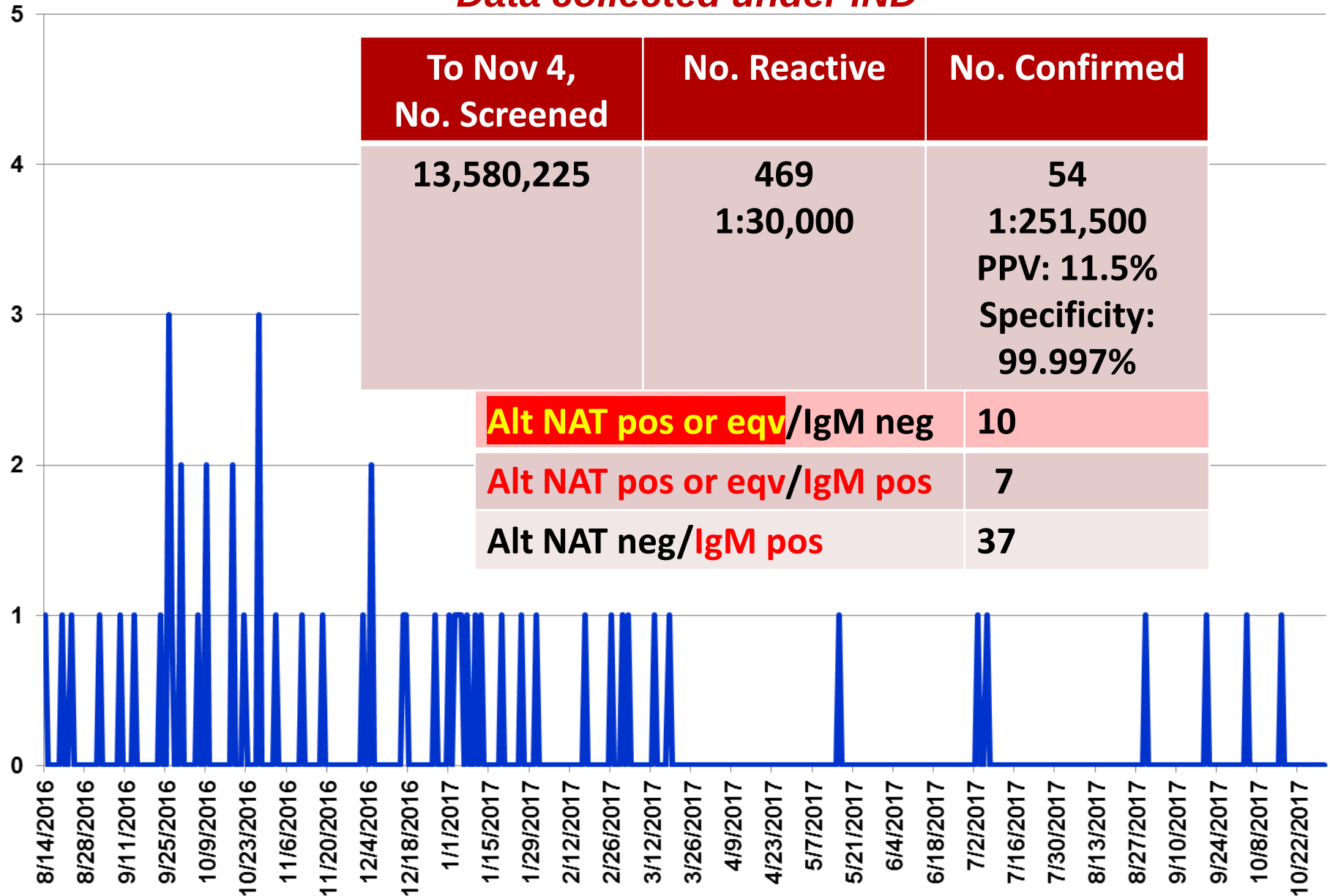
Confirmed positive index donations with conclusive serological results
31-35% were IgM seropositive (n=311)

Classification of MP+/ID only ZIKV NAT yield cases samples relative to IgM Status

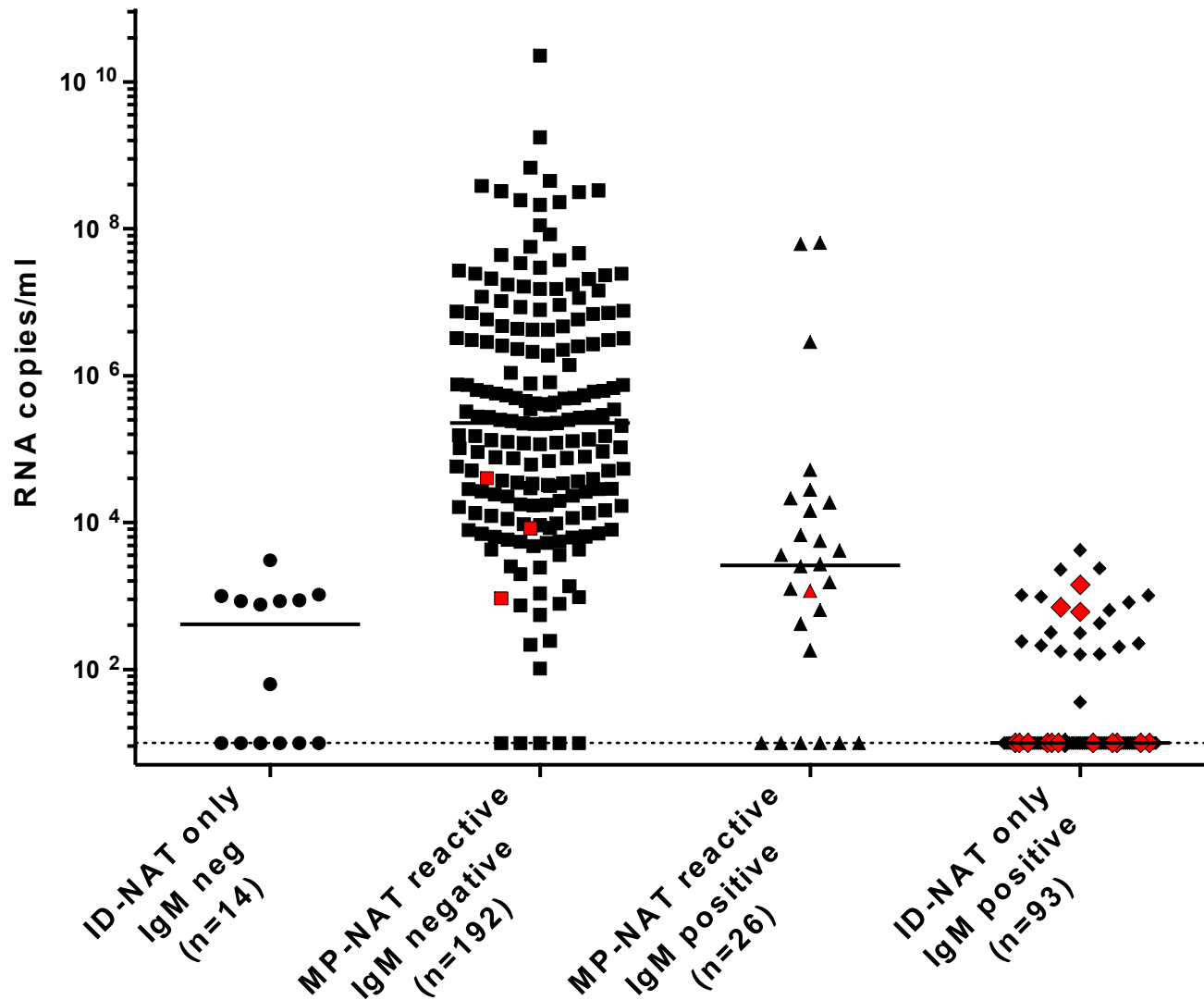
	IgM-		IgM+		Total	ID-NAT Only	(%)	IgM Reactive	(%)
	MP-	MP+	MP-	MP+					
Apr	0	10	1	4	15	1	(6.7)	5	(33.3)
May	1	16	6	5	28	7	(25.0)	11	(39.3)
Jun	4	44	12	7	67	16	(23.9)	19	(28.4)
Jul	4	40	16	1	61	20	(32.8)	17	(27.9)
Aug	4	53	14	4	75	18	(24.0)	18	(24.0)
Sep	0	16	15	3	34	15	(44.1)	18	(52.9)
Oct	2	5	7	2	16	9	(56.3)	9	(56.3)
Nov	0	9	6	0	15	6	(40.0)	6	(40.0)
Dec	0	1	4	3	8	4	(50.0)	7	(87.5)
Total	15	194	81	29	319	96		110	

54 ZIKV Confirmed Positive US Blood Donations

Data collected under IND



Staging of ZIKV NAT yield cases with valid IgM results



Black Symbols = Puerto Rico (n=306)

