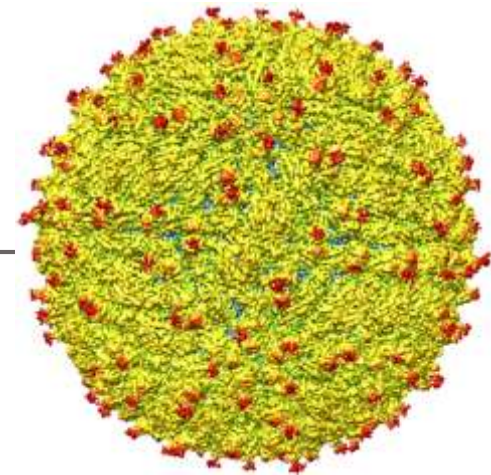
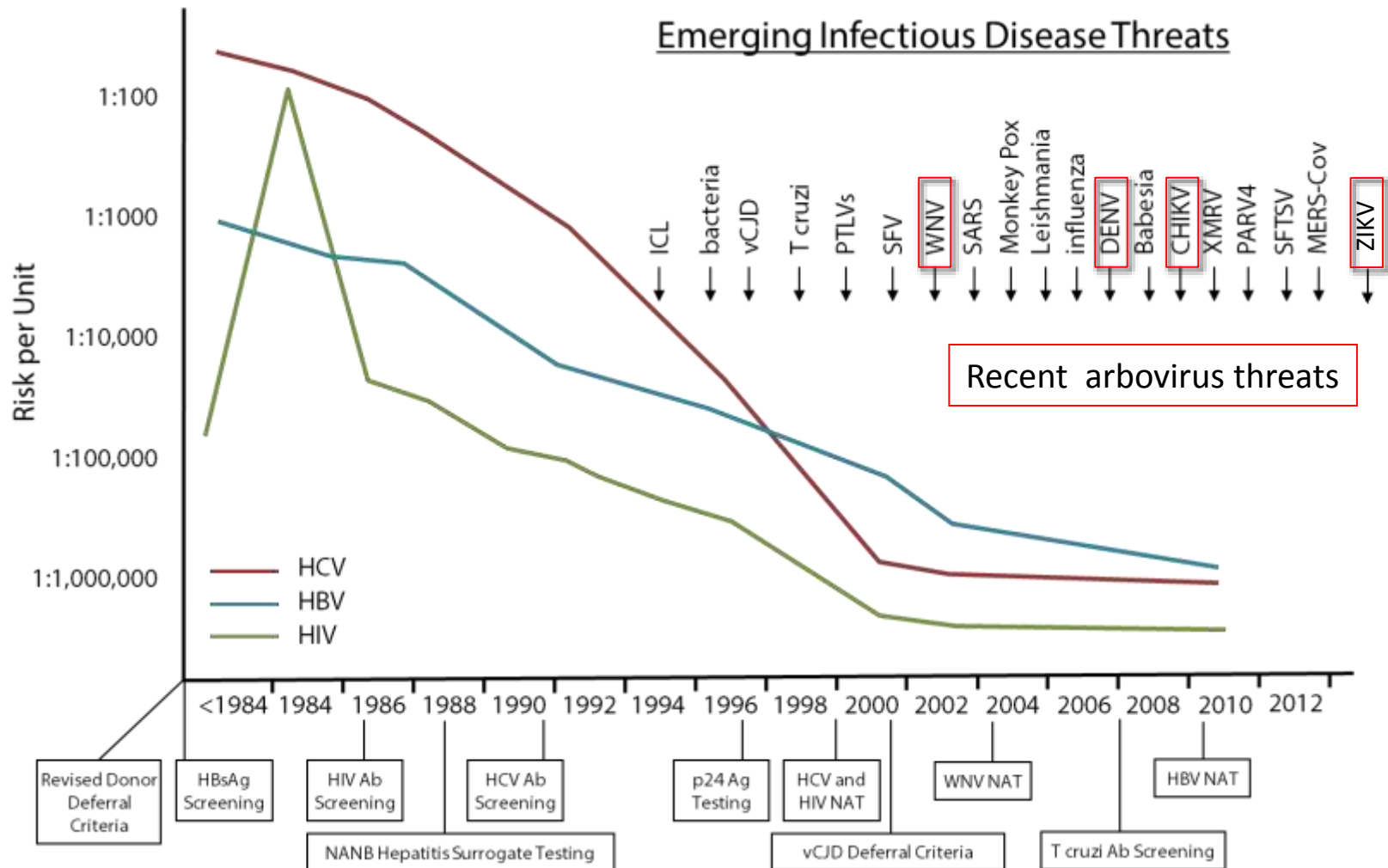


ZIKA VIRUS AND SAFETY OF THE BLOOD SUPPLY

Michael P Busch, MD, PhD
Blood Systems Research Institute
University of California, San Francisco



Risks of major TTVs linked to interventions, and accelerating rate of EIDs of concern to blood safety



“I cannot forecast to you the action of Russia. It is a riddle, wrapped in a mystery, inside an enigma; but perhaps there is a key!”

Winston Churchill



“I cannot forecast to you the action of Arboviruses. They are a riddle, wrapped in a mystery, inside an enigma; but research is the key!”

Lyle Petersen



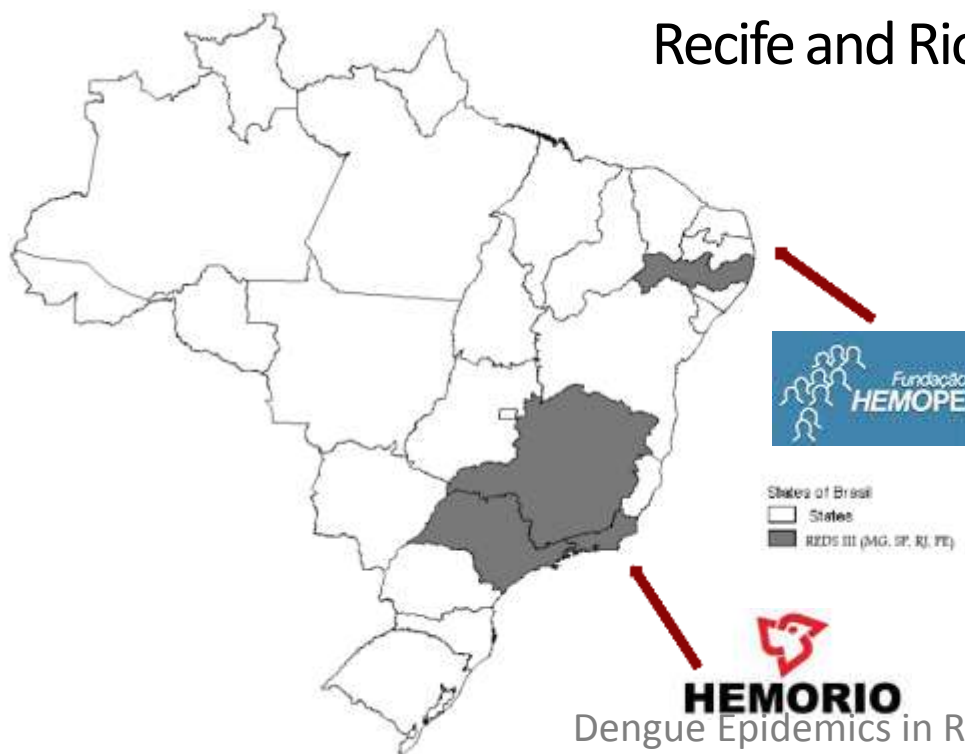
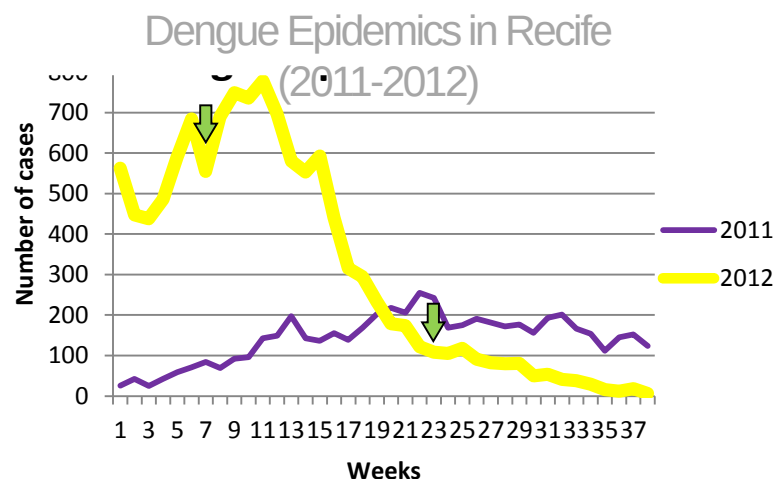
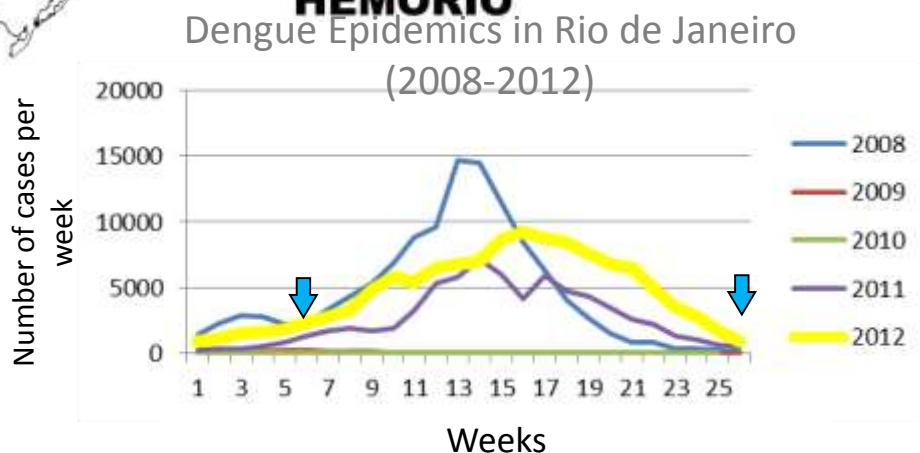
Evaluating an EID threat to blood safety

3 basic questions need to be answered:

- Is it in the blood supply?
 - Necessitates a way to measure the agent in donors during epidemics
 - Estimation of donor risks: prevalence, incidence, durations of detection
 - Estimation of blood component risks
 - Temperature, preparation, storage duration effects on infectivity?
 - Is antibody in the infected donor or co-transfused components protective?
- Is it transfusion-transmitted and what is the risk?
 - Is transmission risk dependent on stage of infection or VL in the donor/component
 - Do recipient antibodies from prior infection protect from TT
- If transmissible by transfusion, does it have a clinical impact in transfused recipients?
 - Is TT disease more or less severe than usual routes of infection

