Virology subgroup: AFTERNOON BREAKOUT SESSION

Topic		Presenter
ZIKV	FU of NAT+ donors	Mars Stone
	Animal infectivity studies	Graham Simmons
HEV	HEV - Latest findings on HEV3 sources and phylogeny	Boris Hogema
	HEV - Data and policy considerations from Japan	Masahiro Satake
HIV	HIV - Donations from HIV+ donors on ART at SANBS	Marion Vermeulin
	HIV – Potential impact of ART & pre-exposure prophylaxis on blood safety	Mike Busch
Brainstorm – New ideas & directions for Subgroup		All

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US Natural History Study of Zika+ Blood Donors

Mars Stone, PhD

Scientist II, Core Director

Viral Reference Lab and Repository Core

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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
- **Where**: Puerto Rico, Oneblood, BSI, NYBC, ARC
- **Sample size**: 130 ZIKV+ donors (80 DENV Ab+; 50 DENV Ab-)
- **Objectives:**
  - Characterize the natural history, persistence, and pathogenesis of ZIKV infection
  - 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
  - Evaluate clinical outcomes post donation
  - Establish a sharable biorepository

Follow-up study of Zika RNA positive blood donors approach

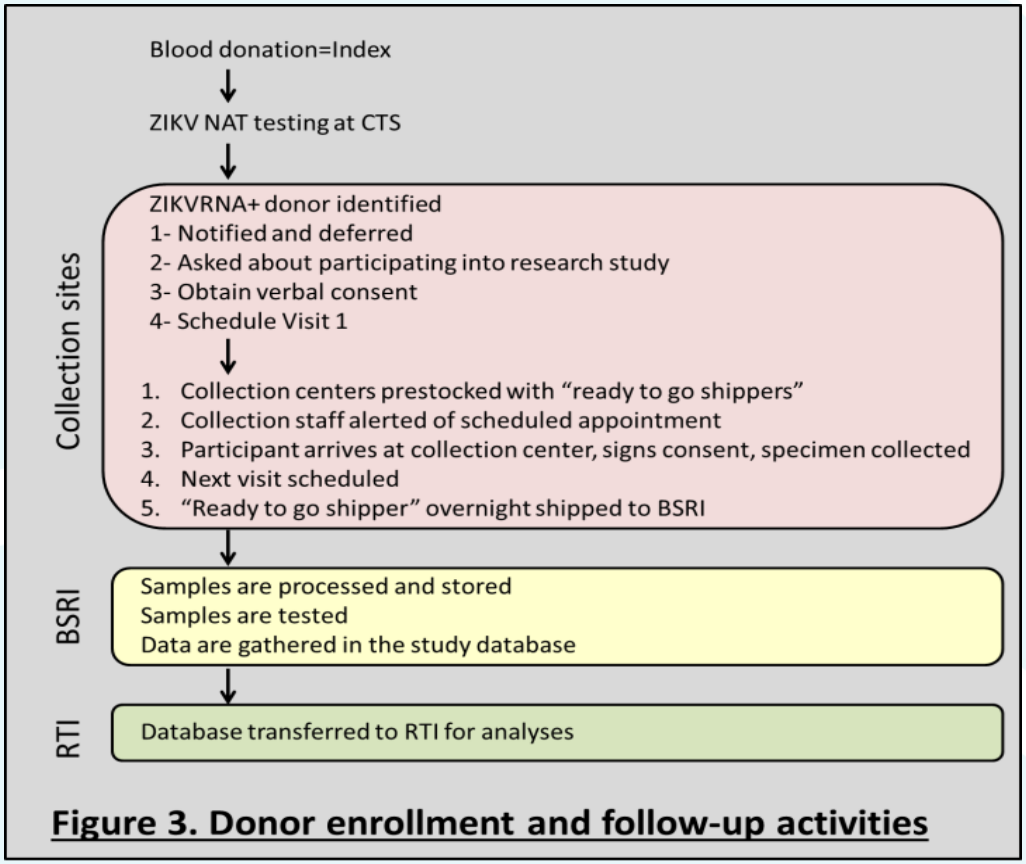
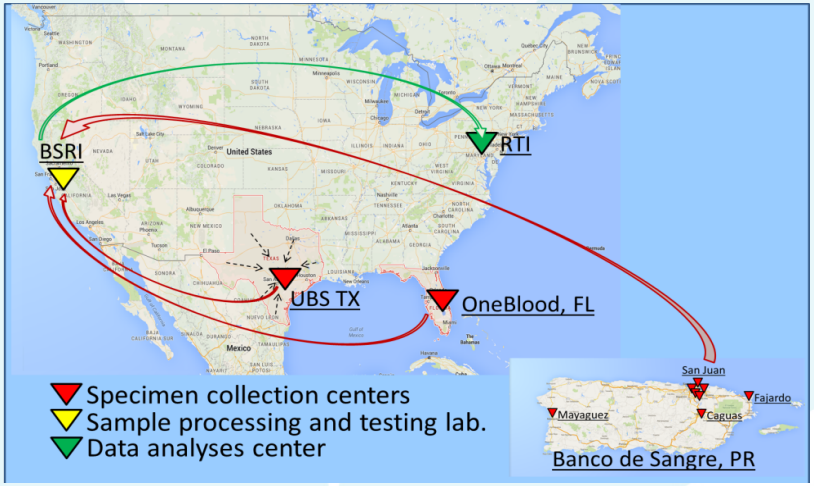


Figure 3. Donor enrollment and follow-up activities



Extended Follow-up

- Characterization of humoral and cellular immunity
- Discriminate recent vs remote infections

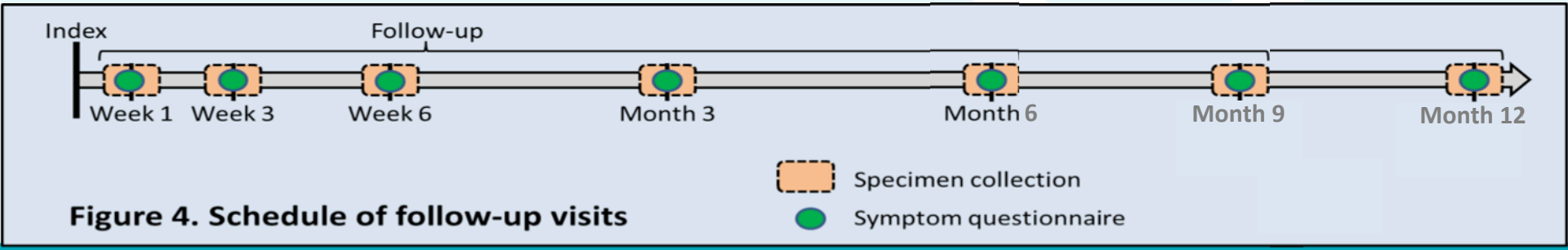


Figure 4. Schedule of follow-up visits

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Follow-up extended to 12 months with focus on reinfection