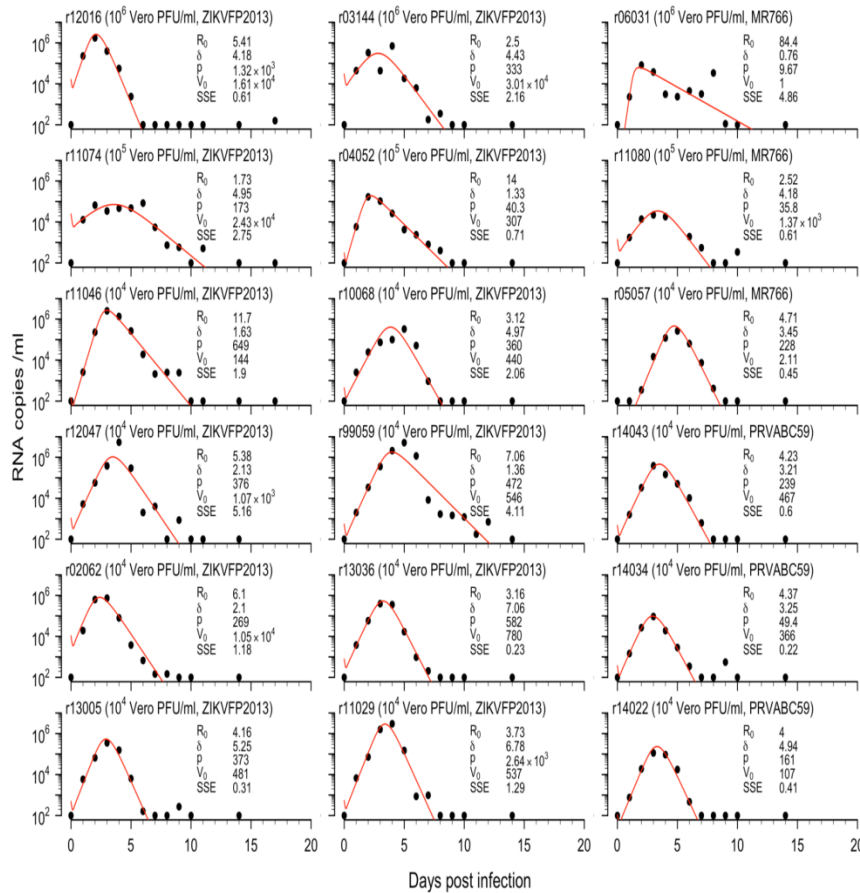
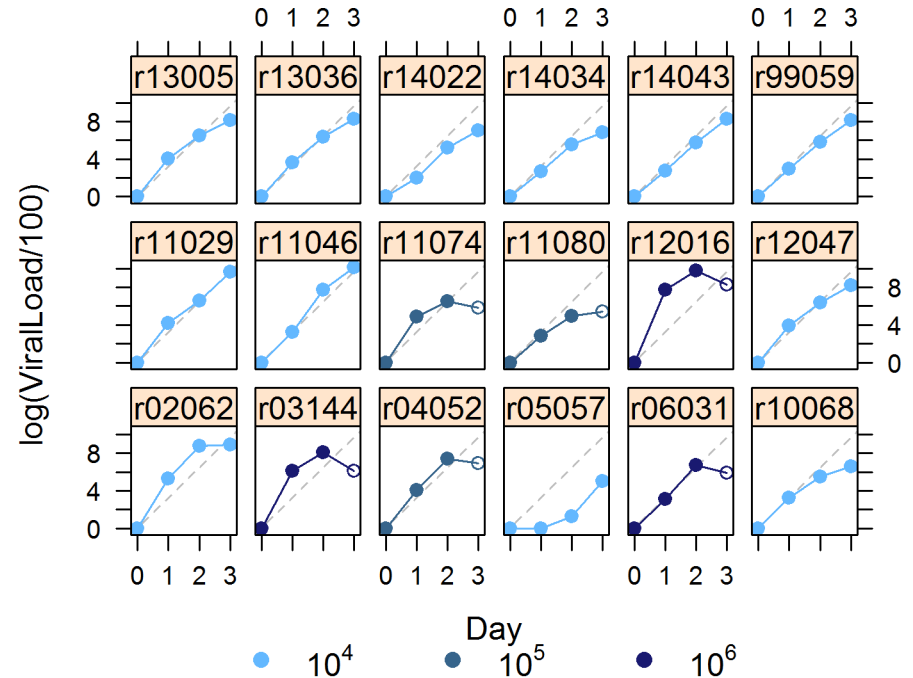
Red Symbols = Continental US (n=19)

Estimate of ID-NAT detection period, pre-IgM seroconversion window period

- We estimated the mean duration of NAT-reactivity of Zika virus in human blood, and subsequently use this estimate to update the annual incidence estimate of ZIKV infections in Puerto Rico in 2016. A three-analysis sequence is used to accomplish this:
 1. Estimate the doubling time of replication of ZIKV in macaques from experimental data (data not shown)
 2. Extrapolating the doubling time estimate found in macaques to humans, we use observed data from blood donors to estimate the distribution (including the mean) of the duration of NAT-reactivity
 3. Use the distribution of the mean duration of NAT-reactivity to update the incidence estimates in Chevalier, *et al.*¹

¹ Chevalier et al. Emerg Inf Dis. 2017;23:790-94.

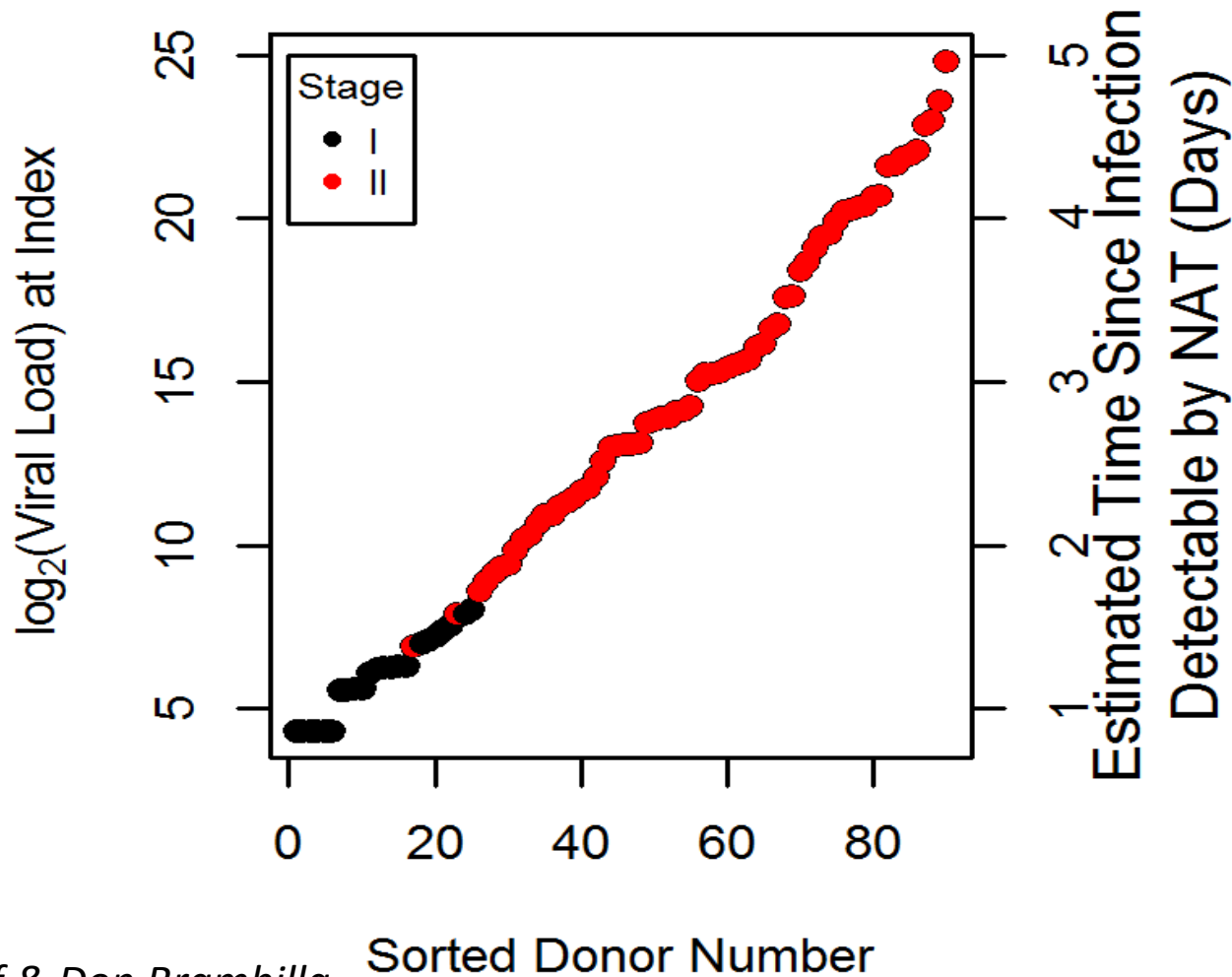
Zika viral dynamics is Rhesus Macaques to estimate doubling time



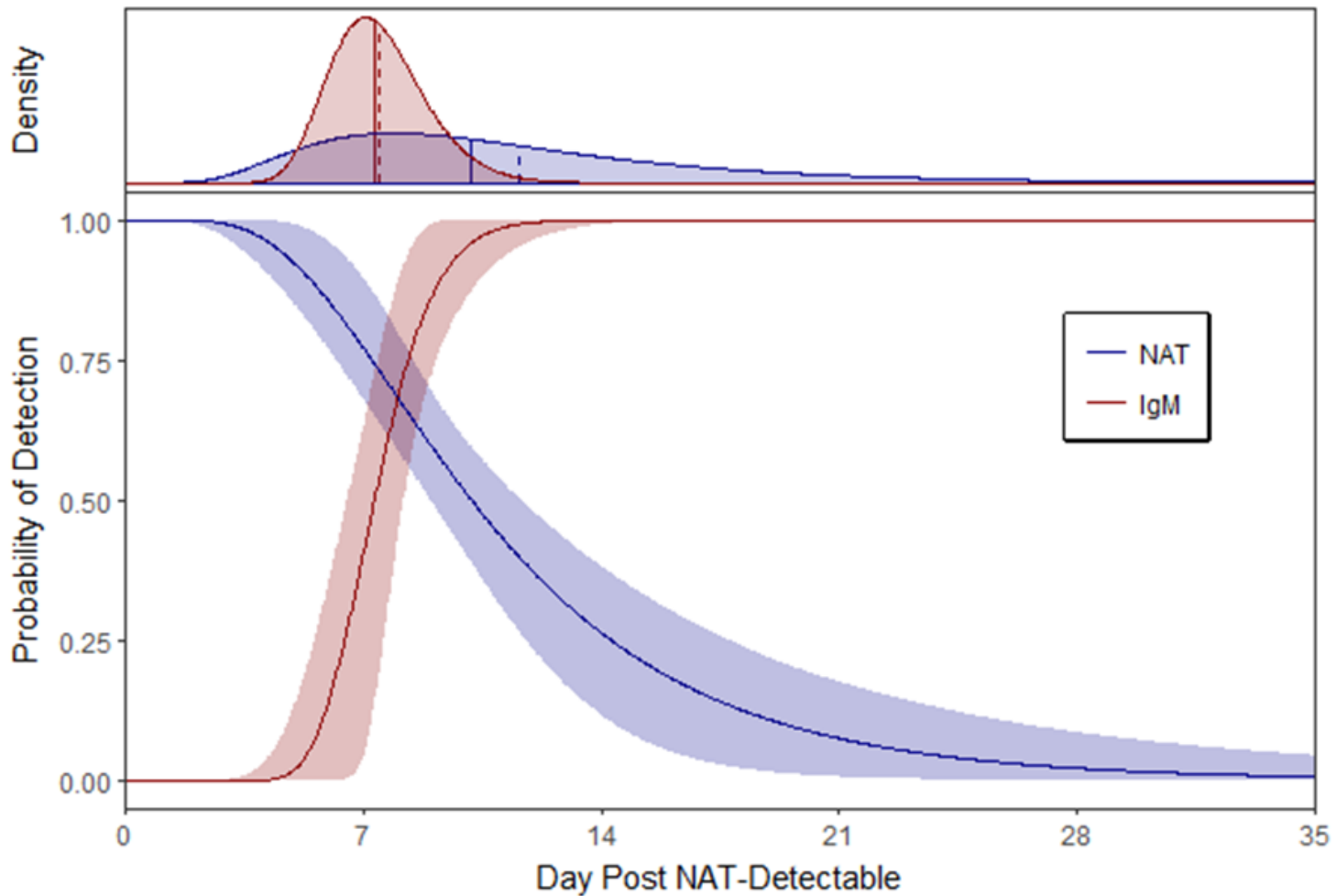
log(Viral Load / 100) by Day for 18 experimentally infected macaques, ID numbers shown in panel banners. Dose (virus particles per mL) is indicated by colors, identified in the legend. Only the filled circles are used in the estimation of the doubling time. The fitted, mean line is shown as the dashed, gray line.