REDS-III Dengue Study Sites and Epidemic Activity

Recife and Rio de Janeiro



Sabino et al, JID 2016

Busch et al, JID 2016

Subject accrual & results of DENV RNA testing

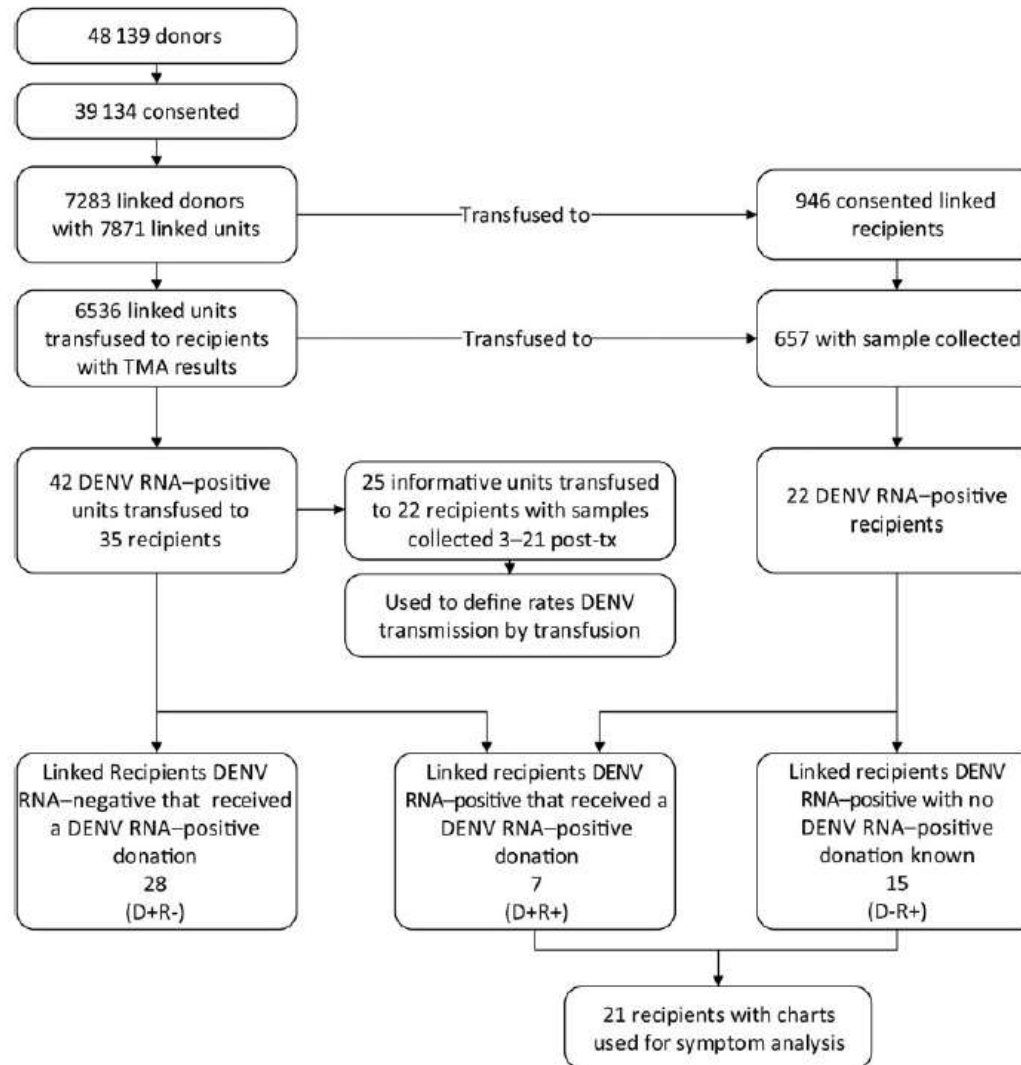
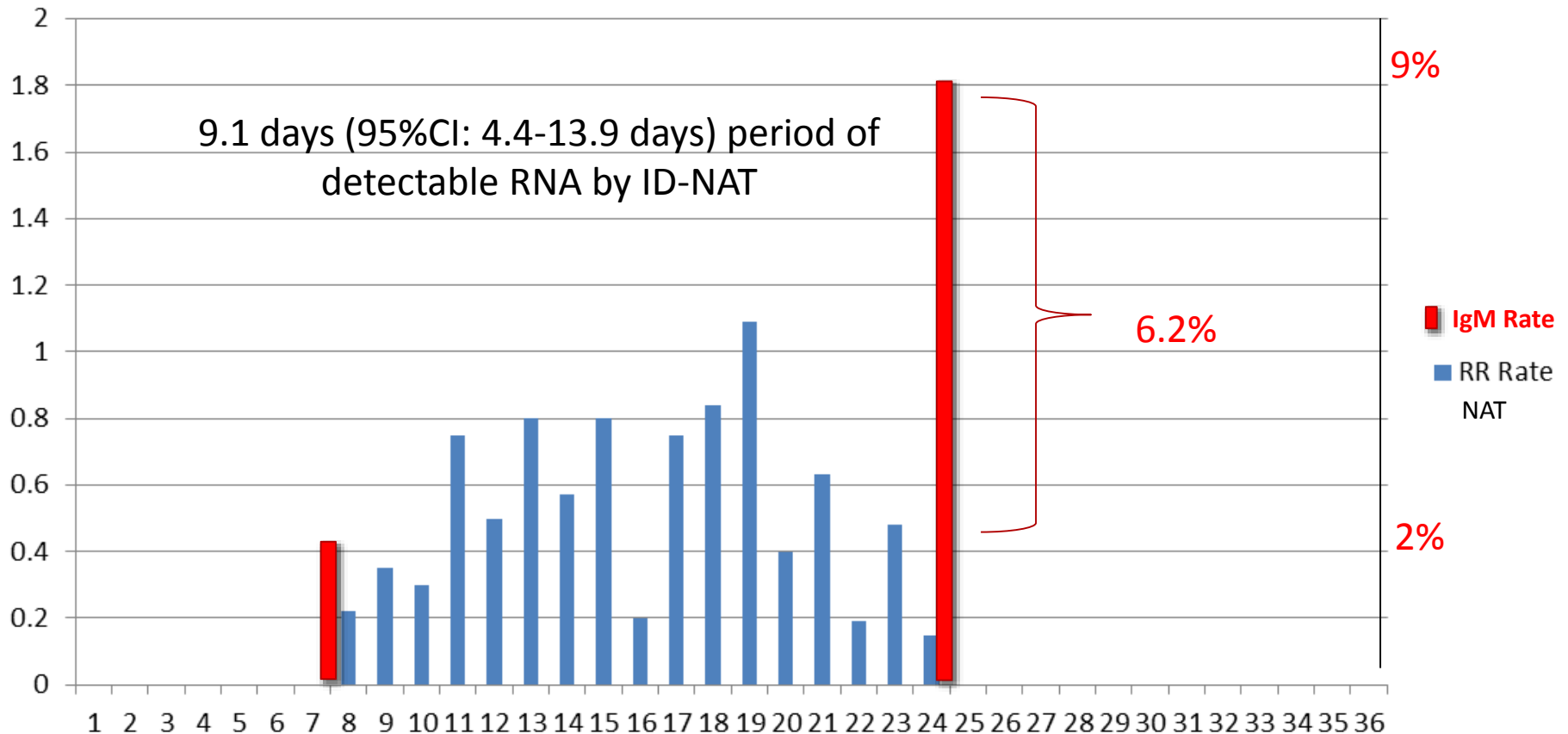


Figure 1. Flow chart defining blood donor and recipient dengue virus (DENV) RNA testing results and selection of subjects for analysis of clinical manifestations. Abbreviations: –, negative; +, positive; D, donor; R, recipient; TMA, transcription-mediated amplification assay.

REDS-III Brazil Dengue Study Rio Donor TMA RR Rates By Week



1 case of clinical dengue diagnosed for every 3 infections
853 cases of reported clinical disease per NAT yield donation

DENV symptoms for case and control patients during the 45-day chart review period

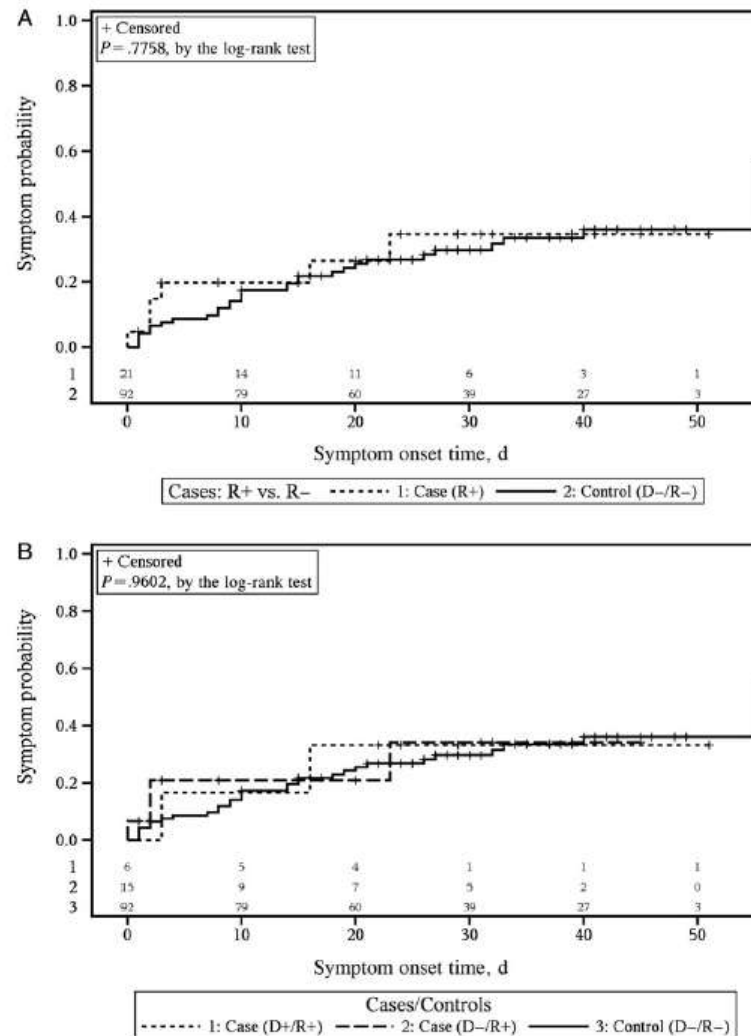
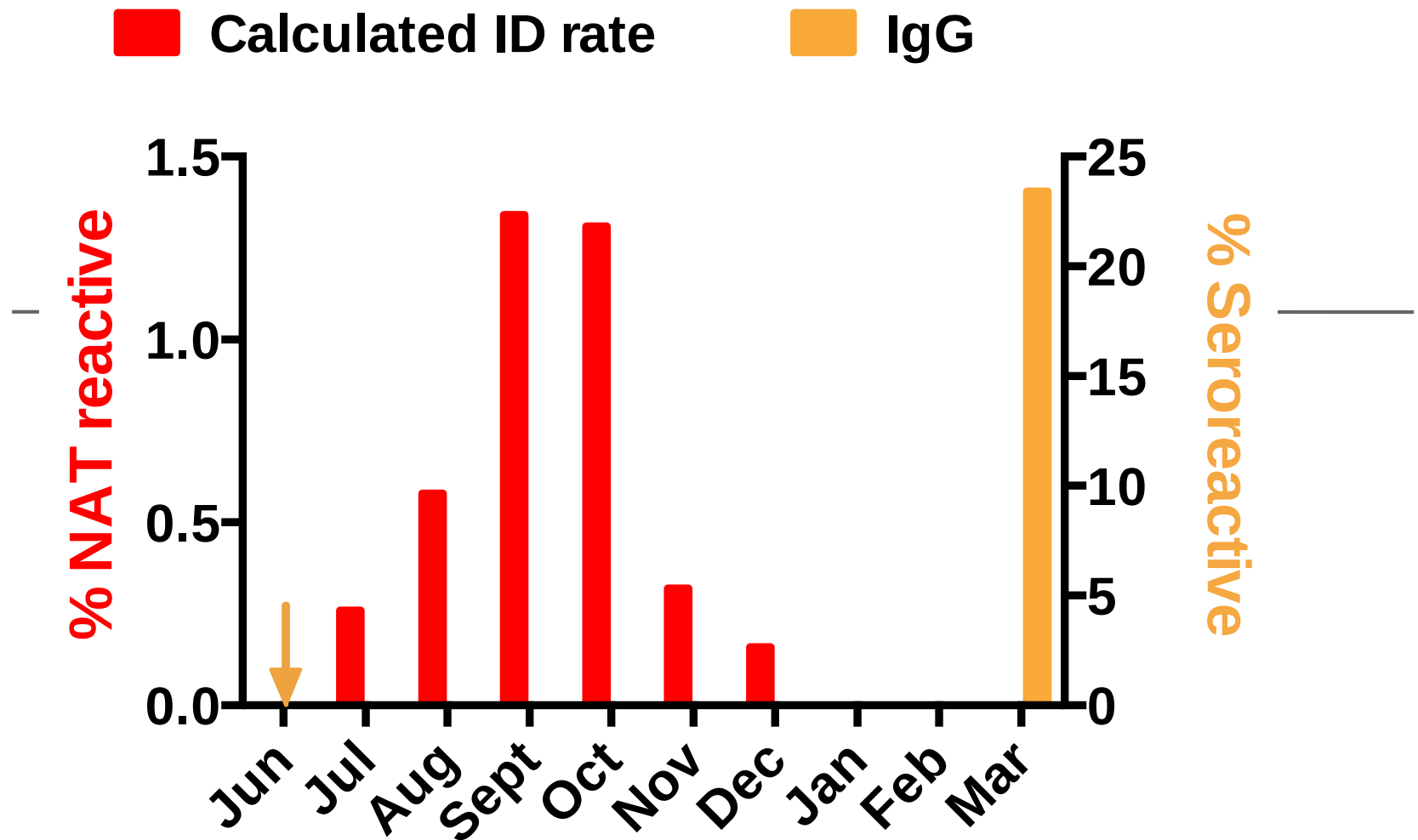
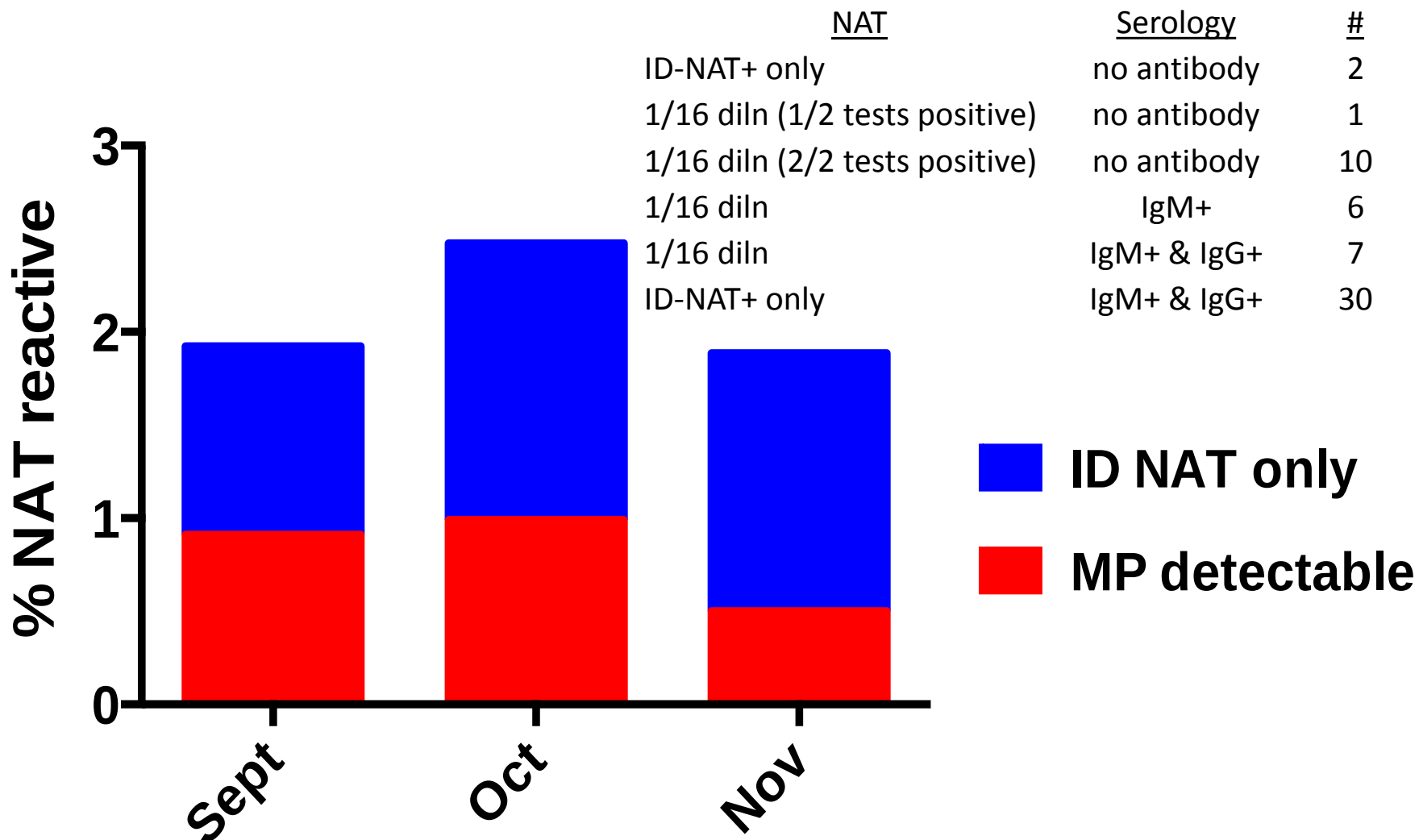