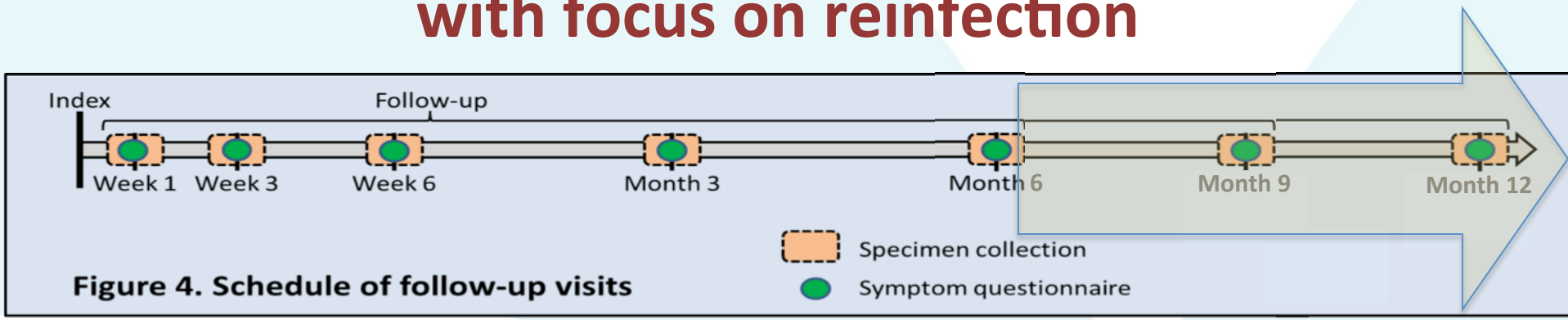
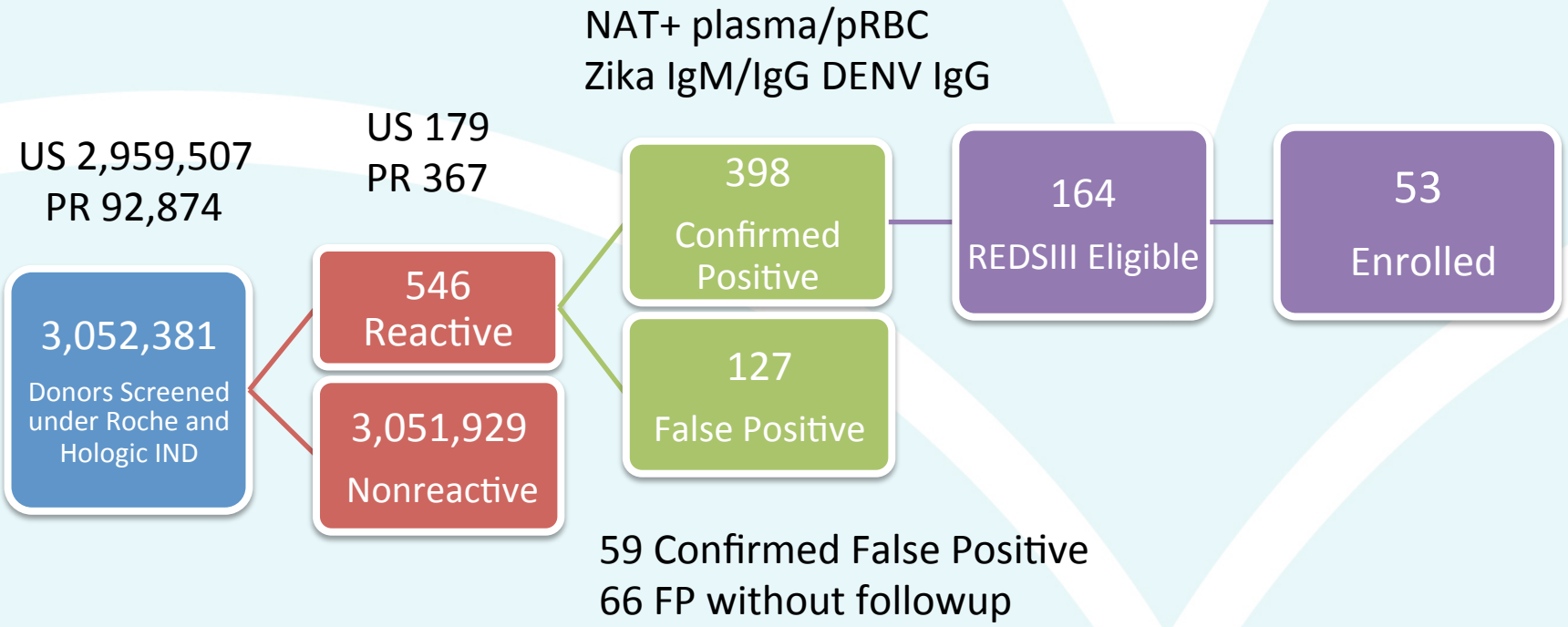


Figure 4. Schedule of follow-up visits

- Allow characterization of humoral and cellular immunity
  - Compare natural vs vaccine immune response
- Discriminate recent vs remote infections
  - Facilitate development of ZIKV incidence assays to discriminate recent vs remote infections
  - Monitoring of pregnant women and travellers
  - Detection of ZIKV reinfections
    - 30-40% exposed in endemic areas in 2016 season
    - Resistant to reinfection = epidemic burn out?
    - Reinfection possible = vaccine efficacy without sterilizing immunity

Enrollment through IND donor screening into REDSIII Natural History Study



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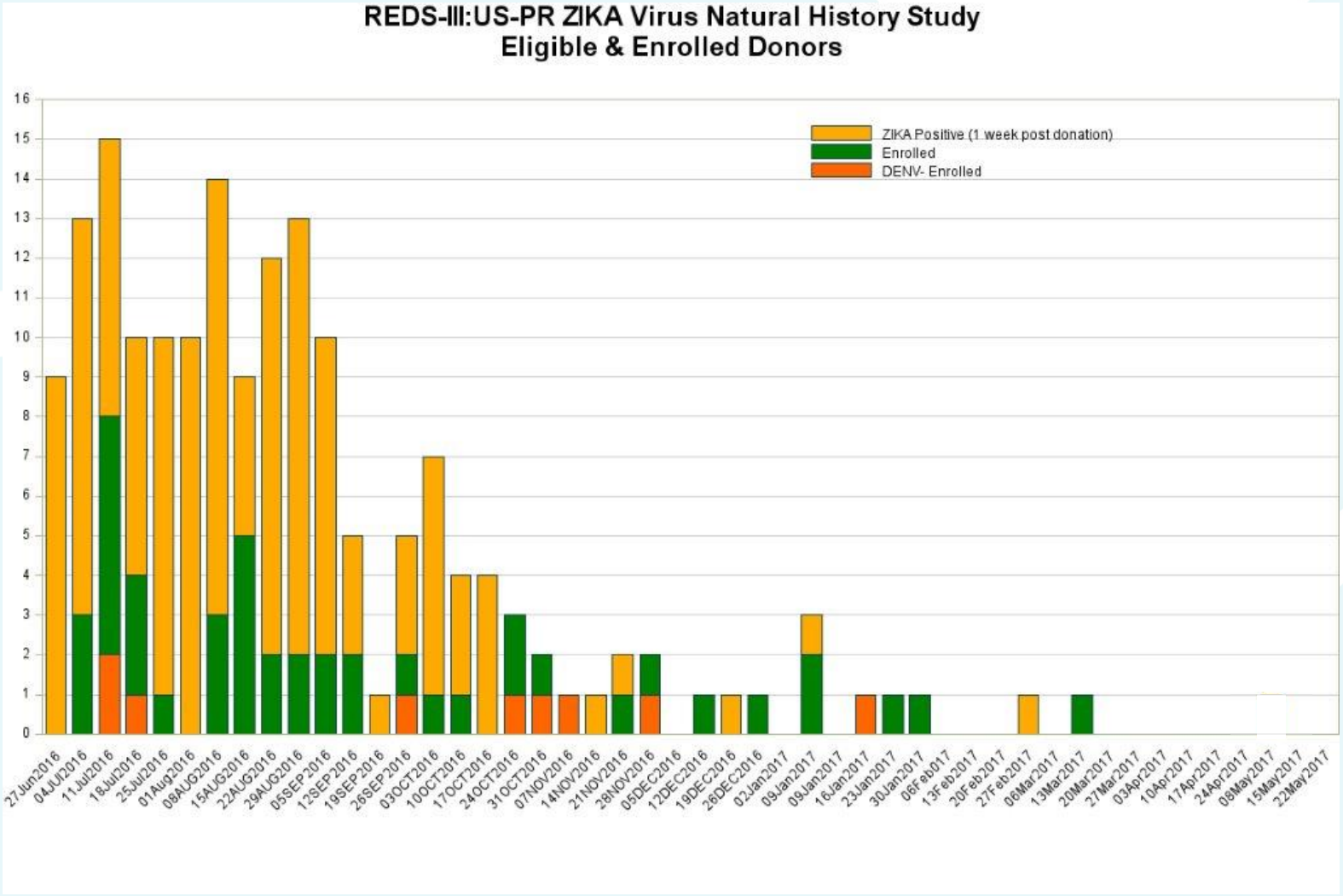
ZIKV+ donors and enrollment into Roche and Hologic IND and REDS-III follow-up studies thru 5/23/17

	Roche	Hologic (non ARC)
Index	450	81
Follow up 1	192	43
Follow up 2	115	20
Follow up 3	3	8
Follow up 4		4
Index with FU to 5/3	444	74
IND %enrollment	43%	58%
REDSIII eligible	166	
REDSIII enrolled	53	
REDSIII %Enrollment	32%	

Enrollment by gender, age and DENV status

	MALES (38)				FEMALES (15)				TOTAL (53)				
DONORS	DENV POS	DENV NEG	DENV EQ	PENDING	DENV POS	DENV NEG	DENV EQ	PENDING	DENV POS	DENV NEG	DENV EQ	PENDING	GRAND TOTAL
TOTAL	32	5	1	0	11	4	0	0	43	9	1	0	**53**
18-25	4	2	0	0	3	1	0	0	7	3	0	0	**10**
26-30	1	1	0	0	0	2	0	0	1	3	0	0	**4**
31-40	3	0	0	0	3	0	0	0	6	0	0	0	**6**
41-50	10	1	1	0	1	1	0	0	11	2	1	0	**14**
51-60	7	1	0	0	2	0	0	0	9	1	0	0	**10**
>60	7	0	0	0	2	0	0	0	9	0	0	0	**9**

Weekly eligible Zika+ and enrollment



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Specimen collection by type and timepoint

DONORS	Symptom Quex	EDTA Tubes	Tempus	Urine	Semen	Saliva	Buccal Swab	Nasal Swab	Total/ week
Week 1	40	270	78	39	11	38	40	40	556
Week 3	47	321	92	46	11	44	47	47	655
Week 6	42	298	85	43	8	40	43	43	602
Month 3	44	301	90	51	8	40	44	44	622
Month 6	37	276	78	40	6	37	40	40	554
Month 9	11	76	21	11	1	10	11	11	152
Month 12									
TOTAL/ specimen	210	210	207	208	49	194	210	210	1498