The estimate (asymptotic SE) for ZIKV Doubling Time is $Z\hat{D}T = \log(2)/\hat{\delta} = 0.223$ (SE = $\log(2)SE_{\hat{\delta}}/\hat{\delta}^2 = 0.015$) days, or 5.35 (0.36) hours.

Viral load and estimated time from infection for NAT yield donors detected pre-IgM seroconversion

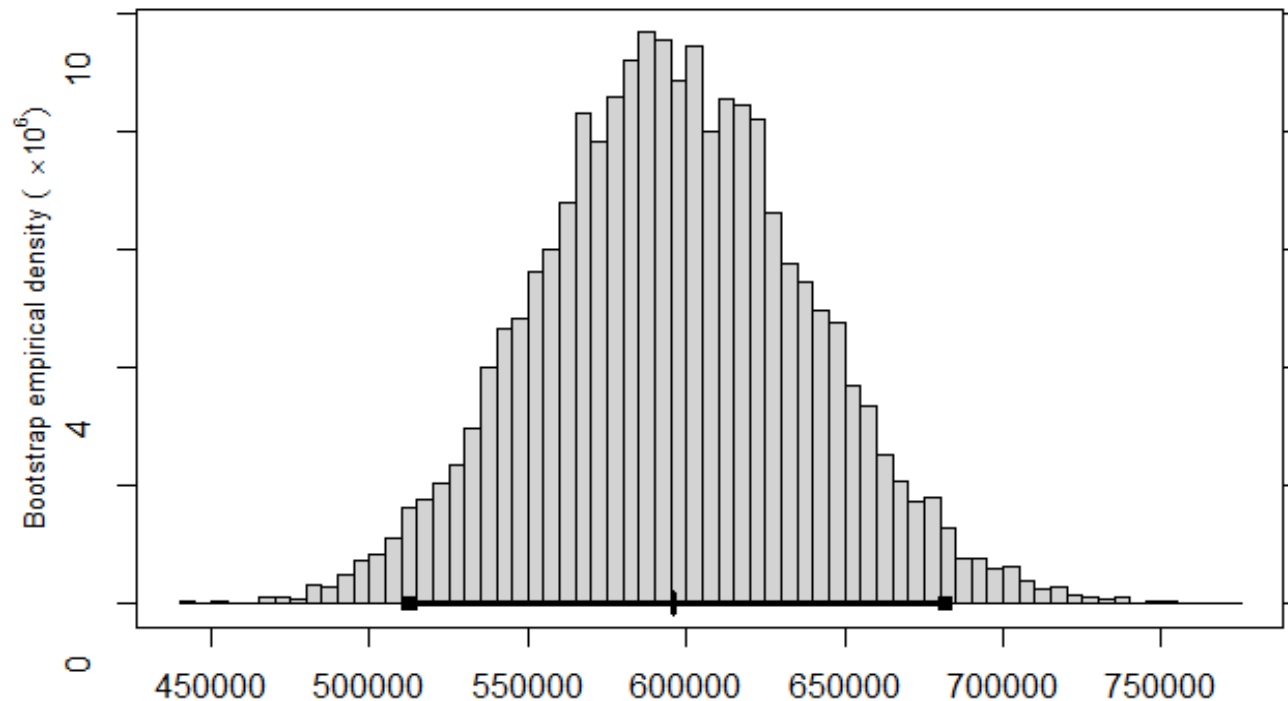


Days Post NAT-Reactivity to IgM



Estimate of Incidence in Puerto Rico

- NAT yield data was transformed into incidence for PR utilizing the empirically based estimate of pre-seroconversion NAT detection period using the methods of Biggerstaff¹



Estimate of the Number of ZIKV Infections, Puerto Rico, 2016

**Analysis yields estimate of 595,938 (95% CI 512,859 - 682,167) ZIKV infections in PR in 2016 .
With population size 3,638,773, estimated incidence for 2016 of 16.4% (95%CI 14.1% - 18.7%)**

¹ Chevalier et al. Emerg Inf Dis. 2017;23:790-94.

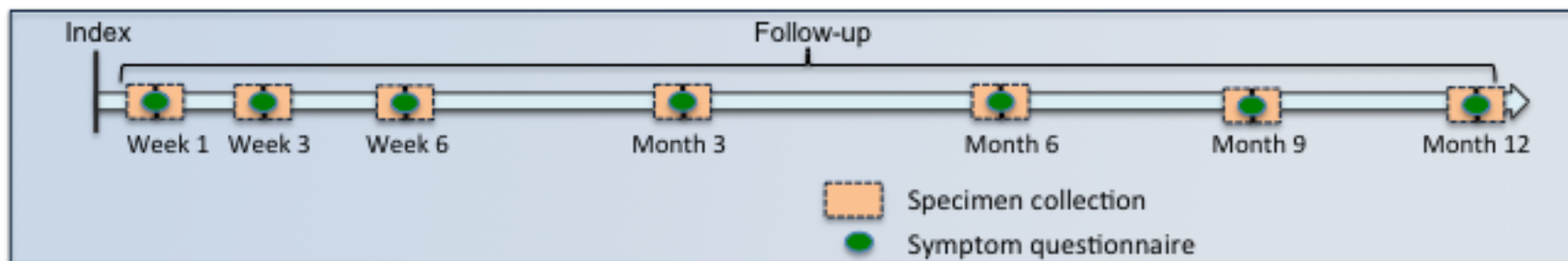
US Natural History Cohort of Zika Virus (ZIKV) RNA Positive Blood Donors

Objectives:

- Evolution of viral and immunological markers over time
- Distribution and compartmentalization in blood and body fluids
- Evaluate the viral and immune mechanisms leading to viral clearance or clinical pathogenesis
- Evaluate clinical outcomes post donation
- Establish a sharable biorepository
 - Characterize the performance of existing and future assays and provide standards for assay development
 - Working with government agencies, industry and academic partners

US Natural History Cohort of Zika Virus (ZIKV) RNA Positive Blood Donors

- **Study Design:** Natural history cohort of ZIKV NAT-positive blood donors followed prospectively for 12 months (index + 7 follow-up visits)
- **When:** Launched in June 2016 with accrual through Sept 2018
 - Extended 1 year in anticipation of 2018 Zika outbreak
- **Where:** Puerto Rico, OneBlood, BSI, NYBC, ARC
- **Sample size:** 130 ZIKV+ donors
 - (80 DENV Ab+; 50 DENV Ab-)



Detection of Zika virus RNA in whole blood of imported Zika virus disease cases up to 2 months after symptom onset, Israel, December 2015 to April 2016

Y Lustig¹, E Mendelson^{1,2}, N Paran³, S Melamed³, E Schwartz⁴
1. Central Virology Laboratory, Ministry of Health, Tel-Hashomer, Israel

Volume 23, Number 5—May 2017

Research Letter

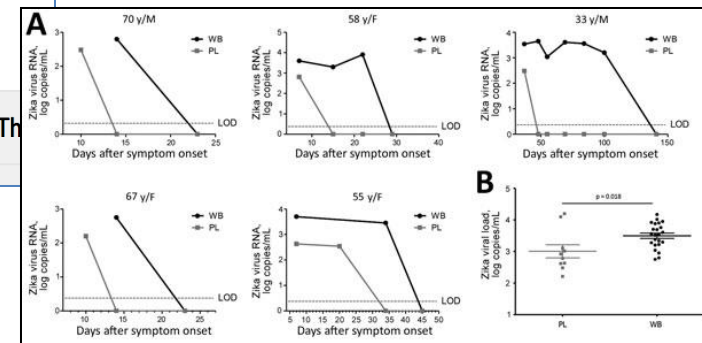
Zika Virus Infection and Prolonged Viremia in Whole-Blood Specimens

Jean Michel Mansuy[✉], Catherine Mengelle, Christophe Pasquier, Sabine Chapuy-Regaud, Pierre Delobel, Guillaume Martin-Blondel, and Jacques Izopet

5 Asymptomatic donors
Plasma - 10 days (range 7–37)
WB -22 days (range 14–100)
VL higher in whole blood

6 symptomatic travelers
Serum - 3 days
WB - 2 months
Urine - 26 days

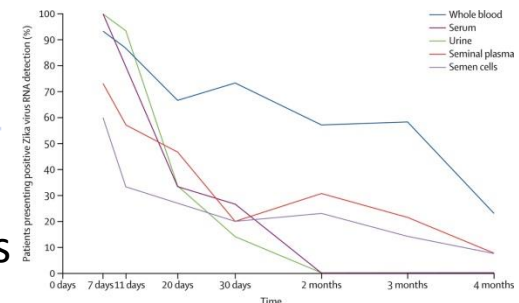
On Th



Effect of acute Zika virus infection on sperm and virus clearance in body fluids: a prospective observational study