Figure 2. Time to occurrence of symptoms consistent with dengue fever. Symptoms status for case and control patients during the 45-day chart review

High Incidence of Chikungunya Virus and Frequency of Viremic Blood Donations during Epidemic, Puerto Rico, 2014



Detection of CHIKV RNA+ donations by Hologic CHIKV/DENV assay applied to 3008 individual donations

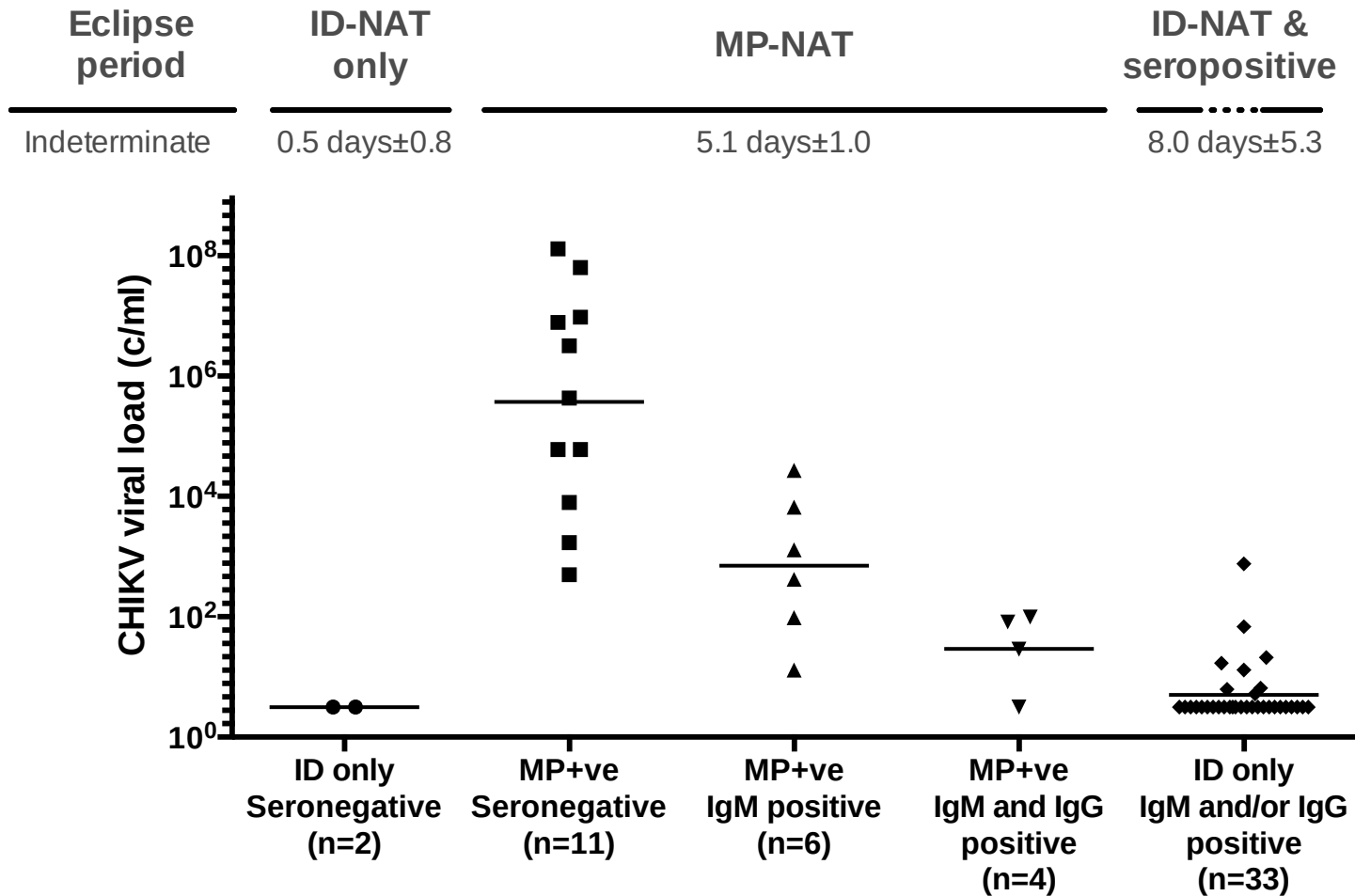


<u>NAT</u>	<u>Serology</u>	<u>#</u>
ID-NAT+ only	no antibody	2
1/16 diln (1/2 tests positive)	no antibody	1
1/16 diln (2/2 tests positive)	no antibody	10
1/16 diln	IgM+	6
1/16 diln	IgM+ & IgG+	7
ID-NAT+ only	IgM+ & IgG+	30

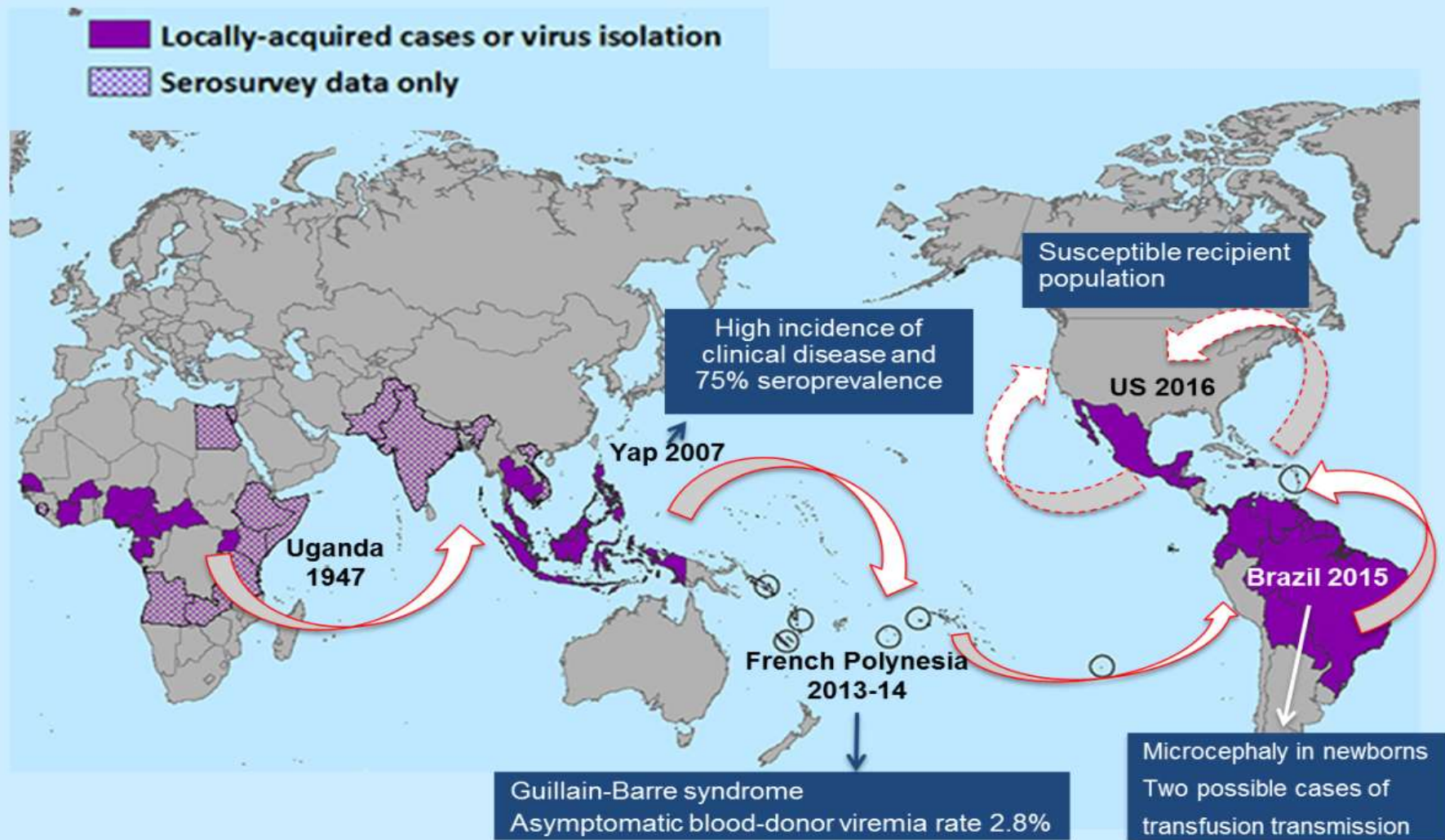
ID NAT only

MP detectable

Dynamics of CHIKV viremia in blood donations



Why is ZIKV an emerging threat to blood safety?