Visit	Plasma (1mL)	Plasma (5mL)	Whole Blood (1ml)	Whole Blood (0.5ml)	PBMC (10^7/ mL)	pRBCs (1.0ml)	Urine (5 mL)	Urine (1mL)	Saliva (1 mL)	DBS (BSRI)	Semen (0.5 - 1 mL)
Week 1	280	147	136	48	372	119	110	195	132	40	30
Week 3	324	175	188	131	492	129	138	230	169	47	31
Week 6	301	165	172	150	508	119	126	215	151	43	21
Month 3	300	145	176	264	460	123	127	220	155	44	22
Month 6	278	146	160	240	544	118	119	200	131	40	15
Month 9	71	41	44	66	112	33	31	55	34	11	4
Month 12											
Total	**1554**	**819**	**876**	**899**	**2488**	**641**	**651**	**1115**	**772**	**225**	**123**

RAPID COMMUNICATIONS

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 Lustig [1], E Mendelson [1,2], N Paran [3], S Melamed [3], E Schwartz [4]
1. Central Virology Laboratory, Ministry of Health, Tel-Hashomer, Israel

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

Volume 23, Number 5—May 2017

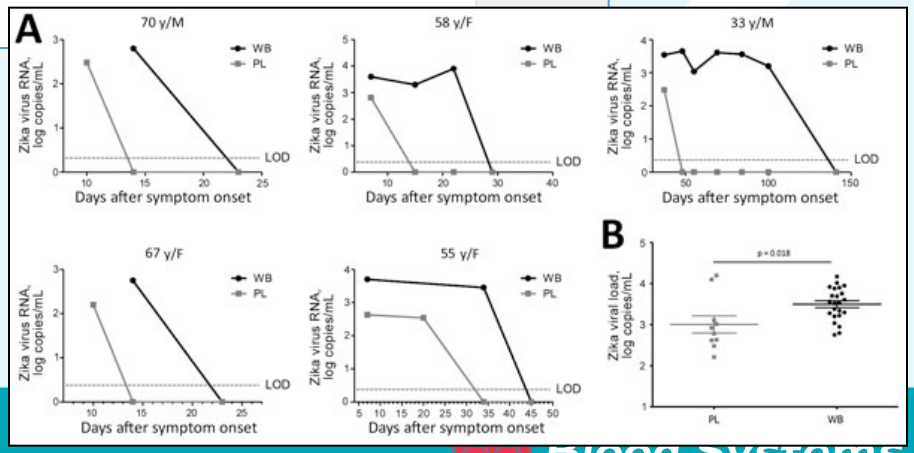
Research Letter

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

On This P

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



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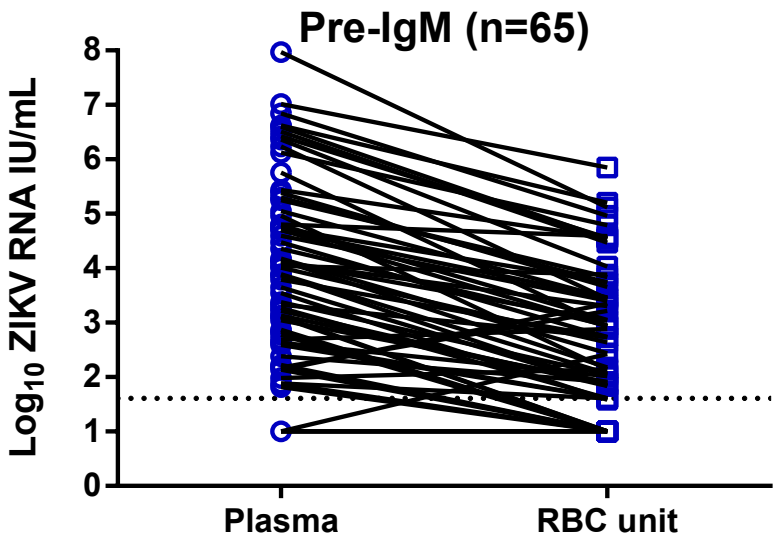
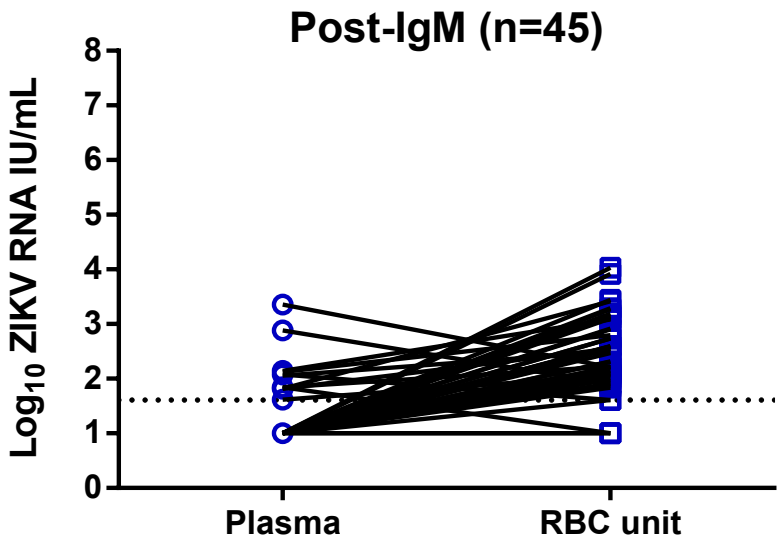
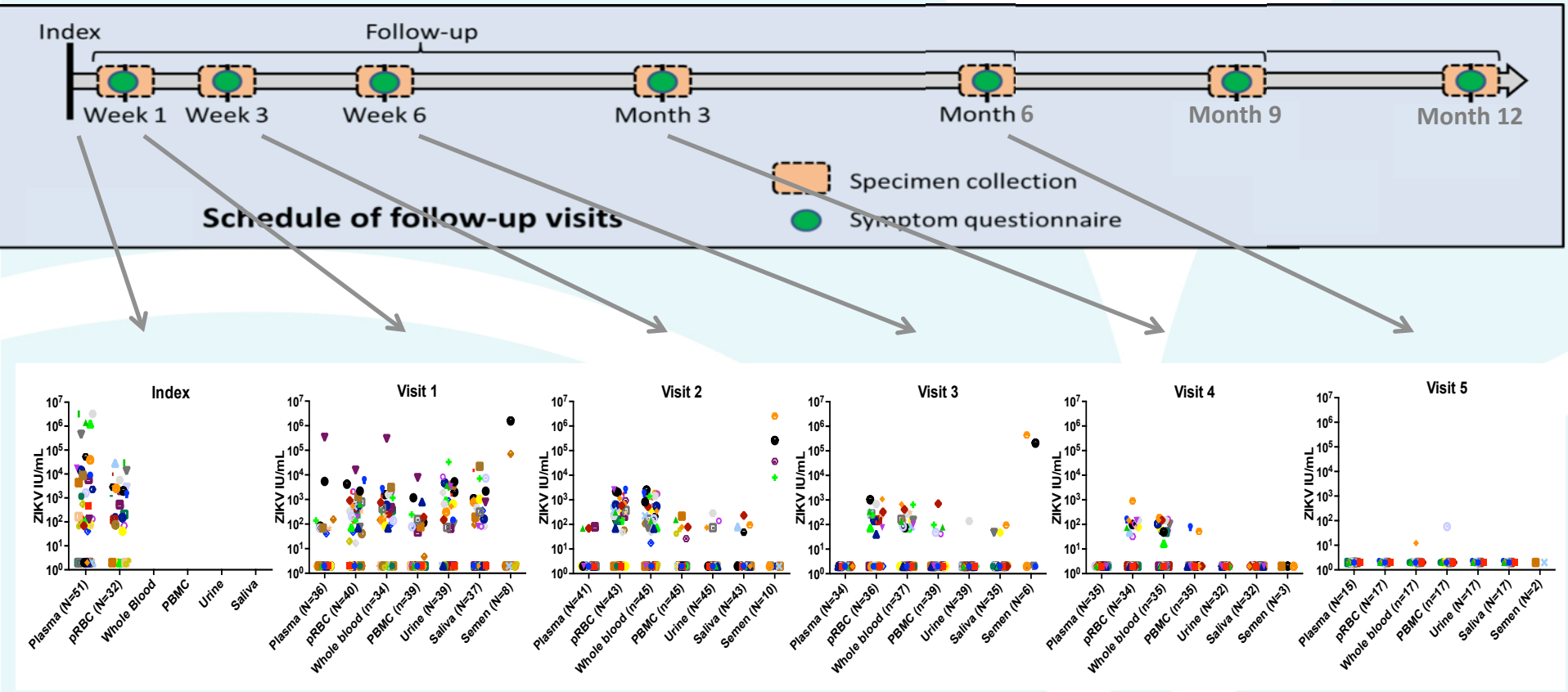
Higher levels of ZIKV RNA in red cells vs plasma in index donation samples after IgM seroconversion