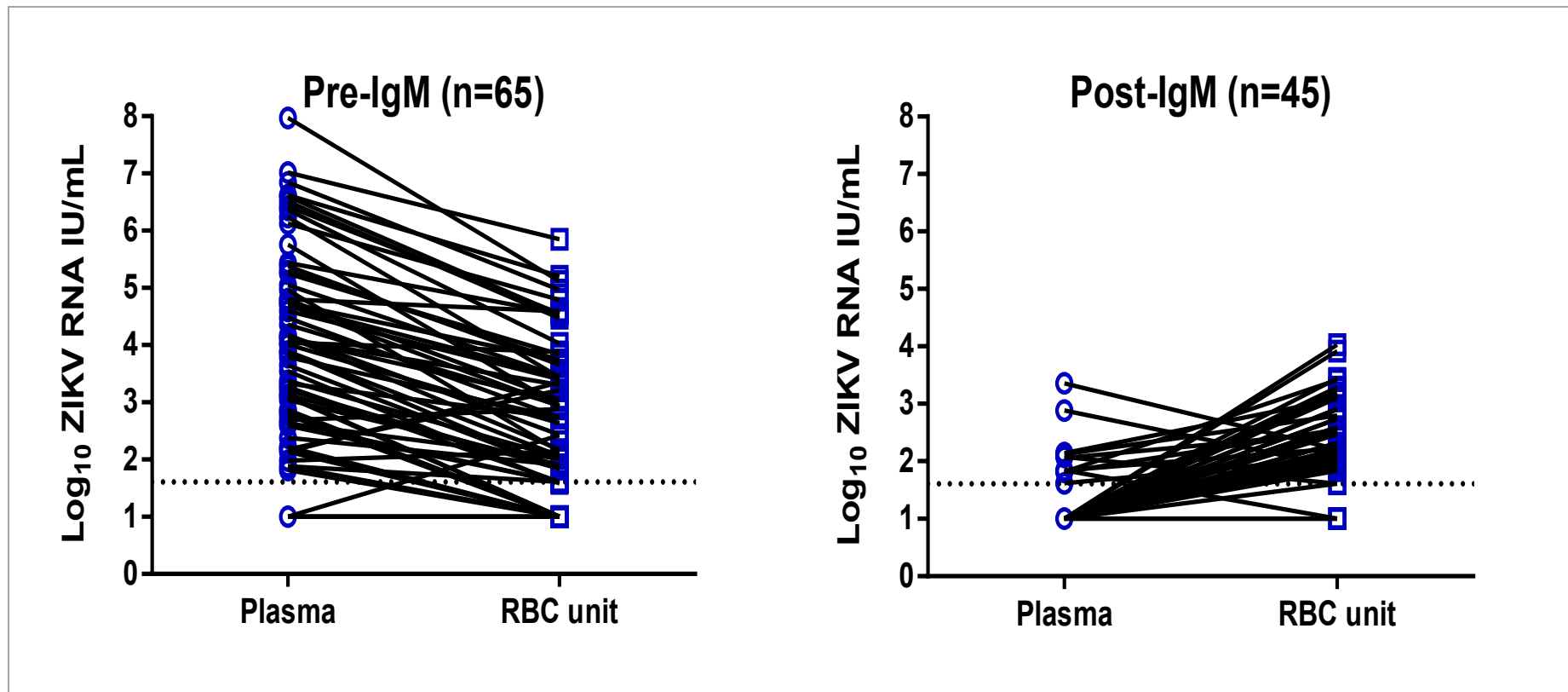
Guillaume Joguët, Jean-Michel Mansuy, Giulia Matusali, Safouane Hamdi, Marie Walschaerts, Lynda Pavili, Stefanie Guyomard, Nadia Prisant, Pierre Lamarre, Nathalie Dejuçq-Rainsford, Christophe Pasquier, Louis Bujan

15 donors w/ acute Zika inf
Whole blood, semen – 4 months



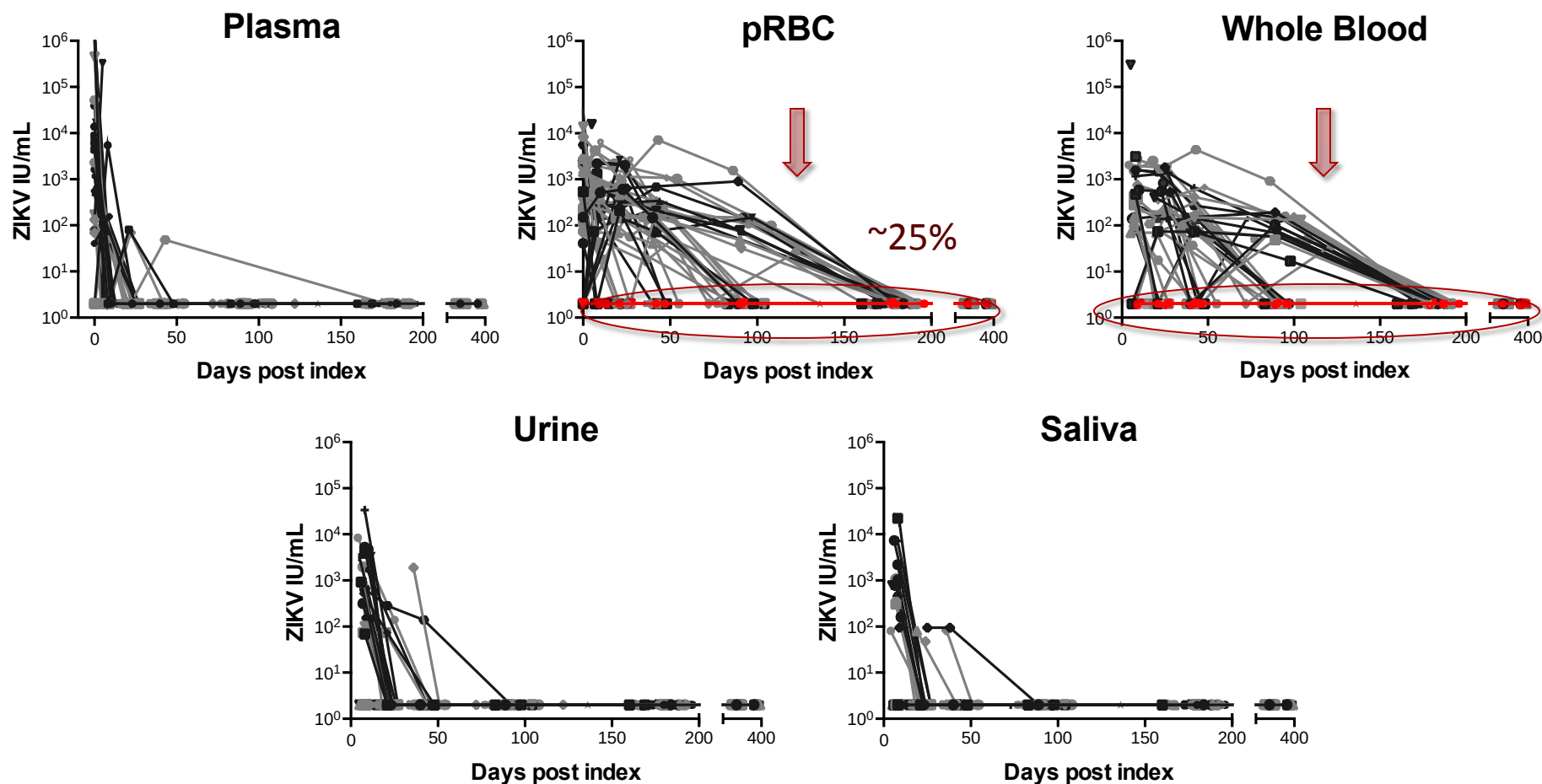
Stone et al., 2017 AABB meeting

Higher levels of ZIKV RNA in red cells vs plasma in IND study donation samples after IgM seroconversion



- Early analysis showed longer persistence of Zika RNA in RBC vs plasma
 - Triggered RBC testing for IND and focus on RBC persistence in the REDS-III longitudinal follow-up study

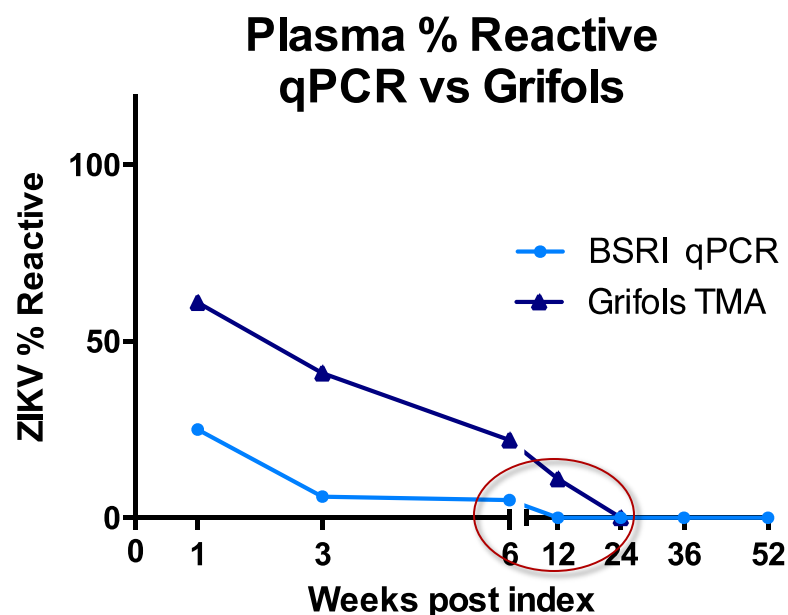
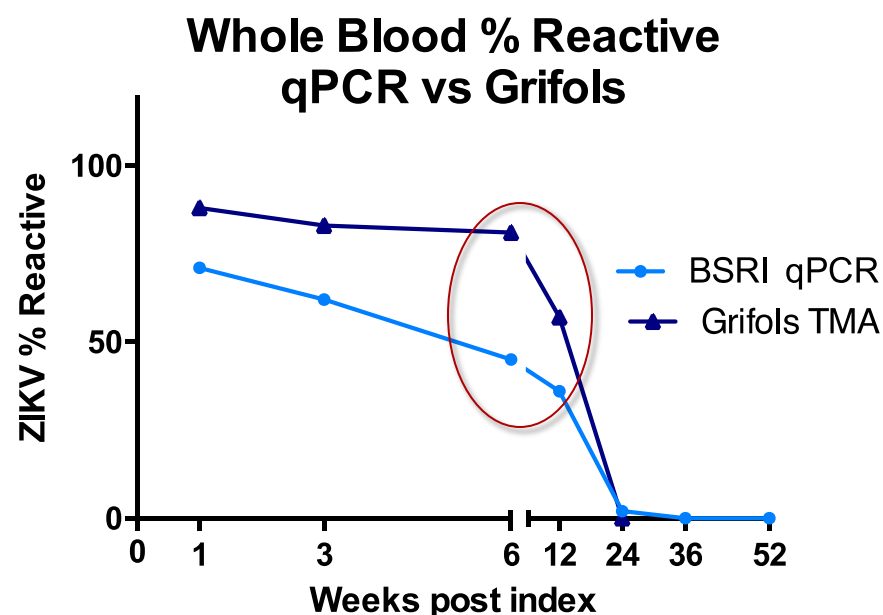
Longer persistence of ZIKV RNA in whole blood and RBC than in plasma and body fluids