Rate of emergence: ZIKV is rapidly spreading through the Americas (adapted from CDC website)

BSRI Blinded ZIKV Panel Collaborative Study

Study management

- 25 member blinded panel was prepared by Blood Systems Research Institute.
- Panel members were coded and shipped to participants; identities of the panels were not known by the personnel performing the testing.
- Results were sent back to BSRI for decoding and summary

Blinded Panel

- 2 viral isolates (2014 Polynesia culture isolate and 2015 Brazilian donor plasma)
- Serial half log dilution in Gemini BioProducts defibrinated human serum
- Negative controls

Participants

- CDC Fort Collins, CO (Laniotti)
- CDC San Juan Puerto Rico (Munoz)
- Blood Systems Research Institute
- Roche Molecular Systems
- Hologic, Inc.
- FDA (Rios)
- Others (EFS, Tahiti, Brazil, UC Davis)

ZIKV panel for NAT assay comparison

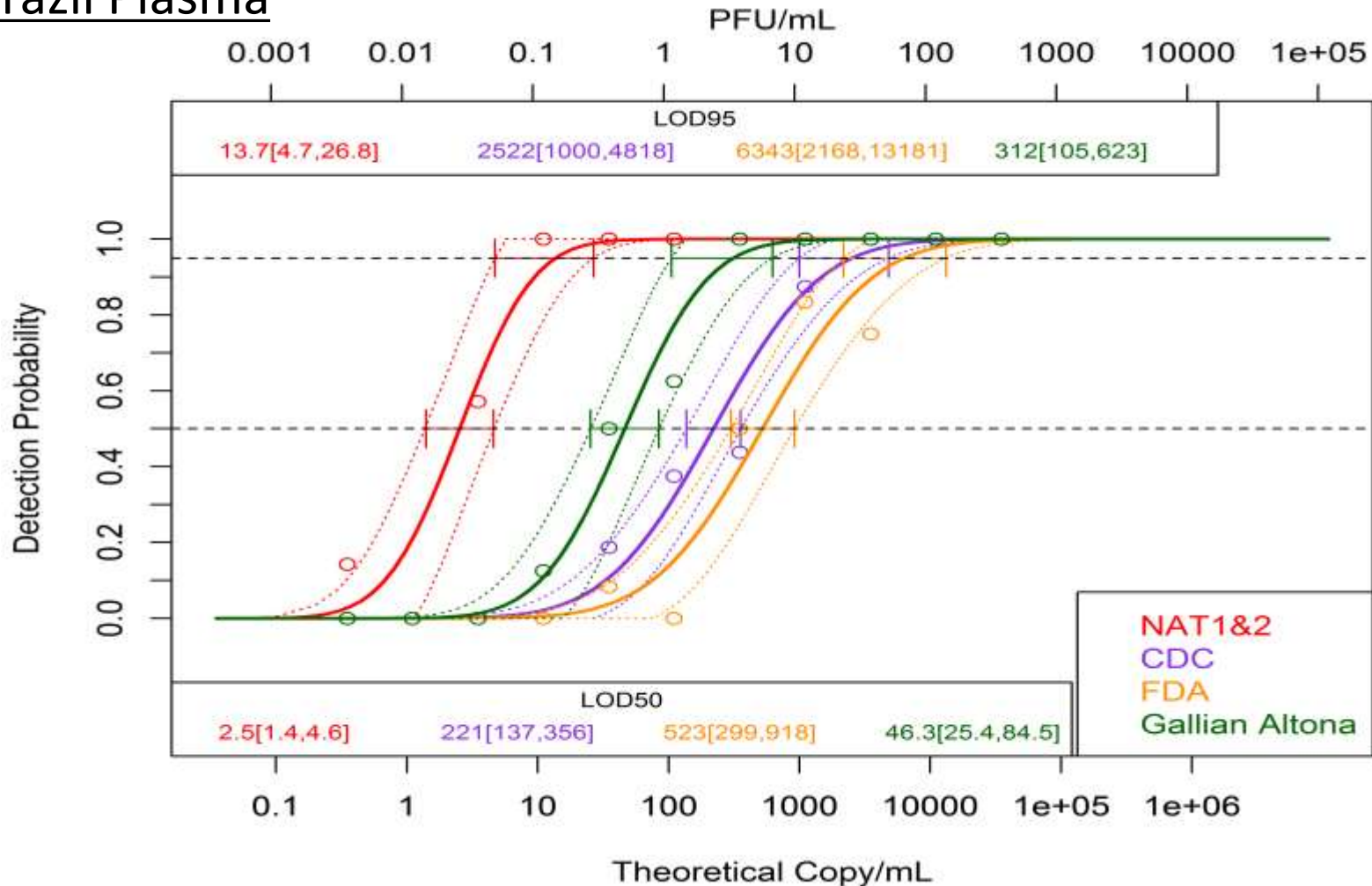
	cp/ml	PFU/ml
PLASMA	3.5E+04	3.81E+02
	1.1E+04	1.21E+02
	3.5E+03	3.81E+01
	1.1E+03	1.21E+01
	3.5E+02	3.81E+00
	1.1E+02	1.21E+00
	3.5E+01	3.81E-01
	1.1E+01	1.21E-01
	3.5E+00	3.81E-02
	1.1E+00	1.21E-02
	3.5E-01	3.81E-03

	cp/ml	PFU/ml
SUPERNATANT	4.4E+04	9.87E+00
	1.4E+04	3.12E+00
	4.4E+03	9.87E-01
	1.4E+03	3.12E-01
	4.4E+02	9.87E-02
	1.4E+02	3.12E-02
	4.4E+01	9.87E-03
	1.4E+01	3.12E-03
	4.4E+00	9.87E-04
	1.4E+00	3.12E-04
	4.4E-01	9.87E-05

NEGATIVE	neg	neg
	neg	neg
	neg	neg

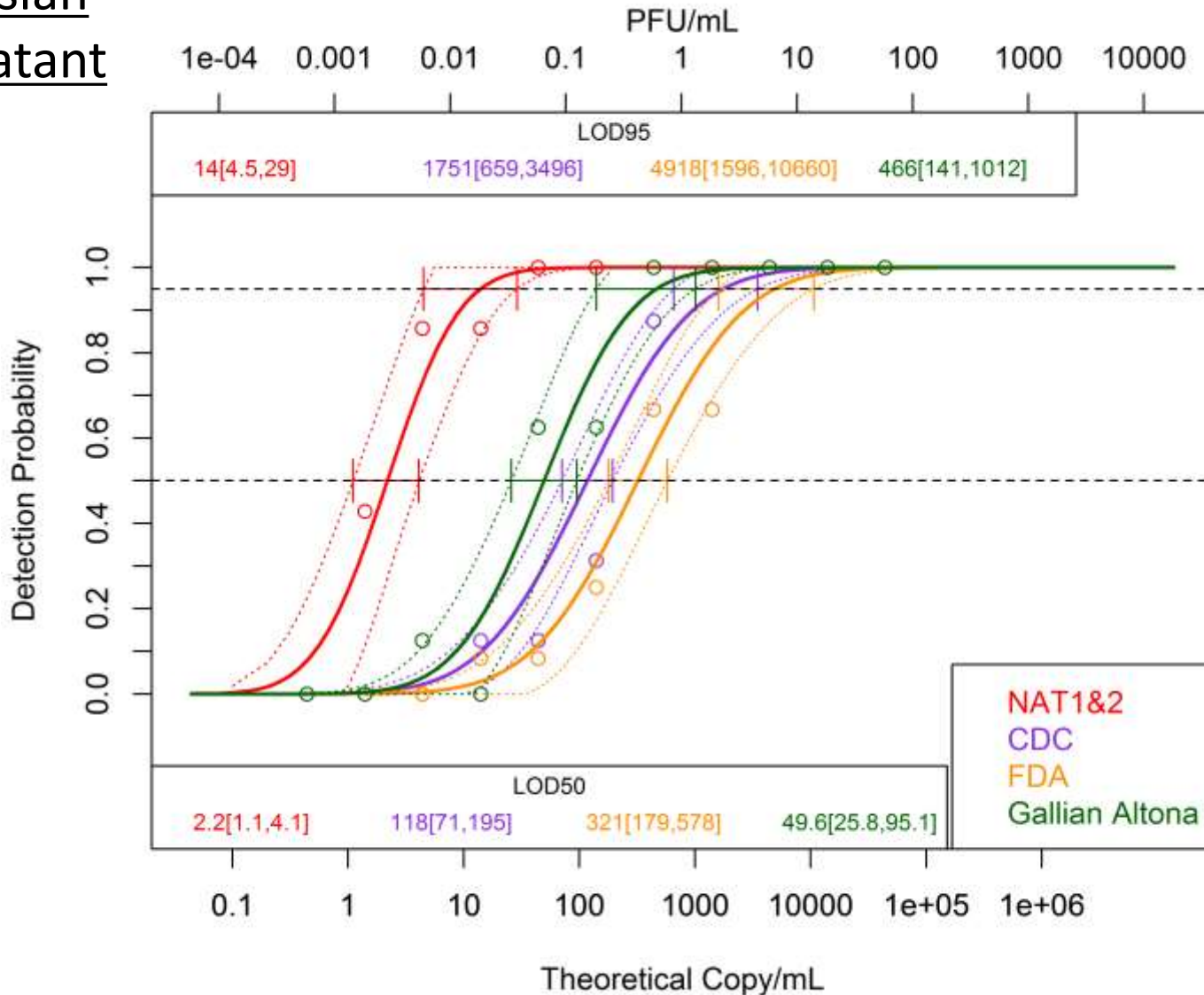
Sensitivity of Donor Screening NAT Assays vs. CDC, FDA and other PCR Assays