ZIKV RNA persists in whole blood, primarily associated with RBCs, much longer than plasma



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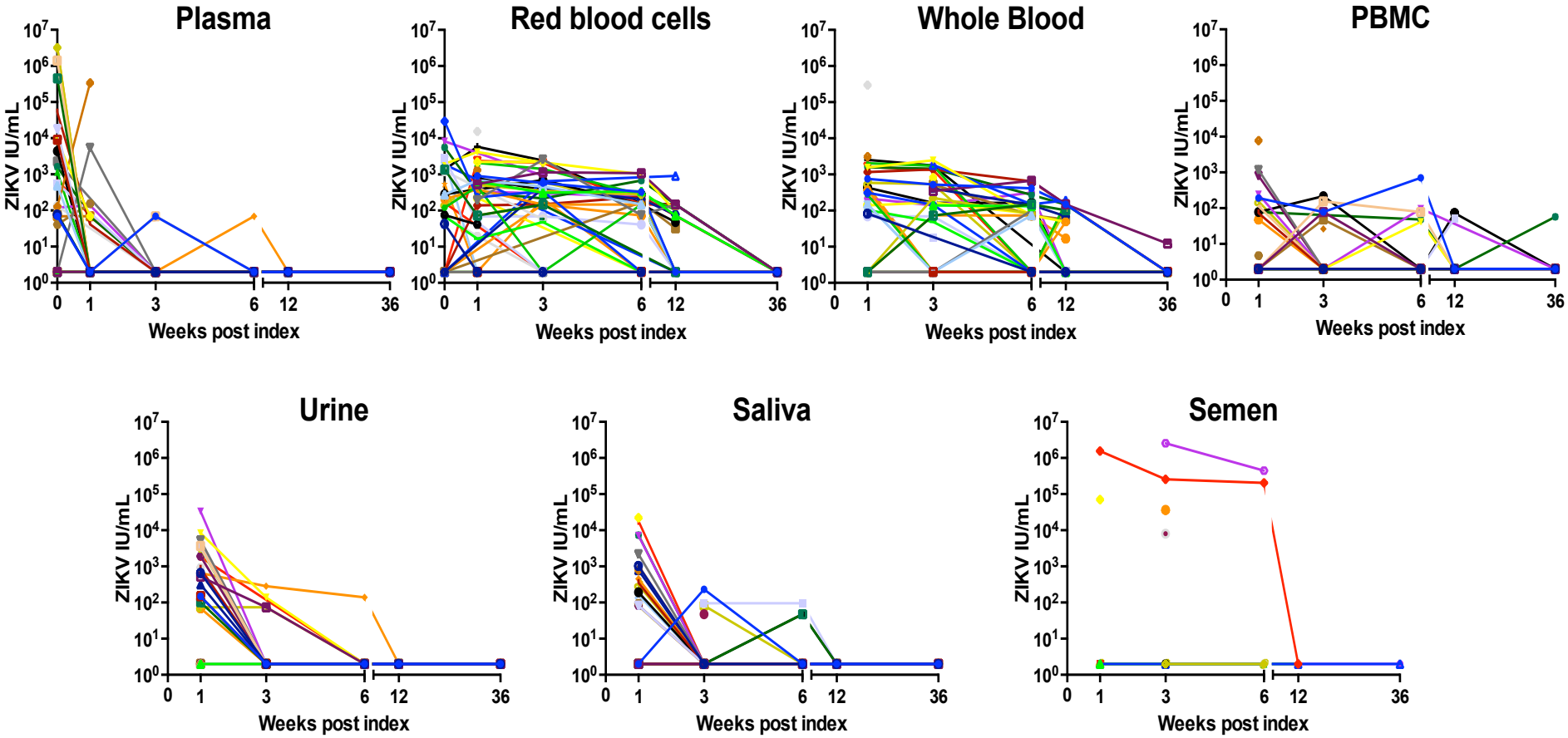
Longer persistence of ZIKV RNA in whole blood and RBC blood compartments than in plasma and body fluids



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Implications of Zika persistence in blood compartments and body fluids

1. For acute symptomatic infection, the use of whole blood extends the period of diagnosis.
2. For asymptomatic infections, virus detection in plasma might be less sensitive within a shorter window than detection in whole-blood specimens.
3. Impact on donation policy: to extend deferral period or consider NAT testing whole blood.
4. Consideration for solid organ donation with potential reservoir for viral replication
5. Could testing whole blood or RBC be used as proxy for persistence in semen and sexual transmission risk
6. **Is RBC-associated ZIKV RNA infectious?**

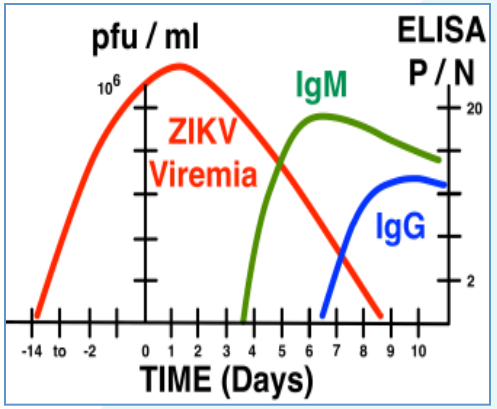
Implications of Zika persistence in blood compartments and body fluids?

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

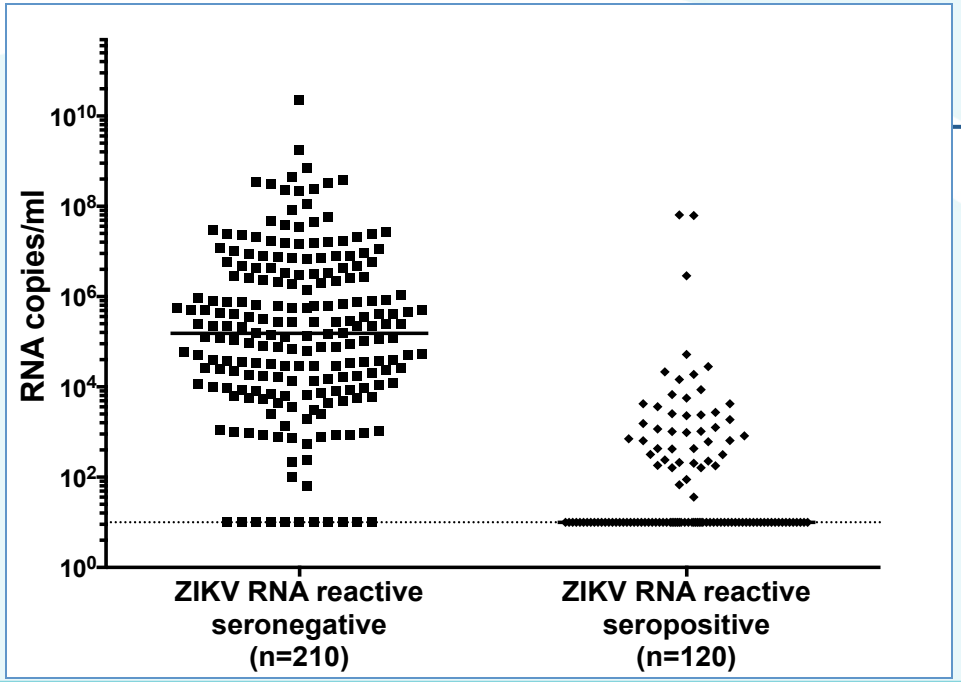
Tentatively concluding:

- ✓ **plasma NAT screening is sufficient**
- ✓ **ZIKV RNA that likley became assoicated with erythroblasts in acute infection is not infectious**