Pre-seroconversion
Post-seroconversion

Increased sensitivity detection of ZIKV RNA in plasma and whole blood by blood screening NAT assays relative to confirmatory qPCR/VL assay

Grifols TMA testing performed only on follow up



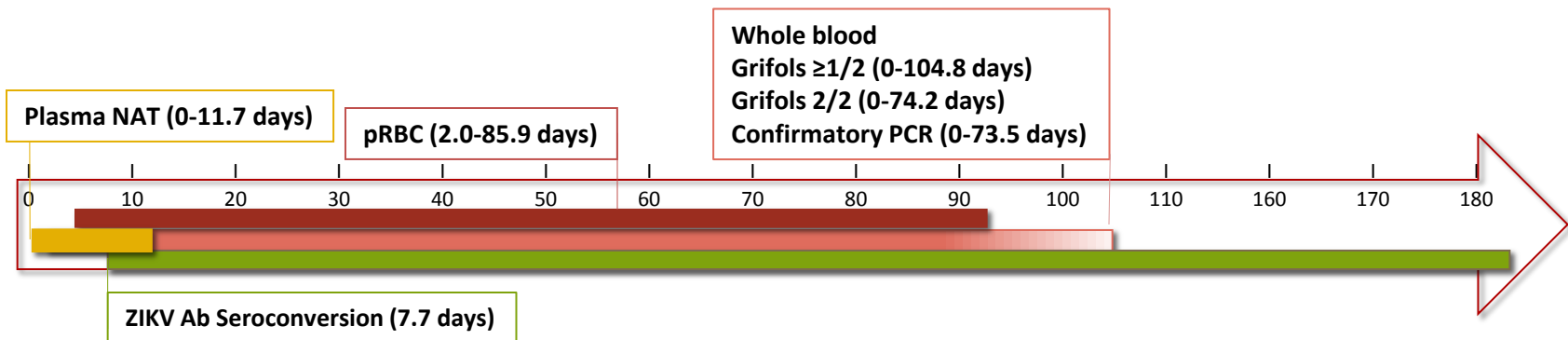
Grifols TMA		BSRI confirmatory qPCR	
LOD95	7.5 (4.1, 11.3)	LOD95	109 [56.4, 176]
LOD50	1.5 (1.1, 2.1)	LOD50	15.8 [11.2, 22.2]

Replicate testing extends the period of reactivity

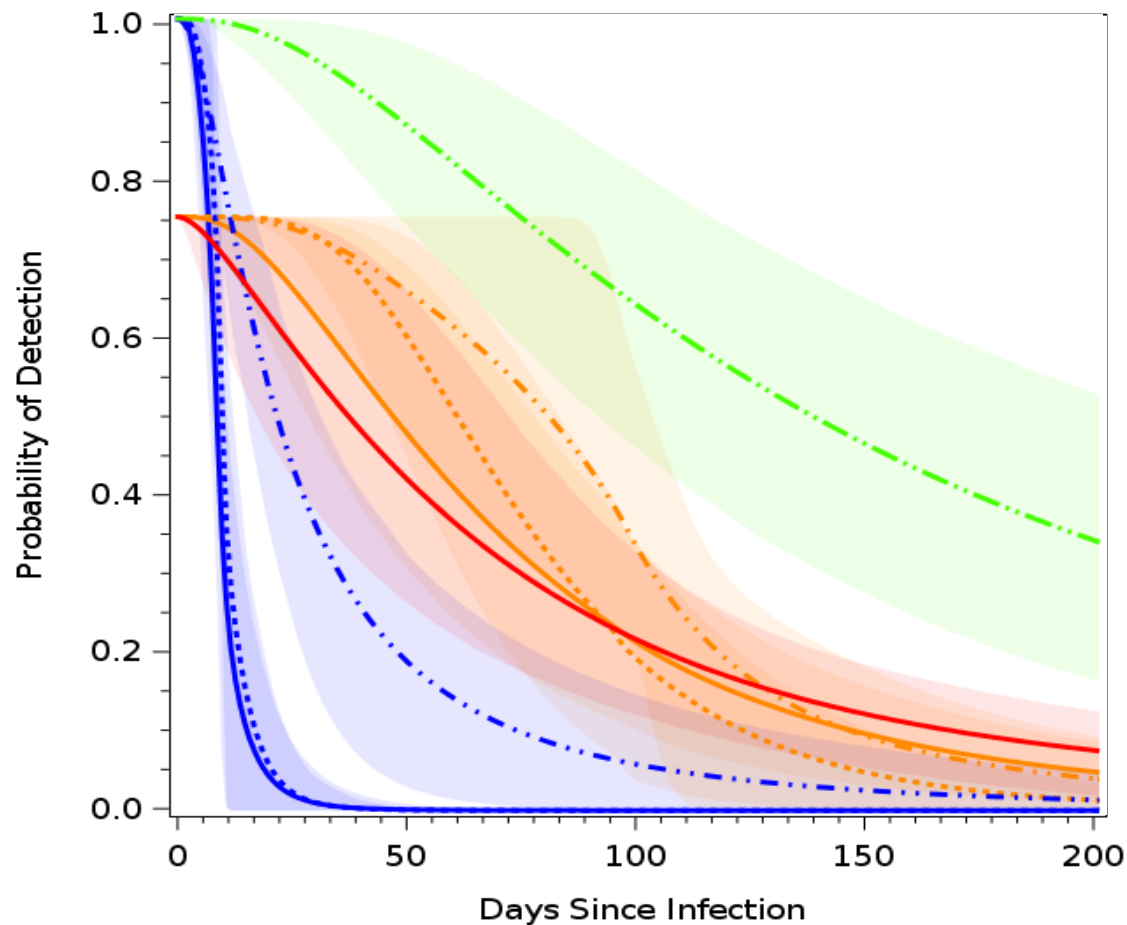
Durations of time to detection and clearance of ZIKV RNA and IgM since NAT+ plasma of 25 donors IgM- at index

Compartment	Assay	Interval*	Mean (CI) days
plasma	MAC-ELISA IgM	IgM detection	7.7 (6.1, 9.2)
RBC	BSRI-PCR	RNA detection	2.0 (0.8, 3.3)

* Since plasma NAT detectable infection



Relative durations of persistence in blood compartments



Plasma
— PCR

Whole Blood
— PCR

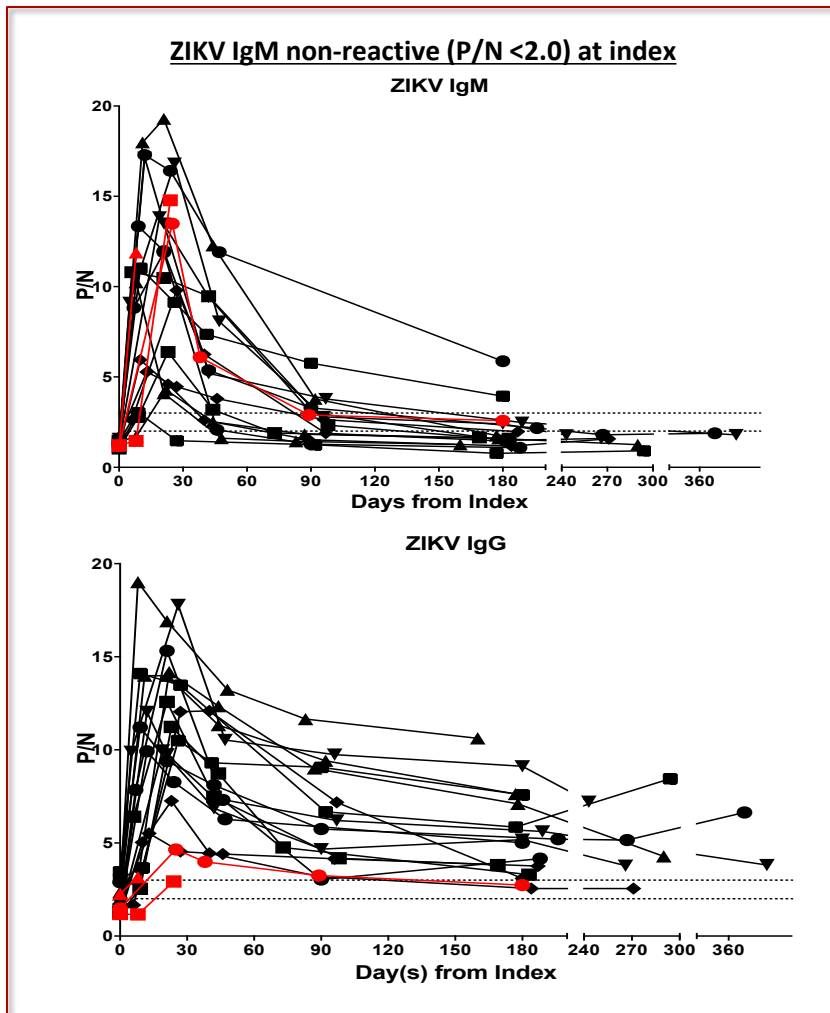
RBC
— PCR

Zika IgM
- . - IgM MAC ELISA

Implications of Zika persistence in blood compartments and body fluids

1. For acute infection, the use of whole blood extends the period of diagnosis
2. Impact on donation policy: to extend deferral period or consider NAT testing whole blood
3. Consideration for solid organ donation with potential reservoir for viral replication
4. Could testing whole blood or RBC be used as proxy for persistence in semen and sexual transmission risk?