Brazil Plasma

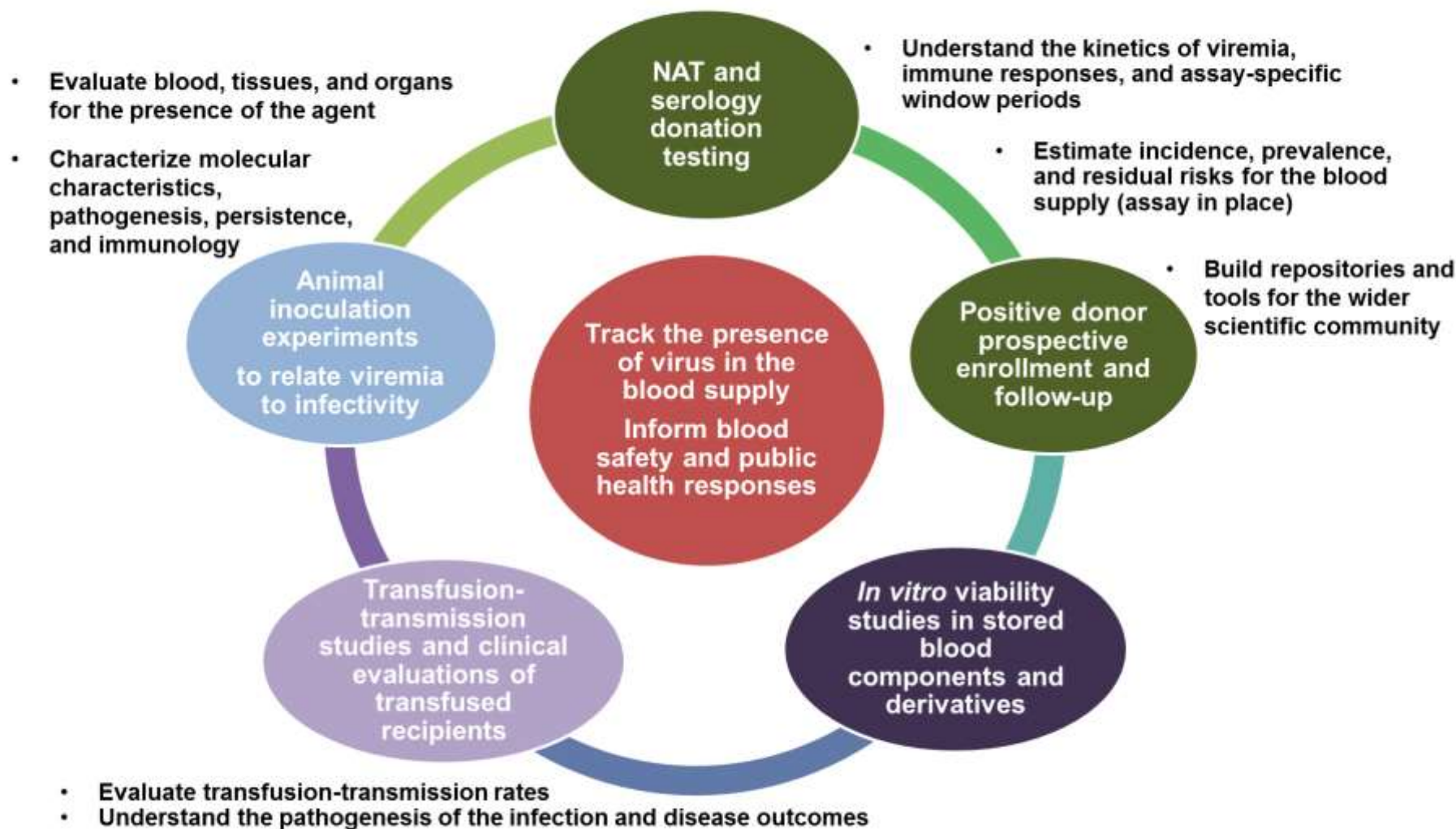


Sensitivity of Donor Screening NAT Assays vs. CDC, FDA and other PCR Assays

Polynesian
Supernatant



Assessing the risk of transfusion-transmission for newly discovered pathogens



NHLBI strategies to support blood safety research on ZIKV

- Leverage the existing Recipient Epidemiology and Donor Evaluation Study-III (REDS-III) blood safety research program
 - US follow-up study of Zika RNA positive blood donors
 - Zika, Chikungunya (CHIKV), and dengue virus (DENV) incidence in Brazilian blood donors
 - Impact of ZIKV Acquisition through Blood Transfusion in Brazil
 - Impact of ZIKV acquisition in Brazilian chronically-transfused patients with Sickle Cell Disease
 - Characterization of blood transfusion-transmission of Zika virus in macaques - under consideration
- Establish a REDS-III ZIKV Oversight Committee that helps develop and monitor these protocols

US follow-up study of Zika RNA positive blood donors

- **Study Design:** Natural history cohort of ZIKV NAT-positive blood donors followed prospectively for 6 months (index + 5 follow-up visits)
- **When:** To be launched in June, 2016
- **Where:** Puerto Rico, South Florida, South Texas
- **Sample size:** 130 ZIKV+ donors (80 DENV Ab+; 50 DENV Ab-)
- **Aims:**
 - Characterize evolution of viral and serological markers to evaluate window periods and assay performance
 - Further study stored blood components to characterize the performance of existing and future assays and provide standards for assay development
 - Evaluate the viral and immune mechanisms leading to viral clearance or clinical pathogenesis over 6 months
 - Evaluate clinical outcomes post donation
 - Establish a sharable biorepository

Follow-up study of Zika RNA positive blood donors

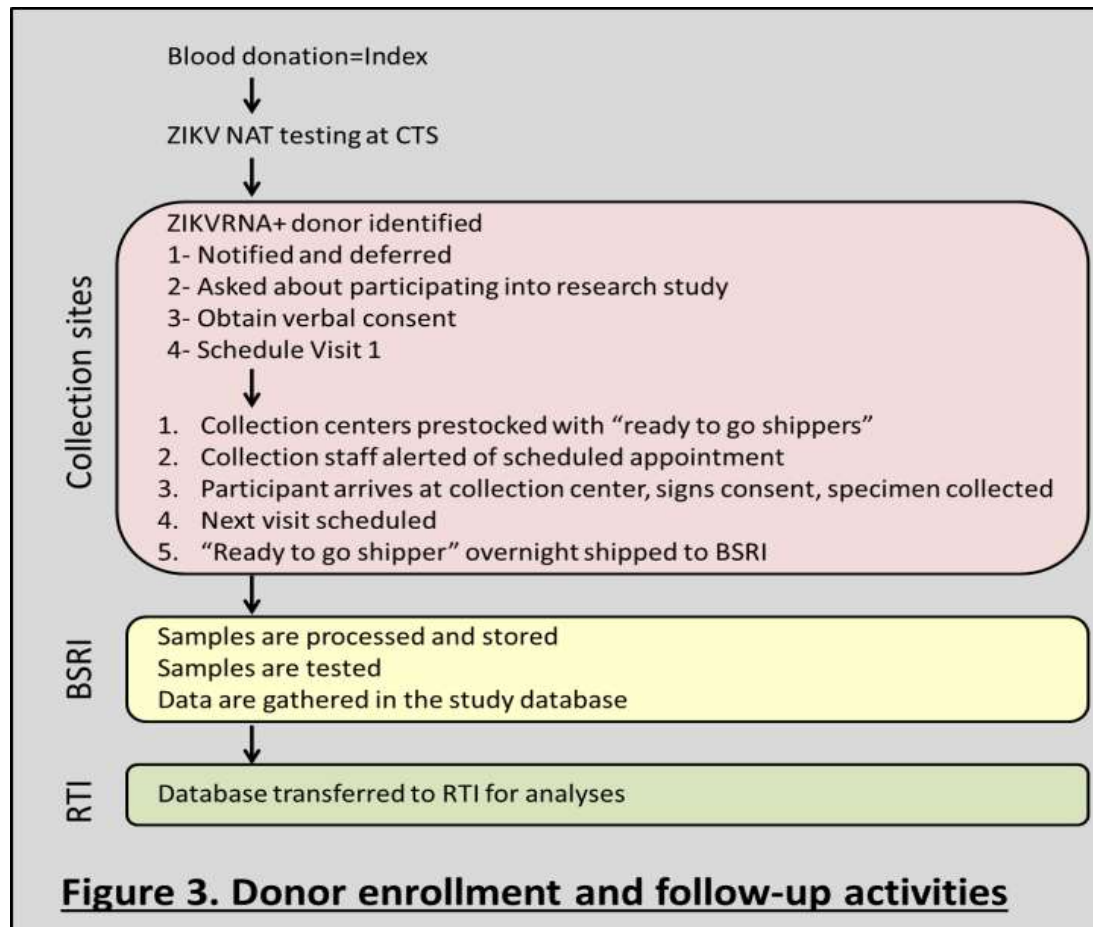
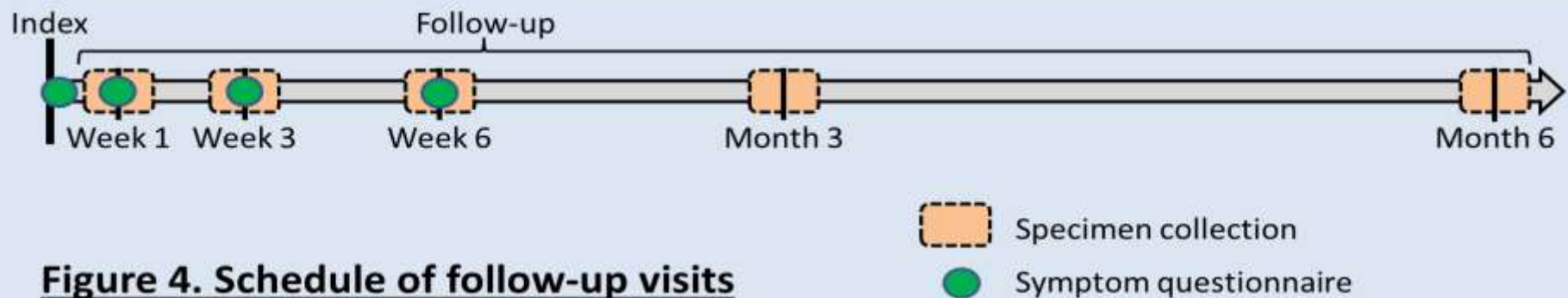


Figure 4. Schedule of follow-up visits

Sample collection and processing

Time-point		Index	week 1	week 3	week 6	month 3	month 6	Total per donor	Total for the study
Days post-index (range)		0	(3-10)	(15-28)	(35-48)	(80-100)	(160-200)		
Samples collected (Collection site activity)	Blood in EDTA tubes (10 mL)	0	7	7	7	7	7	25	2,500
	Blood in Tempus tubes (2.5 mL)	0	2	2	2	2	2		
	Urine (15 mL)	0	1	1	1	1	1		
	Saliva (3 mL)	0	1	1	1	1	1		
	Semen (3.5 mL)	0	1	1	1	1	1		
Samples derived (Processing performed by BSRI)	Plasma (1 mL)	4 (?)	7	7	7	7	7	185	18,500
	Plasma (5 mL)	0	4	4	4	4	4		
	packed blood cells (1 mL)	1 (?)	3	3	3	3	3		
	PBMC (10 ⁷ cells/vial)	0	6	6	6	6	6		
	Tempus tubes	0	2	2	2	2	2		
	Urine (1 mL)	0	5	5	5	5	5		
	Urine (5 mL)	0	2	2	2	2	2		
	Saliva (1 mL)	0	3	3	3	3	3		
	Semen (0.5 mL)	0	1	1	1	1	1		
	Semen (1 mL)	0	3	3	3	3	3		

Sample characterization

Time-point	Type of assay	Assay	Sample type	Input volume (uL)	Infectious agent	Testing lab.
Index	NAT/VL	Cobas ZIKV test	plasma	850	ZIKV	ROCHE IND
		Enhanced CDC ZIKV		500	ZIKV	BSRI IND
		Regular CDC Triplex		100	ZIKV, DENV, CHIKV	BSRI
Follow-up	NAT/VL	Enhanced CDC Triplex	plasma	500	ZIKV, DENV, CHIKV	BSRI
		Regular CDC Triplex	Whole blood	100	ZIKV, DENV, CHIKV	BSRI
		Enhanced CDC ZIKV	Urine	420	ZIKV	BSRI
		Enhanced CDC ZIKV	Saliva	420	ZIKV	BSRI
		Enhanced CDC ZIKV	Semen	200	ZIKV	BSRI
Follow-up	NAT/VL	Arboplex TMA	plasma	750	ZIKV, DENV, CHIKV	HOLOGIC
		Arboplex TMA	Whole blood	750	ZIKV, DENV, CHIKV	HOLOGIC
		ZIKV TMA	Urine	750	ZIKV	HOLOGIC
		ZIKV TMA	Saliva	750	ZIKV	HOLOGIC
		ZIKV TMA	Semen	750	ZIKV	HOLOGIC
Follow-up	NAT/VL	Cobas ZIKV test	plasma	850	ZIKV, DENV, CHIKV	ROCHE
		Cobas ZIKV test	Urine	850	ZIKV	ROCHE
		Cobas ZIKV test	Saliva	850	ZIKV	ROCHE
		Cobas ZIKV test	Semen	850	ZIKV	ROCHE
Follow-up	Serology	IgM	plasma	100	ZIKV	BSRI
		IgG		100	ZIKV	BSRI
		PRNT		100	ZIKV	BSRI

ZIKV, CHIKV, and DENV Surveillance in Brazilian blood donors

Objectives:

- 1) Determine ZIKV, CHIKV and DENV viremic rates through the detection by a research assay of these viruses in minipools (MP) containing 6 donor samples (MP6)
- 2) Use the study results to show that donor testing data can be used as a tool for public health surveillance and to better understand the dynamics of these infections in the Brazilian population
- 3) Transition to real time NAT screening as soon as a donor NAT screening test is implemented in hemocenters in Brazil



4 REDS-III participating Hemocenters
Study period: April 2016 – June 2017
Sample size:

Hemope in Recife, Pernambuco
Hemominas in Belo Horizonte

Hemorio in Rio de Janeiro
Fundacao Pro-Sangue in Sao Paulo

ZIKV CHIKV DENV Surveillance

- Acquire 67 MP6s with sufficient residual volume (at least 0.35 mL) per blood center per week. The 67 MP6 will represent a convenience sample of 402 donations from the first 4 days of each work week (Monday-Thursday) consisting of 16 – 17 MPs per day.
- The MP6 plasma will be transferred to TMA testing tubes by REDS-III study staff at each blood center, frozen, and batch shipped to Dr. Sabino's lab in SP, and subsequently to BSRI/Hologic for ZIKV/CHIKV/DENV TMA testing on a real-time Panther.
- We will generate ZIKV/CHIKV/DENV on 1,072 MP6 per month for the 4 REDS-III blood centers combined (representing 6,432 donations per month) and a total of 16,080 MP6 (96,480 donation samples) for the 15 months of surveillance.