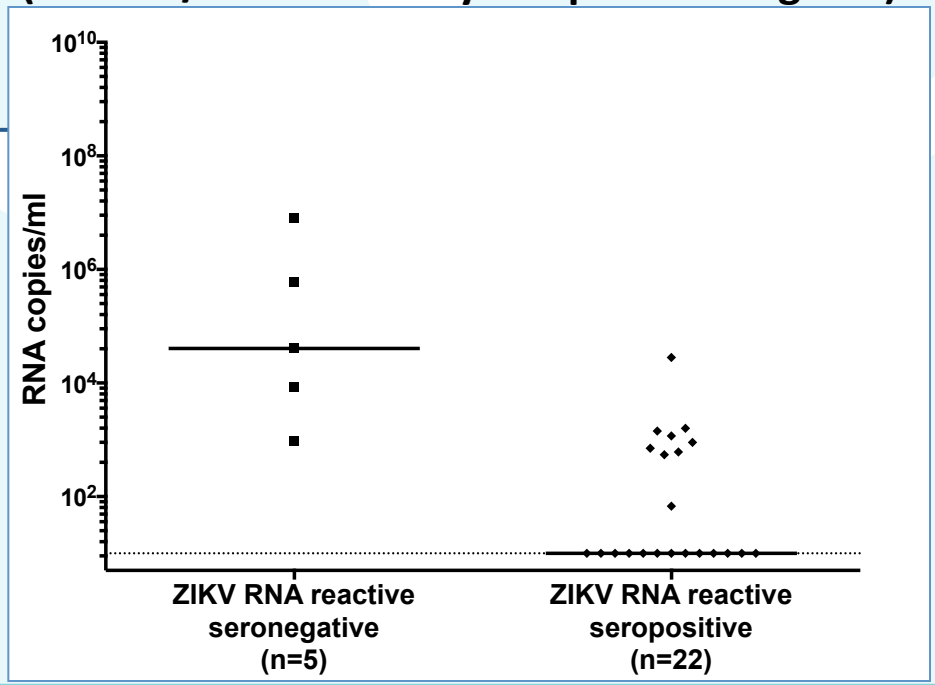
Stages of ZIKV infection at time of index donation in donors detected in epidemic regions vs travellers



Puerto Rico



Continental US
(most w/ travel history to epidemic regions)



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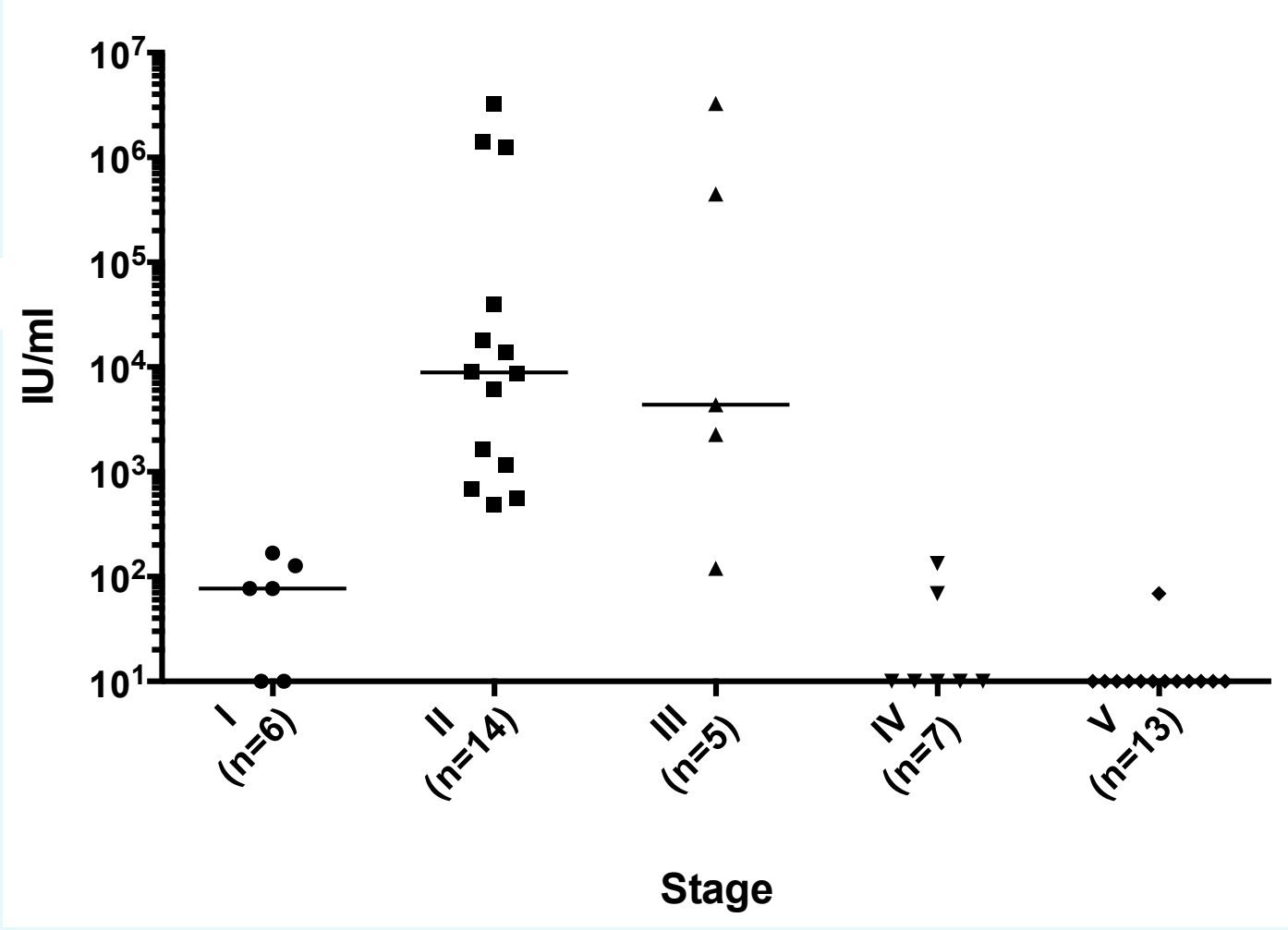
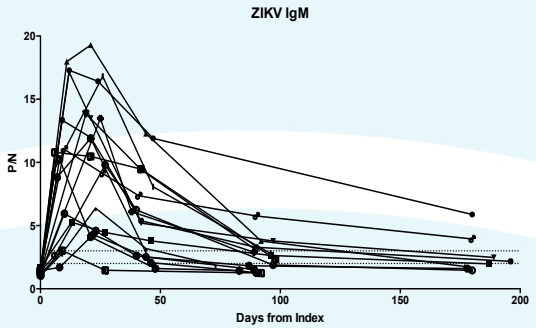
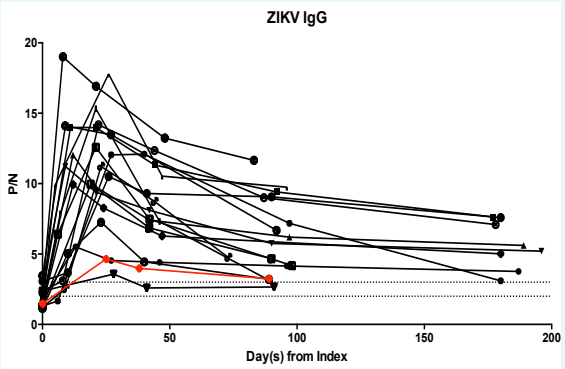
Stage of infection at index donation



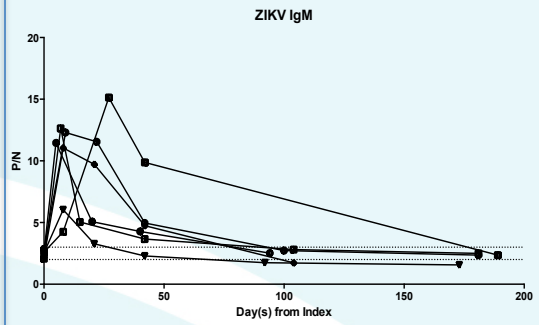
IgM/IgG results on samples from ZIKV NAT + donors with 4-5 follow-ups

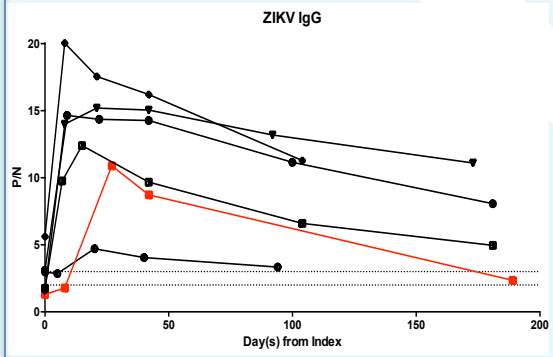
ZIKV IgM non-reactive (P/N <2.0) at index



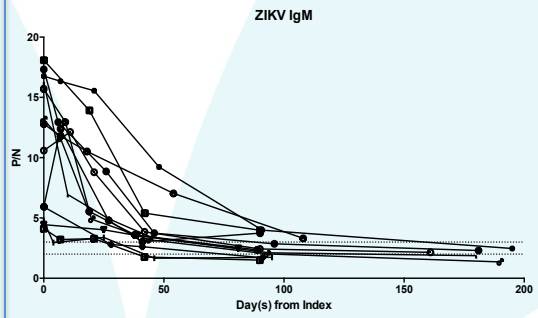


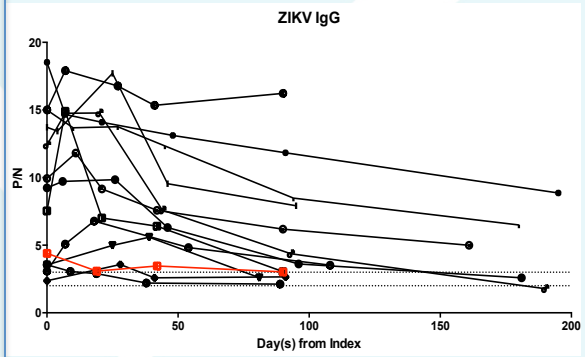
ZIKV IgM Equivocal (P/N 2.0 to <3.0) at index