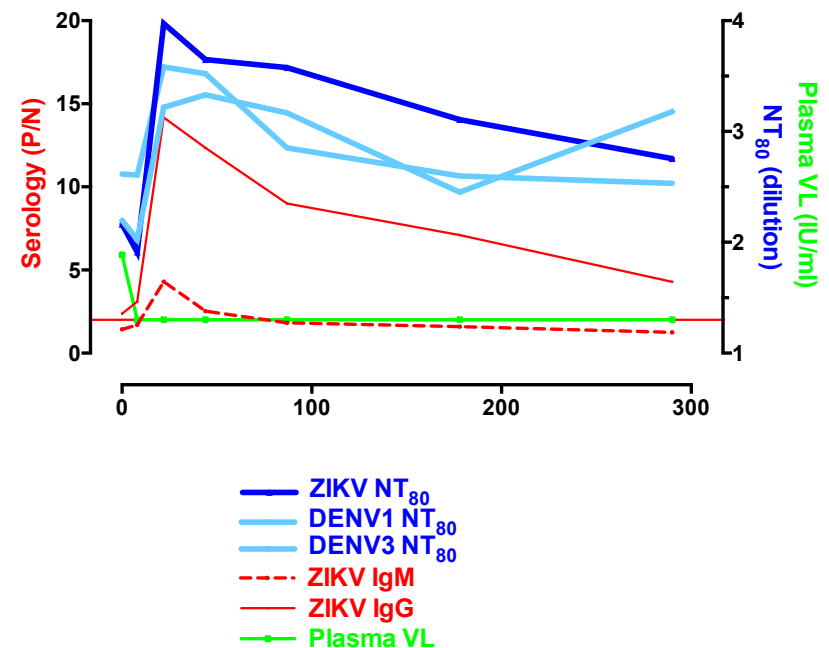
Zika Ab response is brisk but wanes and weaker when DENV Ab-



— DENV IgG negative donor

NAb ZIKV+/DENV+

Stage I



- Strong but waning neutralizing response in primary and secondary infections with strong DENV cross-neutralizing responses

Is RBC-associated ZIKV RNA infectious?

1. No documentation of infectious RBC associated virus after plasma RNA clearance
2. Attempts at inoculating ZIKV RNA+ RBC
 - Onto susceptible cell lines
 - Into IFN- knockout mice
 - Feeding onto Aedes mosquitos
 - Infection of macaques and MID in progress
3. Despite huge epidemics in Latin America and Puerto Rico and French Caribbean Islands (with routine screening), no cases of TT linked to RBC transfusions tested plasma NAT- have been detected

Tentatively concluding:

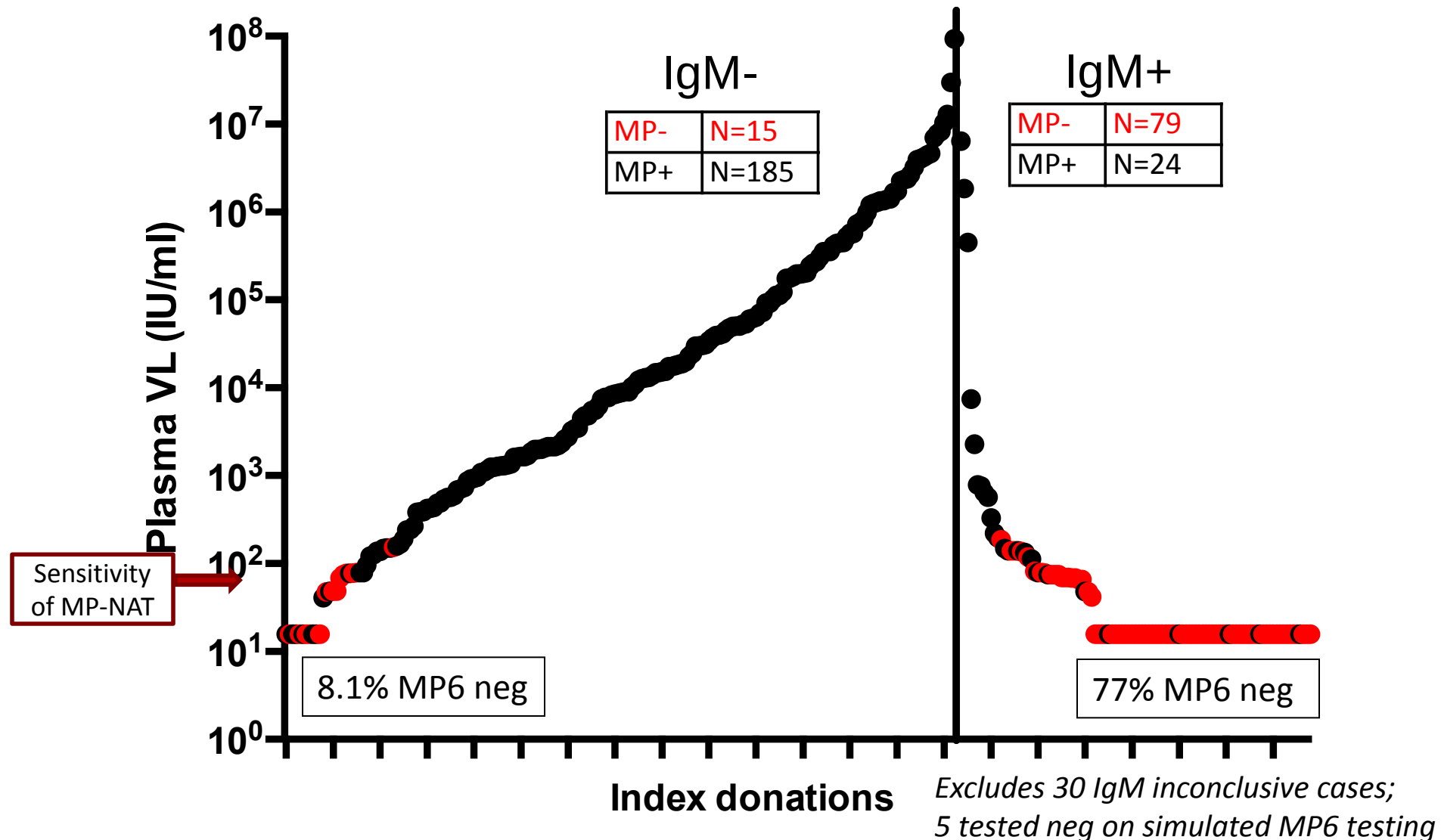
- ✓ ZIKV RNA that likely became associated with erythroblasts in acute infection is not infectious
- ✓ plasma NAT screening is likely sufficient

Analytical sensitivities of blood screening NAT assays when testing performed on Mini-Pools (MPs)

Package Insert (PI) 95% LODs [CIs] adjusted to
MP6 (Roche) and MP16 (Grifols)

	Roche PI		Grifols/Hologic PI
95%LOD	8.1 [6.1, 13.6]	95%LOD	5.9 [4.3, 8.9]
95%LOD MP6	49.0 [36.6, 81.6]	95%LOD MP16	94.4 [68.8, 142.4]

ZIKV confirmed NAT yield donations in Puerto Rico sorted by IgM results & VLs, with reactivity by simulated MP6 (MP+, MP-)



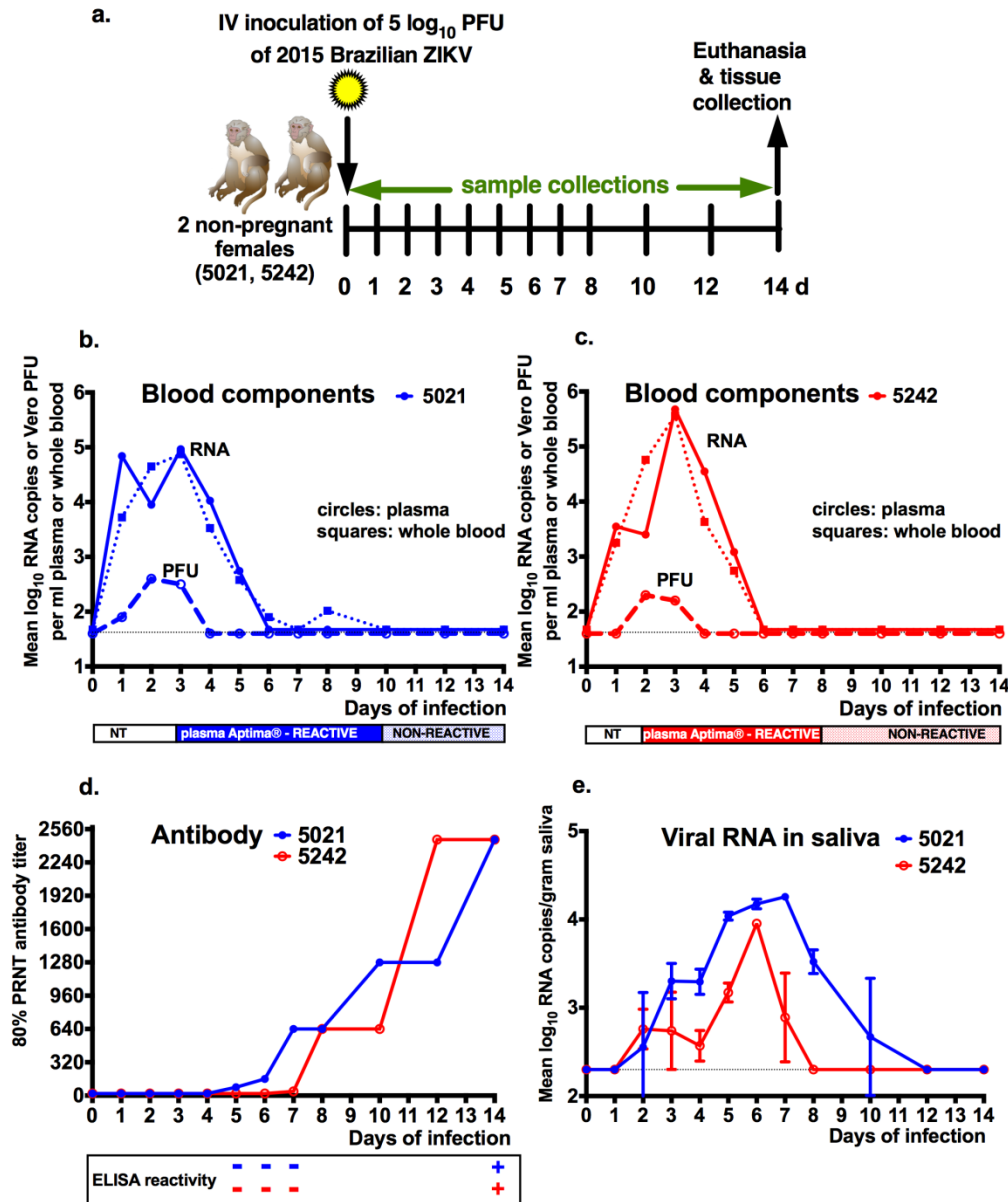
Estimate proportion of the pre-IgM NAT detection period that would be missed by MP-NAT