Impact of ZIKV/CHIKV/DENV Acquisition through Blood Transfusion in Brazil

- **2 different recipient populations**

- Patients at the largest hospital in Latin America, Hospital das Clinicas Sao Paulo (vanguard launch April 2016)
- Patients with SCD who are chronically transfused (to be developed, launch January 2017)

- **Objectives of the Hospital das Clinicas Sao Paulo Study:**

- Determine CHIKV, ZIKV and DENV infection rates among 3500 transfusion recipients by documenting cases of incident viremia of each infection following blood transfusion
- Compare the prevalence of CHIKV, ZIKV and DENV RNA among donation samples given to CHIKV/ZIKV/DENV RNA+ (cases) and RNA- (control) recipients
- Evaluate the occurrence of symptoms consistent with CHIKV/ZIKV/DENV infection among infected case recipients compared to non-exposed/infected control recipients (regardless of route transmission).



Hemope in Recife, Pernambuco
Hemominas in Belo Horizonte

Hemorio in Rio de Janeiro
Fundacao Pro-Sangue in Sao Paulo

Study Overview

Case-control study of recipient acquisition of CHIKV/ZIKV/DENV viremia and consequent clinical outcomes to understand the impact of these transfusion-transmitted arboviruses in a large, newly exposed population.

Two phases of the study:

- Vanguard phase to be conducted in April-May 2016 (N=600)
- Full implementation phase to be conducted during a four-month period in 2017 (N=2900)

In both phases:

Transfusion recipients:

- Pre- and post-transfusion samples (targeting day 3-7 post-transfusion)
- Recipients will be prospectively assessed for CHIKV/ZIKV/DENV symptoms

Objectives

- ***To identify cases of probable transfusion-transmitted CHIKV, ZIKV and DENV***
- ***To understand penetrance of CHIKV, ZIKV and DENV; among infected recipients linked to RNA+ blood components compared to control recipients***

Patient Enrollment – SCD Population

Chronically transfused followed over the course of multiple red cell transfusion episodes – 6 month enrollment period

Location	Hospital	Number of chronically transfused SCD px
Recife, Pernambuco	Hemope	80
Rio de Janeiro	Hemorio	140
Belo Horizonte, Minas Gerais	Hemominas	110
Montes Claros, Minas Gerais	Hemominas	40
Juiz de Fora, Minas Gerais	Hemominas	30
Sao Paulo	ITACI	15

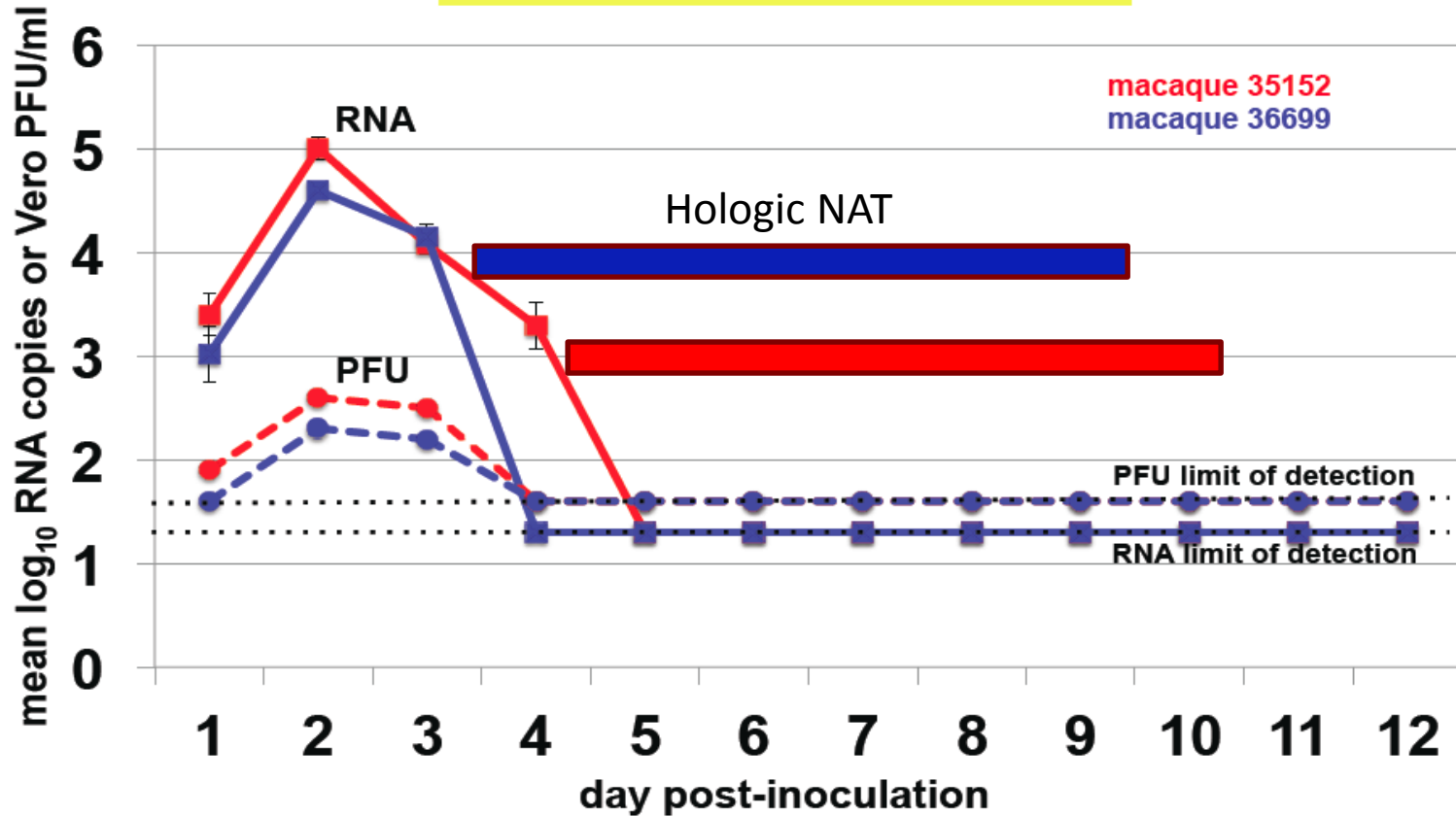
- Targeting to include ~1400 red cell transfusion exposure episodes
- Estimate rates of ZIKV/CHIKV/DENV arbovirus acquisition (RNA-negative pre-transfusion and RNA-positive 5 to 7 days post-transfusion)
- Clinical outcomes assessment in viremic compared to non-viremic SCD patients through symptoms interviews and medical record abstraction.

Characterization of blood transfusion-transmission of Zika virus in macaques - under consideration by NHLBI

- 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
- Proposal submitted to FDA and HRSA to leverage this study by extending monitoring of ZIKV infected macaques to investigate distributions/persistence in tissues and organs of interest

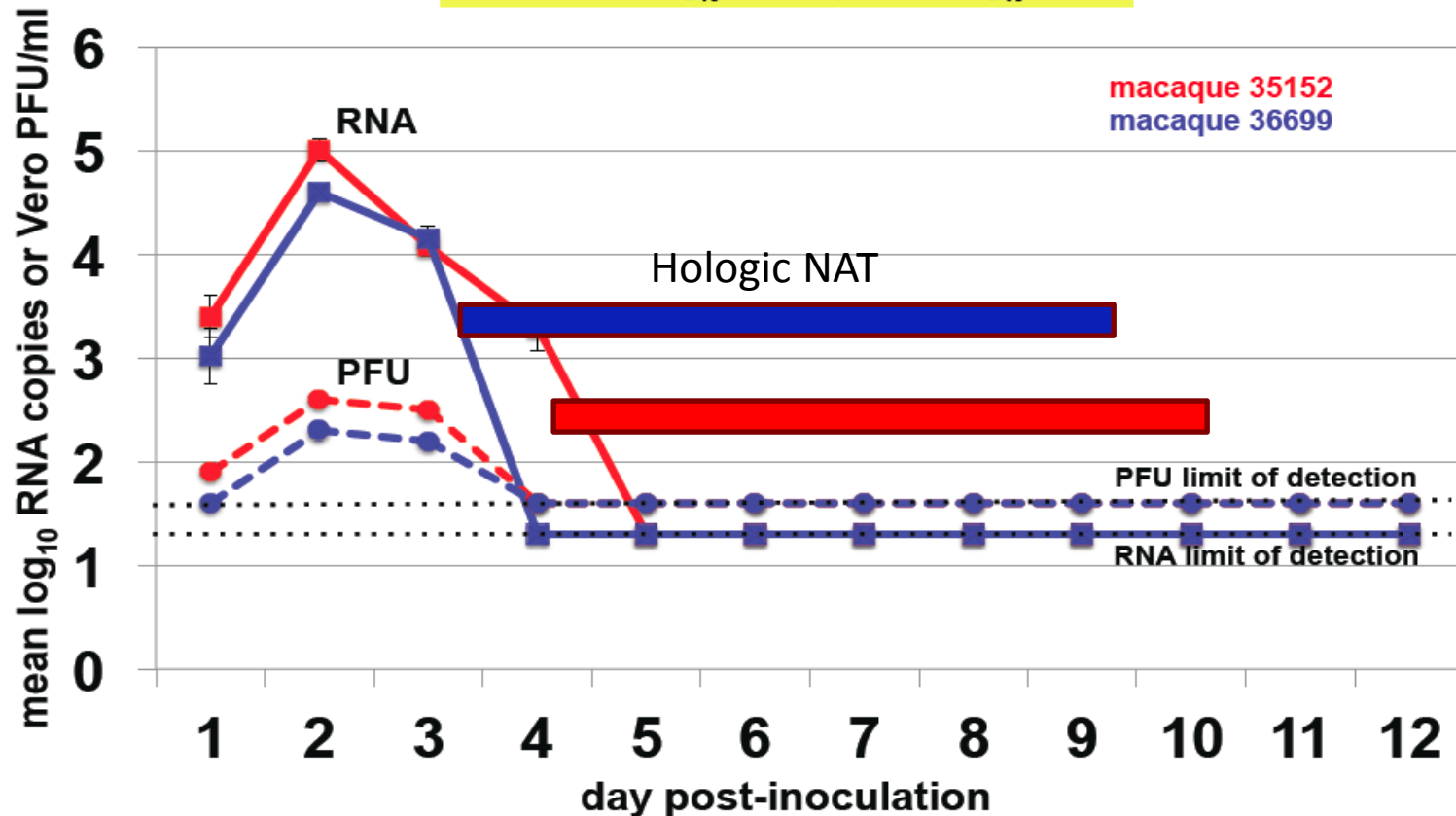
Pilot 1: ZIKV inoculated macaque plasma RNA and PFU Updated 05/24/16

Inoculum: 7.8 log₁₀ RNA copies, 5.0 log₁₀ PFU



Pilot 1: ZIKV inoculated macaque plasma RNA and PFU Updated 05/24/16

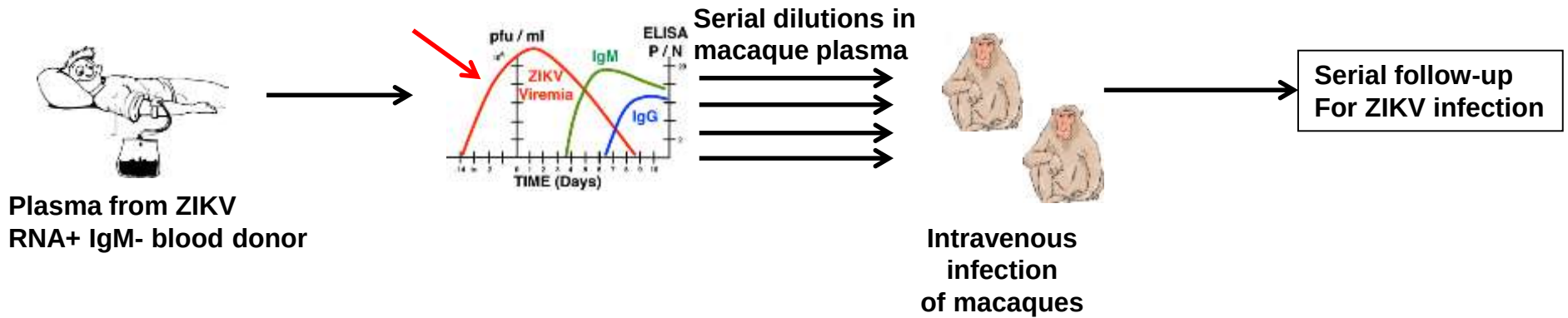
Inoculum: 7.8 log₁₀ RNA copies, 5.0 log₁₀ PFU