ZIKV IgM Reactive (P/N >3.0) at index





DENV IgG negative donor

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Symptoms in ZIKV+ donors sorted stage of infection at index donation

Stage	Visit1		Visit2		Visit3		Visit4		Visit5	
	Symptoms	%	Symptoms	%	Symptoms	%	Symptoms	%	Symptoms	%
I	No Symp	0.00%	B	16.70%						
	ABCDEF	100.00%	C	16.70%	CDE	50.00%				
	CDEF	66.70%	No Symp	0.00%	No Symp	0.00%	DF	33.30%		
	ABCDE	83.30%	CDEF	66.70%	CDF	50.00%	CDF	50.00%	BCDEF	83.30%
	ABCDEF	100.00%	No Symp	0.00%	No Symp	0.00%	CE	33.30%	No Symp	0.00%
	E	16.70%	No Symp	0.00%	No Symp	0.00%	No Symp	0.00%	B	16.70%
II	B	16.70%								
	ABCDF	83.30%								
	No Symp	0.00%								
	ABCDF	83.30%								
	ABCF	66.70%	No Symp	0.00%	No Symp	0.00%				
	No Symp	0.00%	E	16.70%	No Symp	0.00%	D	16.70%		
	ABCDEF	100.00%	No Symp	0.00%	No Symp	0.00%	No Symp	0.00%		
	B	16.70%	No Symp	0.00%	No Symp	0.00%	No Symp	0.00%		
	ACD	50.00%	CDE	50.00%	CD	33.30%	CD	33.30%	CD	33.30%
	ABCDEF	100.00%	No Symp	0.00%	EF	33.30%	No Symp	0.00%	No Symp	0.00%
	CF	33.30%	No Symp	0.00%	F	16.70%	No Symp	0.00%	No Symp	0.00%
	ABCDEF	100.00%	ACDEF	83.30%	CD	33.30%	ACDEF	83.30%	No Symp	0.00%
	BCDEF	83.30%	No Symp	0.00%	No Symp	0.00%	No Symp	0.00%	No Symp	0.00%
	ABCDEF	100.00%	No Symp	0.00%	No Symp	0.00%	D	16.70%	ADEF	66.70%
	BCDF	66.70%	F	16.70%	No Symp	0.00%	CDF	50.00%	DEF	50.00%
	ACDEF	83.30%	CF	33.30%	No Symp	0.00%	D	16.70%	No Symp	0.00%
	No Symp	0.00%	No Symp	0.00%	No Symp	0.00%	No Symp	0.00%	No Symp	0.00%
III	C	16.70%	D	16.70%						
	CD	33.30%	ACD	50.00%	C	16.70%				
	BCDEF	83.30%	EF	33.30%	No Symp	0.00%	No Symp	0.00%		
	No Symp	0.00%	No Symp	0.00%	No Symp	0.00%	No Symp	0.00%		
	No Symp	0.00%	No Symp	0.00%	No Symp	0.00%	No Symp	0.00%	E	16.70%
	AC	33.30%	No Symp	0.00%	No Symp	0.00%	No Symp	0.00%	C	16.70%
	ABCDEF	100.00%	ABCDEF	100.00%	BCDEF	83.30%	BEF	50.00%	No Symp	0.00%
IV	No Symp	0.00%								
	No Symp	0.00%								
	BDF	50.00%								
	No Symp	0.00%	F	16.70%						
	F	16.70%	No Symp	0.00%	No Symp	0.00%				
	No Symp	0.00%	No Symp	0.00%	No Symp	0.00%				
	F	16.70%	No Symp	0.00%	No Symp	0.00%	No Symp	0.00%		
	EF	33.30%	CEF	50.00%	EF	33.30%	F	16.70%		
	D	16.70%	No Symp	0.00%	No Symp	0.00%	No Symp	0.00%		
	A	16.70%	No Symp	0.00%	CD	33.30%	No Symp	0.00%		
	ABCDEF	100.00%	CDE	50.00%	ACDE	66.70%	No Symp	0.00%		
	ACDF	66.70%	No Symp	0.00%	No Symp	0.00%	DF	33.30%	No Symp	0.00%
	No Symp	0.00%	No Symp	0.00%	No Symp	0.00%	No Symp	0.00%	No Symp	0.00%
	E	16.70%	No Symp	0.00%	No Symp	0.00%	No Symp	0.00%	No Symp	0.00%
	BCDEF	83.30%	ABCDEF	100.00%	ABCDF	83.30%	ABCDF	83.30%	ABCDEF	100.00%
	ABCEF	83.30%	No Symp	0.00%	No Symp	0.00%	No Symp	0.00%	E	16.70%
	No Symp	0.00%	No Symp	0.00%	No Symp	0.00%	No Symp	0.00%	No Symp	0.00%
	BCDEF	83.30%	CF	33.30%	No Symp	0.00%	DF	33.30%	F	16.70%
	No Symp	0.00%	No Symp	0.00%	No Symp	0.00%	AF	33.30%	A	16.70%
	No Symp	0.00%	No Symp	0.00%	No Symp	0.00%	A	16.70%	No Symp	0.00%
		132		45		32		36		26

Pre-IgM (Simmons Stage I/II)

- 15/23 (65%) with >3/6 symptoms
- 4/23 (17%) asymptomatic

Symptoms:

A=Fever
B=Rash
C=Joint Pain or Bone Pain
D=Body Pain or Muscle pain
E=Painful Eyes or Red eyes
F=Headache

Post IgM/IgG (Simmons Stage III/IV

- 7/27 (30%) with >3/6 symptoms
- 10/27 (38%) asymptomatic

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Sharing samples from ZIKV biorepository

Enabling development and optimization of serology assays and pathogenesis, vaccine and cellular immunological studies

Recipient	Sample Use
FDA	RBC infectivity; regulatory assessment of EUA510k assays
NIAID	Distribution for Zika Vx evaluation
CDC	Zika DBS Ab newborn screening
WHO	International standards for Zika and CHIK
MSD	Novel ultra-sensitvie immunoassay for Zika virus NS1
Ortho	Zika/DENV Serology
Abbott	Zika/DENV Serology
Roche	Zika/DENV Serology
Johnston ASU	immunosignature technology for Zika diagnostics
Elledge - Harvard	phase display analysis
Jerome - U Wash	Zika/Dengue western blot assay
Omniarray	Arboviral IgM/IgG multiplex assay
Polyak - UW	Novel Zika Ab assay
Luminex	Multiplex arboviral serology
Immunetics	Multiplex arboviral POC
Ragon Institute HMPF DVR	pan-flavivirus peptide microarray
Burbelo - NIH	Zika LIPS
Wang - UHI	Zika/Dengue NS1 ELISA and western blot assays

Working with government agencies, industry and academic partners

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Weekly Detection Rate of ZIKV RNA in Blood Donated in Puerto Rico Since April 3, 2016