- Based on doubling time (DT) of ZIKV RNA in the pre-IgM WP of 5.35 hours from macaque infection data, and differential sensitivities of ID vs MP NAT (MP6 for Roche; MP16 for Grifols).
- Estimate for mean duration from initial NAT-detectability to IgM-positivity is 7.45 days (95% CI 6.66 - 8.28 days), while mean NAT-detectability duration including pre-IgM and post-IgM stages is 11.73 days (95% CI 10 - 14.46 days).
- The ~7.5 day (180 hour) pre-IgM ramp-up stage would be reduced by 2.5-4 DTs or 13.4-21.4 hours
 - $2.5 \times 5.35 = 13.4$ hrs for Roche with MPs of 6 (7.4% increased risk)
 - $4 \times 5.35 = 21.4$ hrs for Grifols with MPs of 16 (11.9% increased risk)

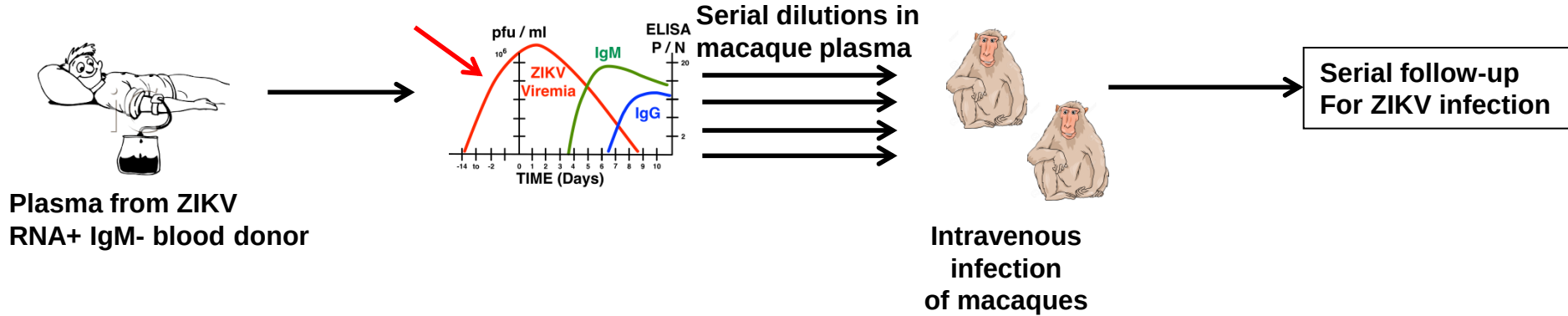
Characterization of blood transfusion-transmission of Zika virus in macaques

- Characterization of transfusion-transmission of ZIKV in macaques (collaboration between REDS-III Central Lab (BSRI) and the UC Davis Primate Center)
- Aims:
 - Dynamics of acute ZIKV TT infection in a macaque model
 - Characterization of minimal infectious dose for ZIKV in pre and post-Ab SC stages of infection
 - Characterization of the effect of pathogen-reduction on transmission— specifically at high viral loads
- FDA and HRSA leveraging this study by extending monitoring of ZIKV infected macaques to investigate distributions/persistence in tissues and organs of interest

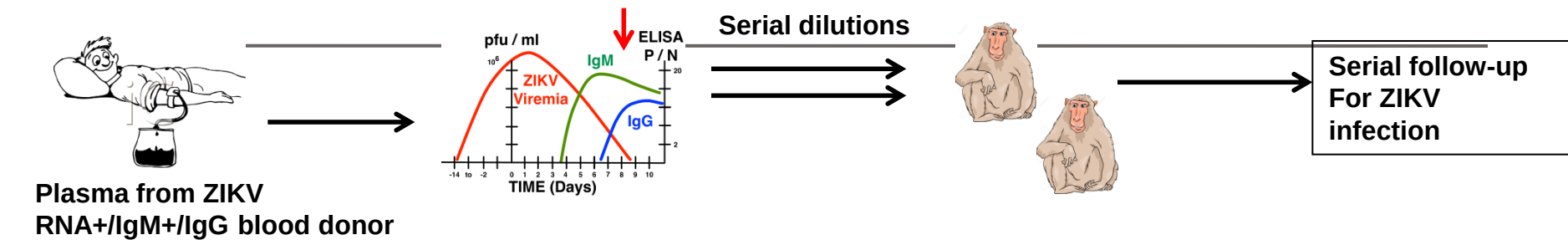
Acute ZIKV Infection Dynamics in Blood in Macaques



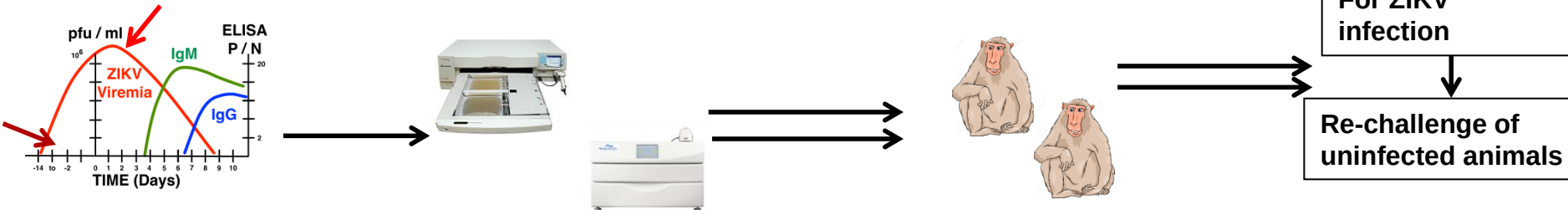
Aim 2A. Minimal infectious dose in ramp-up phase



Aim 2B Minimal infectious dose in the presence of ZIKV antibodies



Aim 3. Analysis of efficacy pathogen reduction technologies



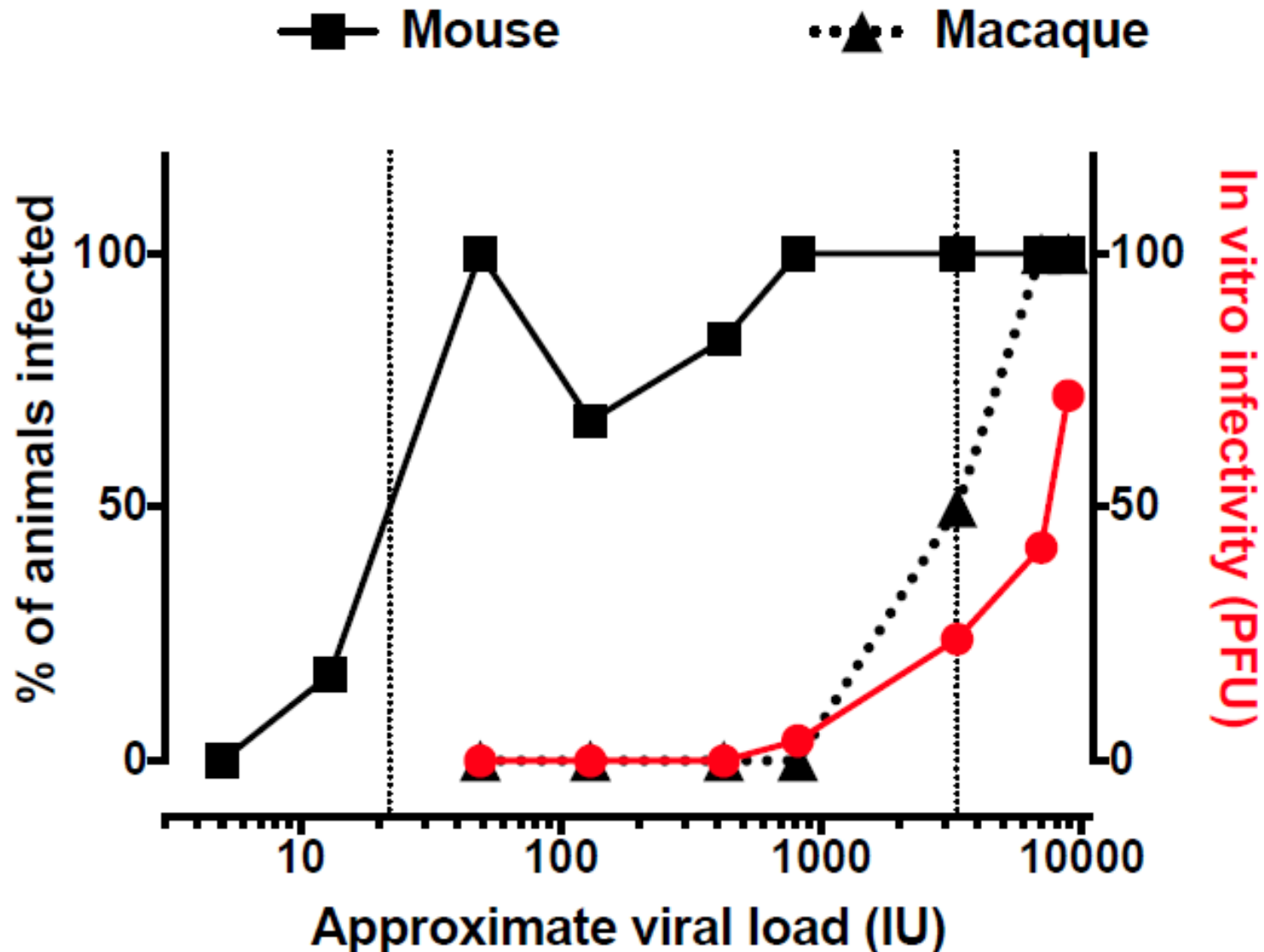
Infectivity in macaques

Dilution in macaque plasma	Viral load (IU/ml)	In vitro infectivity (PFU/ml)	Mouse infectivity adjusted to be equivalent of 1ml	Macaque infectivity using 1 ml
A	228500	648	Not Tested	Not Tested
B	68500	396	Not Tested	Not Tested
C	27900	270	Not Tested	Not Tested
D	8900	72	6/6 infected	1/1 infected
E	~7000	~65	6/6 infected	1/1 infected
F	3325	24	6/6 infected	1/2 infected
G	813	4	12/12 infected	0/2 infected
H	423	Below LOD	10/12 infected	0/2 infected
I	130	Below LOD	4/6 infected	0/1 infected
J	49	Below LOD (~0.2)	6/6 infected	0/1 infected
K	ND (~13)	Below LOD (~0.04)	1/6 infected	ND
L	ND (~5)	Below LOD	0/6 infected	ND

3325-8900 RNA copies/24-72 PFU infectious in macaques

Equates to 17–45 RNA copies/ml in 200 ml plasma

Summary of ZIKV Infectivity in Vero cells, immunodeficient mice and macaques



Conclusions

Use of MP-NAT with triggering to ID-NAT during autochthonous ZIKV outbreaks is appropriate because:

- Window-period infections are detectable only by ID-NAT for a very short period
- Blood is likely not infectious at very low VLs and once ZIKV neutralizing antibodies are detectable
- Infectious antibody-negative period will have generally elapsed before those with travel-associated infections give blood
- It is reasonable to anticipate very few local transmissions in the CONUS
- In Puerto Rico, although ~80% of population still ZIKV susceptible, MP-NAT is sufficient to detect incipient outbreaks followed by conversion to ID-NAT

The next ZIKV: MAYARO? Oropouche?