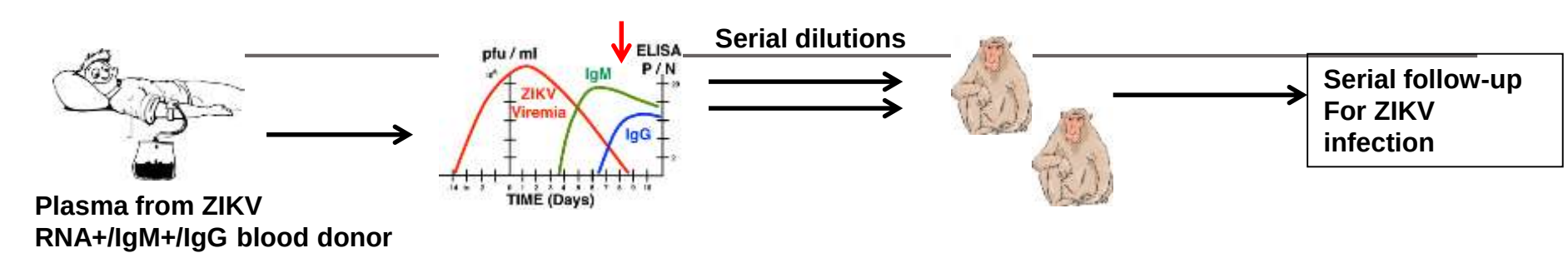
Aim 1: Evaluate the dynamics of acute Zika virus infection in a macaque model. We will explore ZIKV dynamics during acute infection in macaques using sensitive blood screening NAT and viral load (VL) assays and serological assays. This will include characterization of the length of the eclipse phase, viral doubling time during the ramp-up phase, and persistence of virus in blood compartments following seroconversion.

Characterization of blood transfusion-transmission of Zika virus in macaques

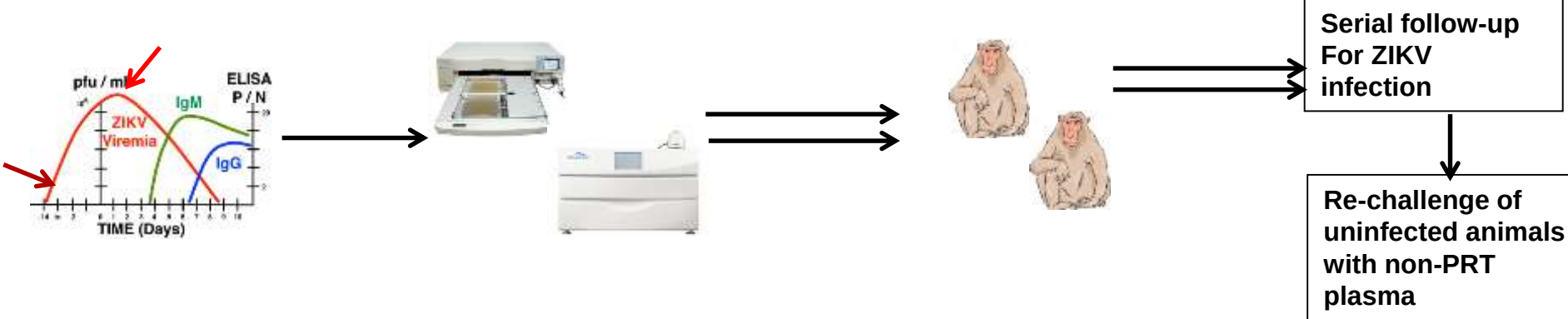
Aim 2A. Minimal infectious dose in ramp-up phase



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



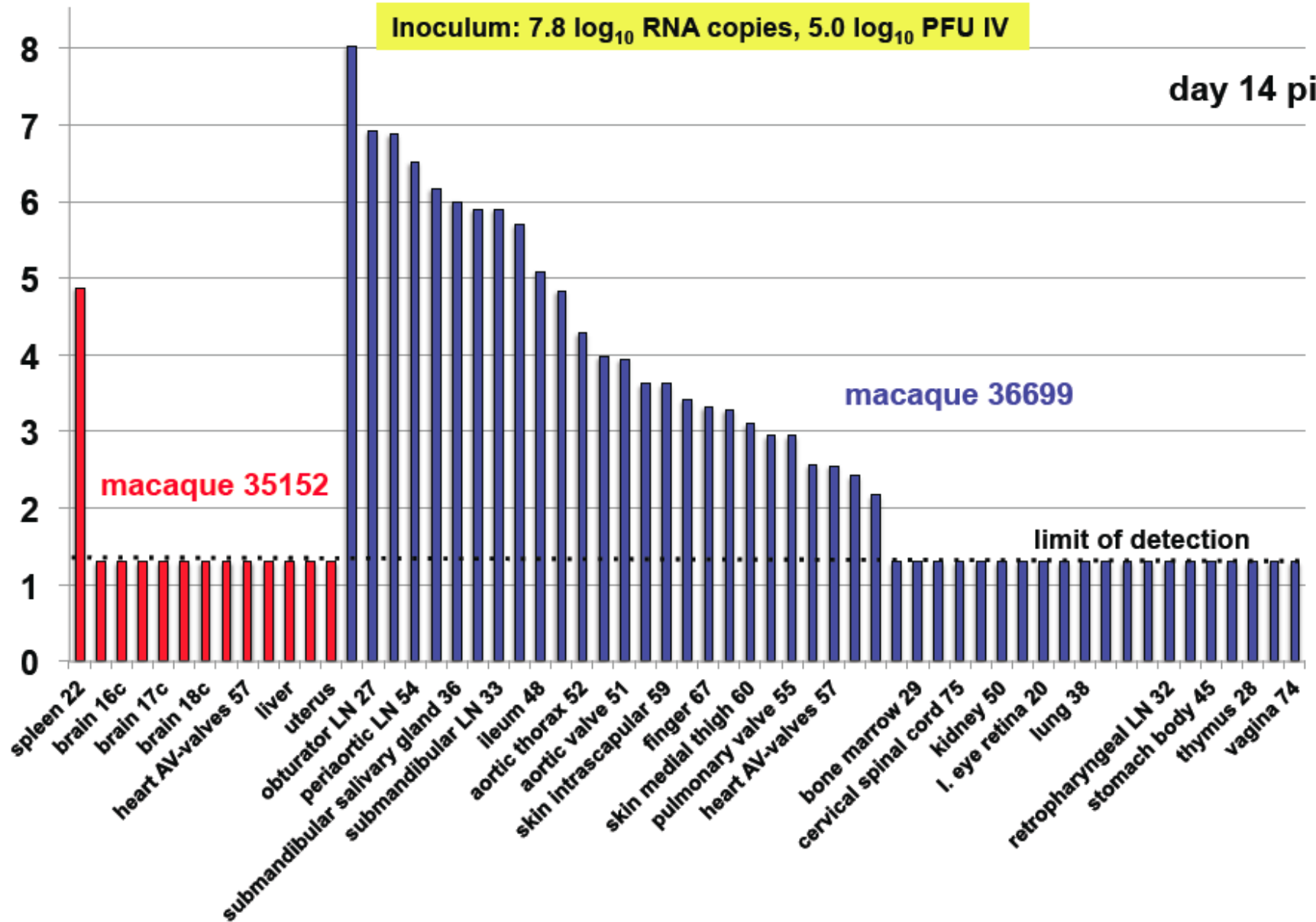
Aim 3. Analysis of efficacy pathogen reduction technologies



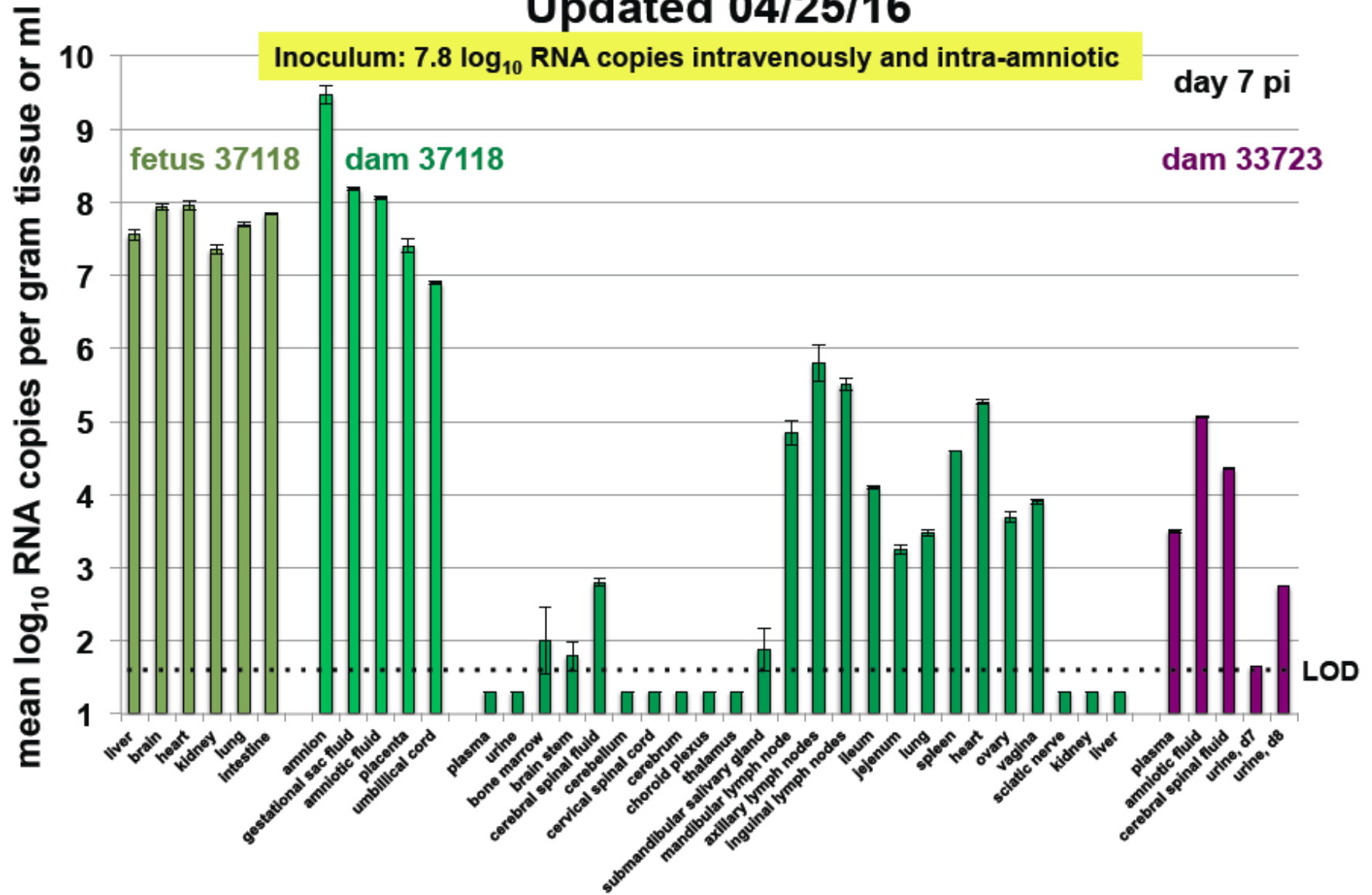
Pilot 1: ZIKV inoculated macaque organ RNA levels
Updated 05/24/16