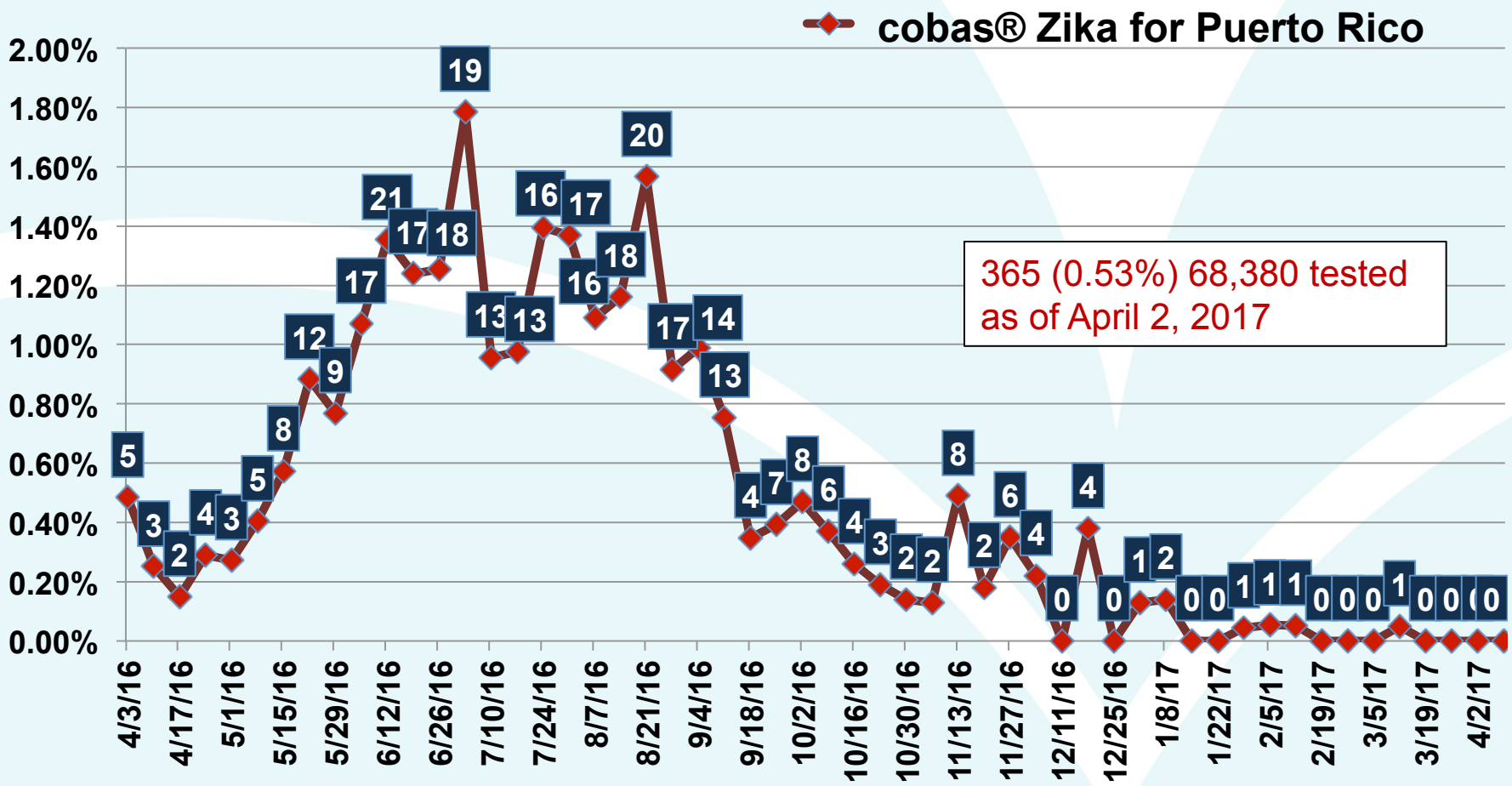

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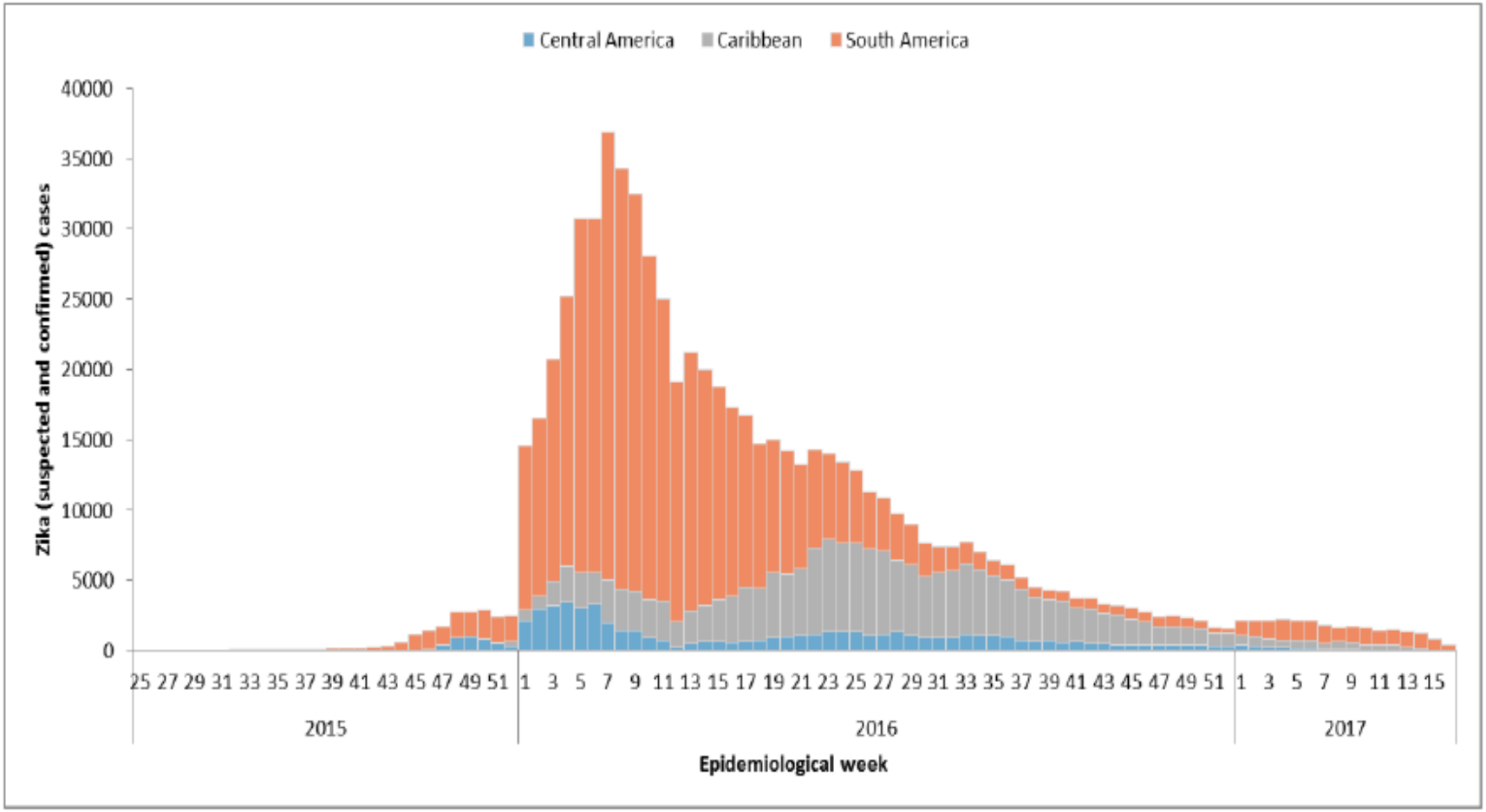
Figure 2. Distribution of suspected and confirmed Zika cases by epidemiological week and sub-region. Region of the Americas, 2016 – 2017 (as of EW 16).[15]



Source: Data provided by countries and territories and reproduced by PAHO/WHO

Sharp drop in the number of new cases
Importance of rapid response
Epidemics may resolve quickly leaving scientific questions unanswered

Blood Systems Research Institute

Acknowledgements

- Blood Systems Research Institute (REDS-III CL)
  - Mike Busch
  - Marion Lanteri
  - Graham Simmons
  - Sonia Bakkour
  - Tzong-Hae Lee
  - Brian Custer
  - Thema Gonzalez
- REDS-III Brazil Program
  - Ester Cerdeira Sabino, the Fundaçao 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: Bello Horizonte - Minas Gerais (Fundaçao Hemominas), Recife - Pernambuco (Fundaçao Hemope), Rio de Janeiro (Fundaçao Hemorio), and São Paolo (Fundaçao Pro-Sangue).
- REDS-III Data Coordinating center, RTI International
  - Don Brambilla
  - Marian Sullivan
- NHLBI
  - Simone Glynn
- REDS-III Contract
- Roche
  - Susan Galel
  - Lisa Pate
  - Tony Hardiman
- Hologic/Grifols
  - Jeff Linnen
  - Kui Gao
- CTS
  - Phillip Williamson
- 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 Petersen, CDC
  - Brad Biggerstaff, CDC

National Heart Lung and Blood Institute

REDS-III

Blood Systems Research Institute

ZIKV – Animal infectivity studies

Graham Simmons

Blood Systems Research Institute, San Francisco, CA

University of California, San Francisco

- **Calculation of minimal infectious doses**
  - Is MID above LOD of NAT assays/levels of pathogen reduction?
- **Transmissibility of components**
  - Is persistent RBC-associated viral RNA infectious?
- **Prevention of TT**
  - Do donor humoral responses prevent TT?
  - Pathogen reduction systems.
- **Pathogenicity of TT infection**
  - Are standard routes of transmission (e.g mosquito-borne) more pathogenic?