Alphaviruses: Serological Evidence of Human Infection in Paraguay (2012–2013)

Fátima Cardozo,^{1,*} Brenda Konigheim,^{2,*} Guillermo Albrieu-Llinás,² María Elisa Rivarola,²
Javier Aguilar,² Alejandra Rojas,¹ Agustín Ignacio Quaglia,² Malvina Paez,¹ Yvalena Guillén,¹
Adrián Díaz,² María Asunción Vallejos,³ Lilian Herebia,³ María Lidia Recalde,⁴
Marta Silvia Contigiani,² and Laura Mendoza¹

Abstract

Introduction: Alphaviruses can produce febrile illness and encephalitis in dead-end hosts such as horses and humans. Within this genus, the Venezuelan Equine Encephalitis virus (VEEV) complex includes pathogenic epizootic subtypes and enzootic subtypes that are not pathogenic in horses (except subtype IE, Mexican strains), although they can cause febrile symptoms in humans. The Rio Negro virus (RNV—VEEV subtype VI) circulates in Argentina, where it was associated with undifferentiated febrile illness. Mayaro (MAYV) and Una (UNAV) viruses belong to a different group, the Semliki Forest virus complex, with confirmed circulation.

Objective: The present study aimed to determine RNV, MAYV, and UNAV seroprevalences by plaque reduction neutralization test in 652 samples of Paraguayan individuals mainly from the Central Department, between years 2012 and 2013.

Methods: Samples with antibodies titer >1:20 against RNV were also tested for Mosso das Pedras—subtype IF, subtype IAB, and Pixuna (PIXV)—subtype IV viruses that belongs to VEEV antigenic complex.

Results: The overall seroprevalence of RNV was 3.83%, and for UNAV it was 0.46%, and no neutralizing antibodies were detected against MAYV in the studied population. Two of the twenty-seven heterotypic samples were positive for PIXV. The 50.1% of neutralizing antibody titers against RNV were high (equal to or greater than 1/640), suggesting recent infections. The effect of age on the prevalence of RNV was negligible.

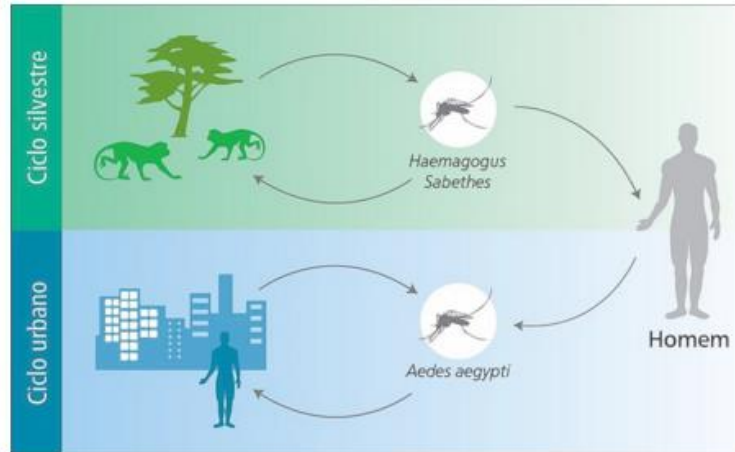
Conclusions: These results bring new information about neglected alphaviruses in South America, and these data will serve as the basis for future studies of seroprevalence of other VEEV, and studies to search potential hosts and vectors of these viruses in the region.

Keywords: alphaviruses, Paraguayan human population, serological evidence, Una virus, Venezuelan Equine Encephalitis virus.

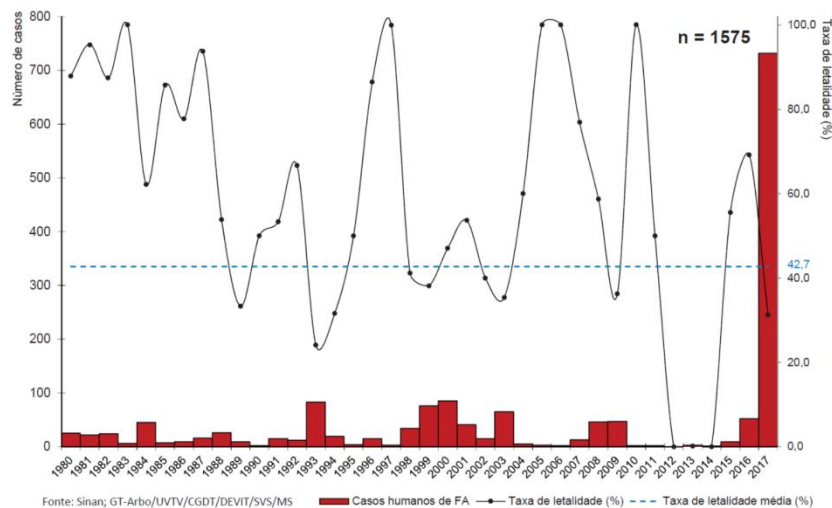
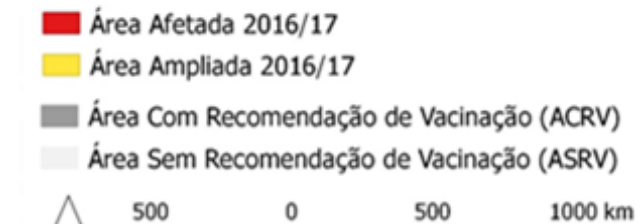
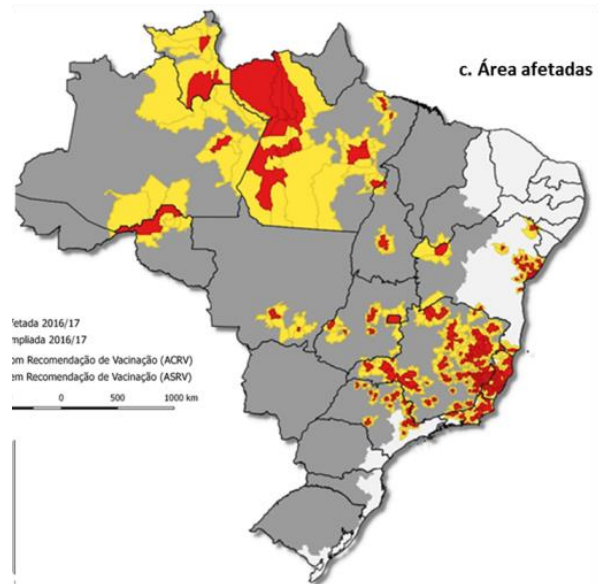


Figure A1. Distribution of Mayaro virus (MAYV) in the Central and South America (3, 5–7).

Risk associated with the current YFV outbreak in Brazil and risk of propagation like ZIKV?



2017/2018 – Maior surto de febre amarela silvestre nas últimas cinco décadas
2017 = 779 casos confirmados



Argentina, France report imported yellow fever cases from Brazil

by NEWS DESK

🕒 February 26, 2018

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Since July 2017, [Brazilian health authorities have reported](#) 545 confirmed yellow fever cases and 164 deaths.



Image/CIA

In January, [the Netherlands reported](#) on a imported yellow fever case in the country in a traveler to Brazil. the patient who has since recovered was a 46-year-old unvaccinated male patient, who traveled to and stayed in Maripora, São Paulo State, Brazil since mid December, was confirmed positive for yellow fever upon return to the Netherlands on Jan. 8.

Now both Argentina and France are reporting imported yellow fever cases in travelers to Brazil in recent weeks.

Two women from Buenos Aires, Argentina were confirmed positive for yellow fever, [according to a G1 Globo report \(computer translated\)](#). The two contracted the disease in Brazil, in the city of Ilha Grande, in Rio de Janeiro.

In addition, The French Ministry of Health confirmed a yellow fever case in a unvaccinated woman who traveled through Minas Gerais recently, [according to an em.com report \(computer translated\)](#).

French *Aedes albopictus* are able to transmit yellow fever virus

([Euro Surveill.](#) 2016 Sep 29; 21(39): 30361. PMID: PMC5069433)

Acknowledgements

- **Blood Systems Research Institute**

- Mars Ston
- Marion Lanteri
- Graham Simmons
- Sonia Bakkour
- Roberta Bruhn
- Zhanna Kaidarova
- VRLRC/Stone Lab

- **REDS-III Data Coordinating Center, RTI**

- Don Brambilla
- Marian Sullivan
- Chris McClure

- **NHLBI**

- Simone Glynn

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- Susan A. Galel
- Lisa Lee Pate
- Tony Hardiman

- **Hologic/Grifols**

- Jeff Linnen
- Kui Gao

- **CTS**

- Phillip Williamson

- **OneBlood**

- Rita Reik

- **Banco de Sangre, PR**

- Jose Orlando Alsina

- **UC Davis**

- Koen Van Rompay
- Lark Coffey

- **REDS-III ZIKV Oversight Committee**

- Jay Epstein, FDA
- Hira Nakhasi, FDA
- Matt Kuehnert, CDC
- Lyle Petersen, CDC
- Brad Biggerstaff, CDC

Funding: NHLBI REDS-III, CDC, BARDA