Inoculum: 7.8 log₁₀ RNA copies, 5.0 log₁₀ PFU IV

day 14 pi



Pilot 2: ZIKV inoculated macaque vRNA levels Updated 04/25/16



Acknowledgements

- REDS-III Central Laboratory, Blood Systems Research Institute
 - Brian Custer
 - Marion Lanteri
 - Graham Simmons
 - Mars Stone
- REDS-III Brazil Program
 - Ester Cerdeira Sabino, the Fundação Faculdade de Medicina and Hospital das Clinicas of the Medical School of the University of São Paulo with participation of 4 blood centers located in: Belo Horizonte - Minas Gerais (Fundação Hemominas), Recife - Pernambuco (Fundação Hemope), Rio de Janeiro (Fundação Hemorio), and São Paulo (Fundação Pro-Sangue).
- REDS-III Data Coordinating center, RTI International
 - Don Brambilla
 - Marian Sullivan
- UC Davis
 - Koen Van Rompay
 - Lark Coffee
- REDS-III Chair
 - Steve Kleinman
- REDS-III ZIKV Oversight Committee
 - Jay Epstein, FDA
 - Hira Nakhasi, FDA
 - Matt Kuehnert, CDC
 - Lyle Peterson, CDC
 - Brad Biggerstaff, CDC
- NHLBI
 - Simone Glynn
 - Allison Cristman
 - Kelli Malkin
 - Shimian Zou



Inferred TT

- Assume we enroll 3000 recipients and 200 test RNA+ post-tx and negative pre-tx (cases) during the full protocol phase of the study.
- Assume these 200 cases received 600 units (mean of 3 units per recipient) and 180 test RNA+
- Then of 200 RNA+ cases we have 180 probable TT cases and 20 community acquired infections.
- We will evaluate the 200 RNA negative recipient controls and test all of the donor samples.
- Assume these controls were transfused with 600 units and 20 donations (3.3%) test RNA+
- Assume 3.3% (260) of 2600 RNA negative recipients who received 7800 units would also test RNA+
- Based on these numbers we would have the following in the table:
- The inferred transmission rate would be $= 180/460 = 39\%$

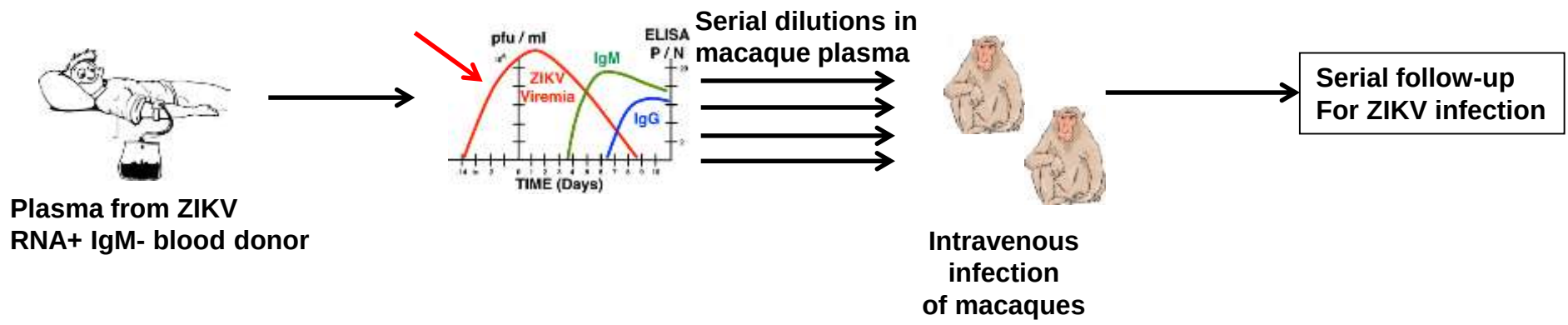
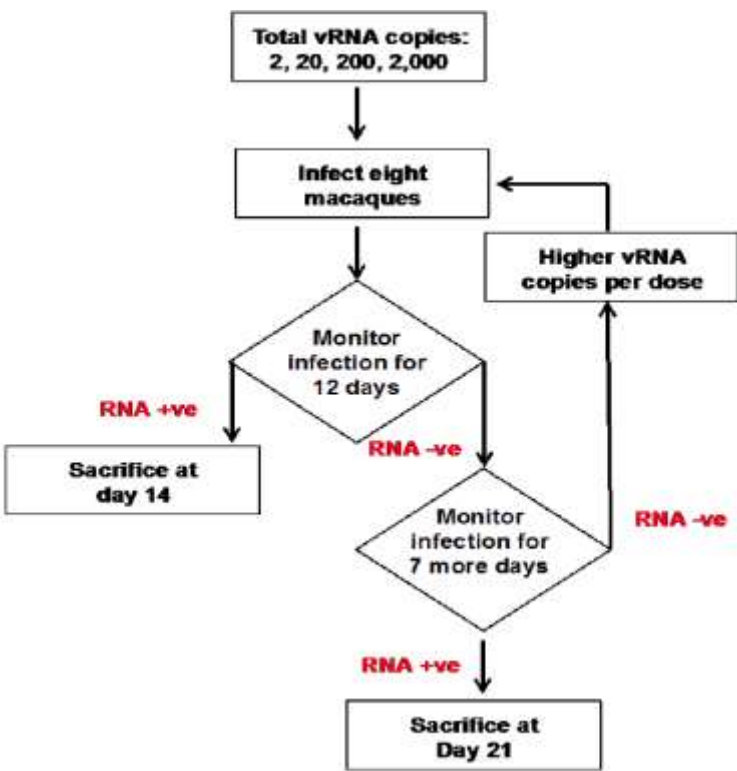
	Recipients RNA+	Recipients RNA-		Total
	Cases	Controls	Units not tested	
Total # of recipients	200	200	2600	3000
Units received	600	600	7800	9000
Positive units	180 [tested]	20 (3%) [tested]	260 [estimated]	460

Risk of ZIKV Transmission by Blood Transfusion

- an estimated 80% of ZIKV infections are asymptomatic
- infection may lead to severe clinical outcomes (i.e., microcephaly, GBS)
- pre-symptomatic period varies from 3 to 12 days
- viremia is reported to range from 10^3 - 10^7 copies/ml
- 2.8% of samples from asymptomatic blood donors in French Polynesia were ZIKV RNA positive
- two possible cases of transfusion-transmission in Brazil
- intrauterine transmission now well established with serious sequestrated transmission occurs at significant rate

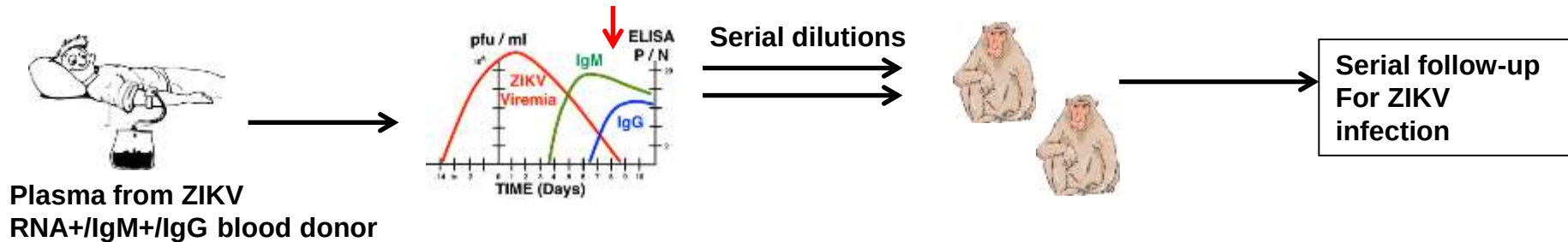
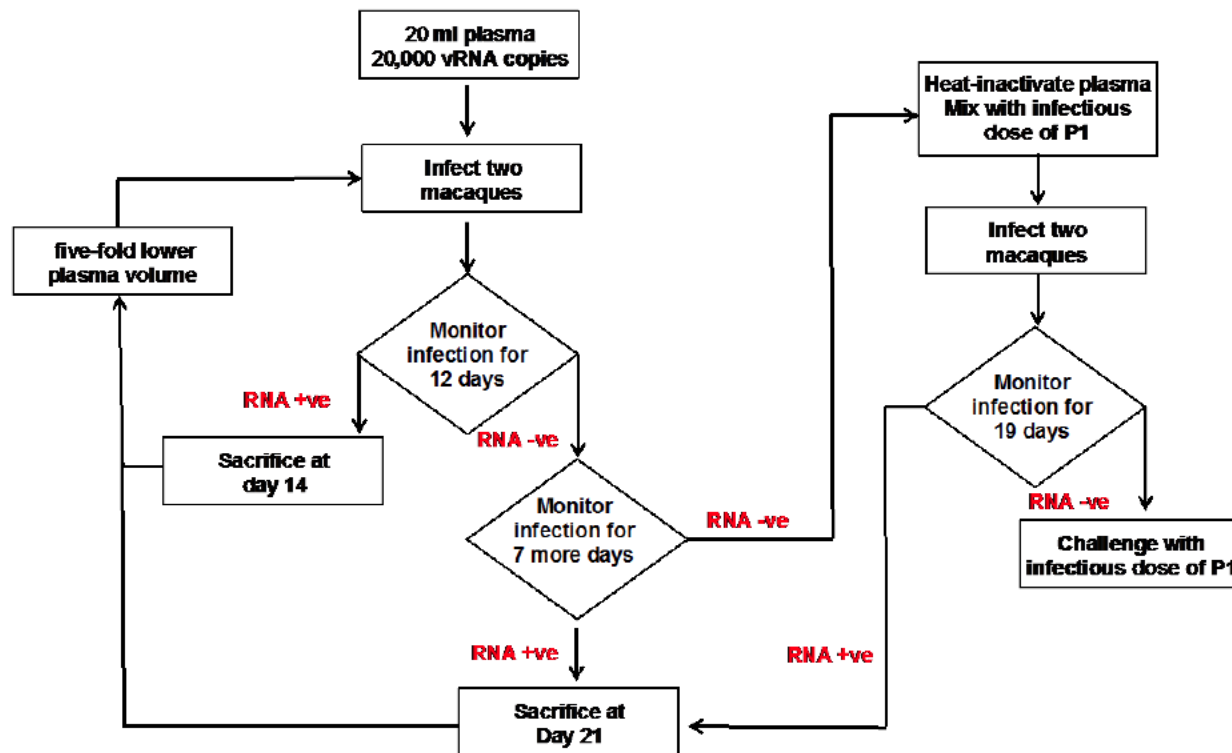
Characterization of blood transfusion-transmission of Zika virus in macaques

Minimal infectious dose in ramp-up phase

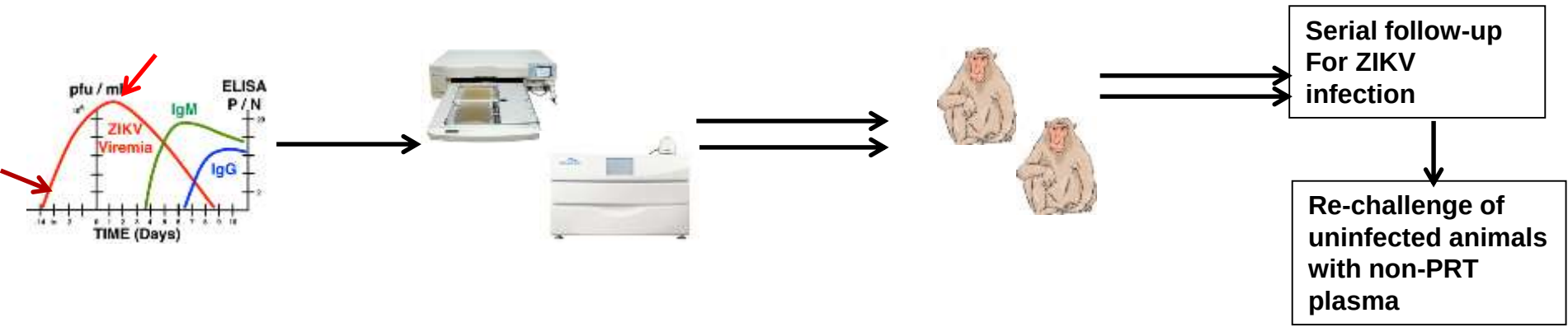


Characterization of blood transfusion-transmission of Zika virus in macaques

Minimal infectious dose in the presence of ZIKV antibodies



Efficacy of pathogen reduction technologies



PRT system	Number of macaques	
	Ramp-up period plasma	Peak viremia plasma
INTERCEPT	2	2
Mirasol	2	2
No PRT	0 ^a	1
TOTAL	4	5
a. Controls for ramp-up period plasma will be provided by Aim 2.1		