- **Calculation of minimal infectious doses**
  - Requires highly sensitive models
  - Realistic model to humans??
- **Transmissibility of components**
  - Allows sufficient volumes and route of delivery
- **Role of donor immune responses**
  - Sensitive AND immunocompetent
- **Pathogenicity of TT infection**
  - Reproduces human pathogenicity

- **Immunocompetent mice**
  - Adults
  - Neonates
- **Immunosuppressed mice**
  - Interferon αβ receptor 1 K/O
  - IFN αβ receptor 1/IFN γ receptor dual K/O
  - **Anti-IFN αβ receptor 1 antibody treated**
- **NHPs**
  - **Rhesus**, cynomologous macaques, marmosets

Available animal models for Zika

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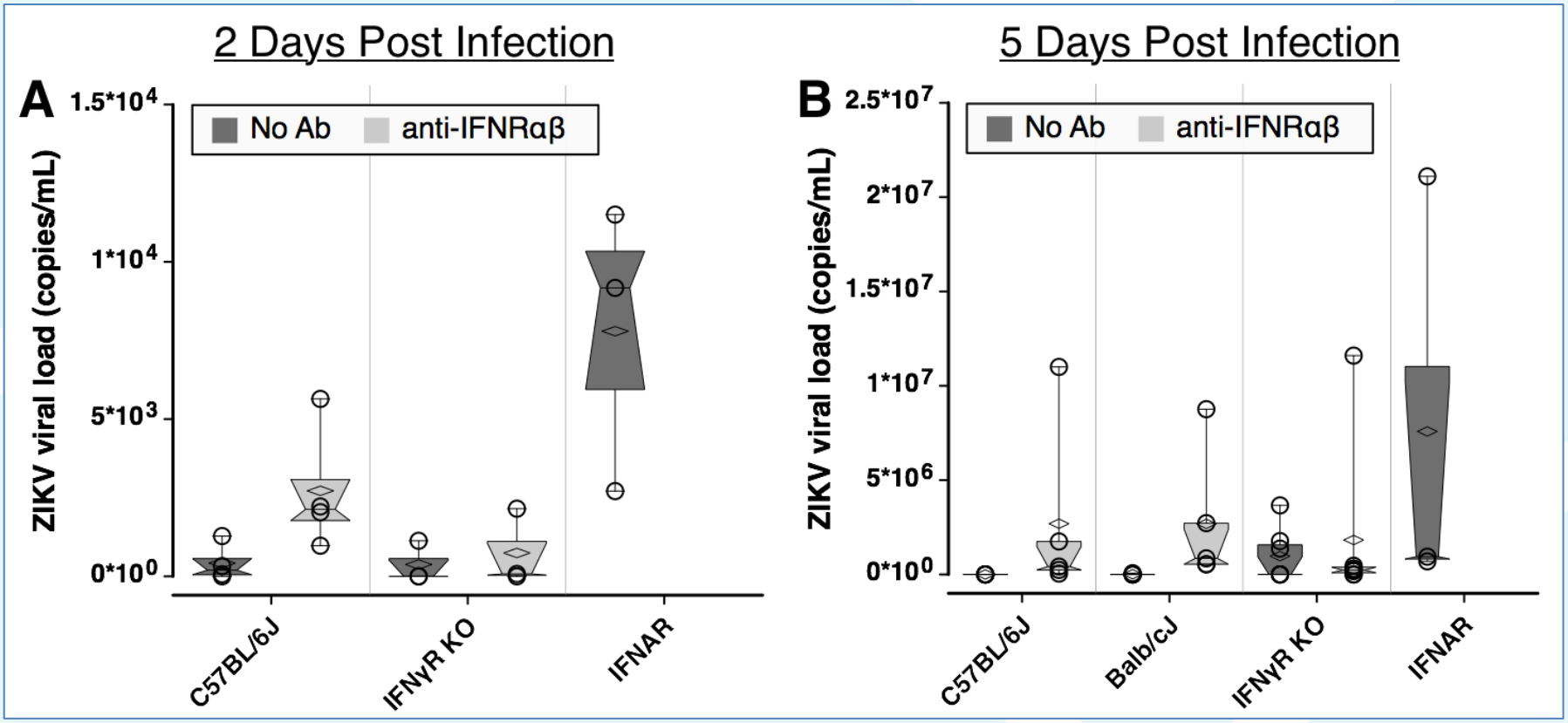

Examples of murine models

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Animal	Assay	Day post infection												
		0	1	2	3	4	5	6	7	8	9	10	11	12
Macaque 1	CDC RT-PCR	-	+	+	+	+	+	-	-	-	-	-	-	-
	Infectivity	-	+	+	+	-	-	-	-	-	-	-	-	-
	Hologic TMA	NT	NT	NT	+	+	+	+	+	+	+	+	-	-
Macaque 2	CDC RT-PCR	-	+	+	+	+	+	-	-	-	-	-	-	-
	Infectivity	-	-	+	+	-	-	-	-	-	-	-	-	-
	Hologic TMA	NT	NT	+	+	+	+	+	+	+	-	-	-	-

Studies performed at CNPRC

Example of NHP model

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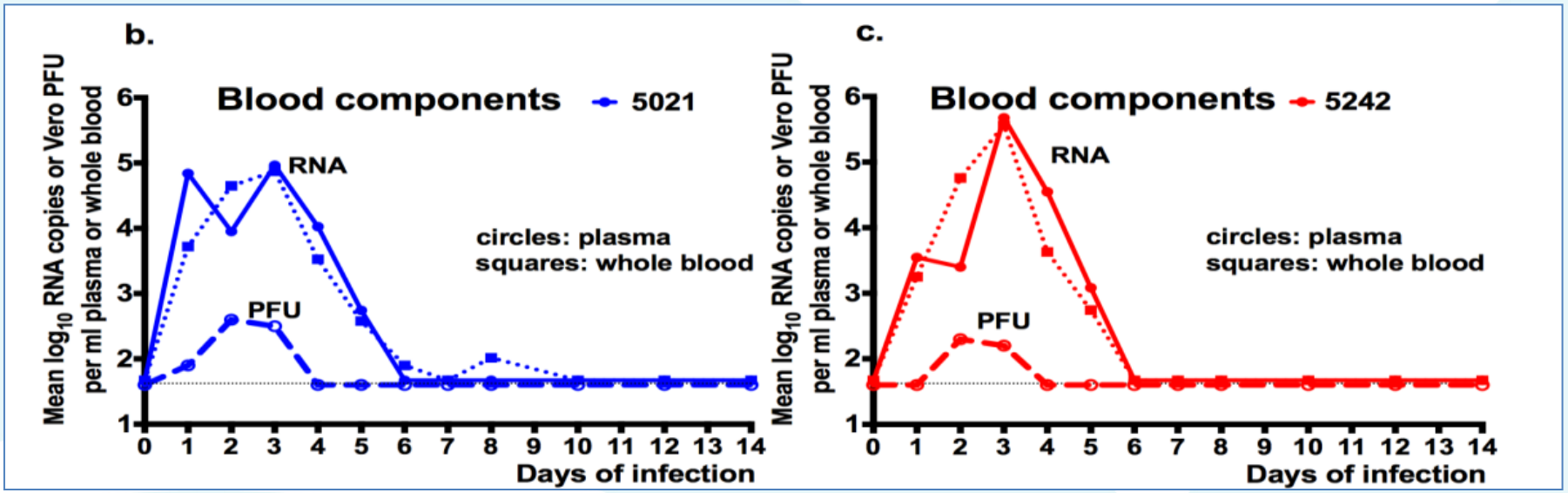

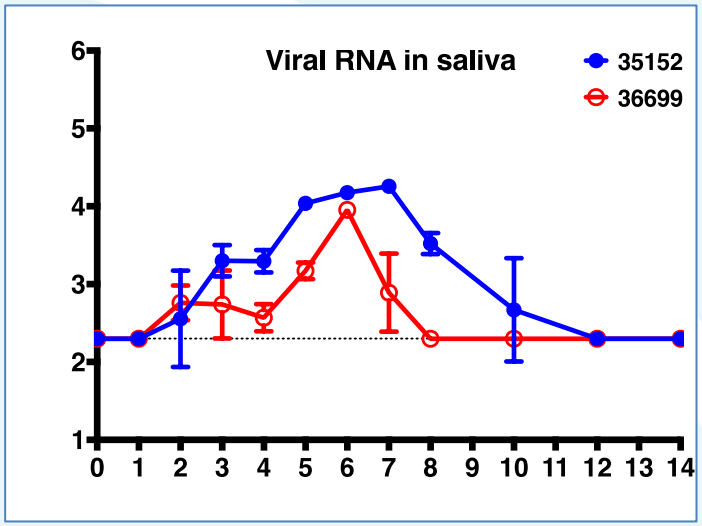

Additional sample types

Blood Systems Research Institute

TRANSFUSION COMPLICATIONS

Probable transfusion-transmitted Zika virus in Brazil

Maria L. Barjas-Castro,[1] Rodrigo N. Angerami,[2] Mariana S. Cunha,[3] Akemi Suzuki,[3] Juliana S. Nogueira,[3] Iray M. Rocco,[3] Adriana Y. Maeda,[3] Fernanda G.S. Vasami,[3] Gizelda Katz,[4] Ilka F.S.F. Boin,[5] Raquel S.B. Stucchi,[5] Mariângela R. Resende,[2,5] Danillo L.A. Esposito,[6] Renato P. de Souza,[3] Benedito A. da Fonseca,[6] and Marcelo Addas-Carvalho[1]

Plasma from probable TT case of Zika. RNA positive, infectious virus positive Zika IgM/IgG negative, DENV IgG low.

Performed half-log serial dilutions in Pooled WNV seronegative ACD macaque plasma. Flash frozen.

Calculation of minimal infectious dose

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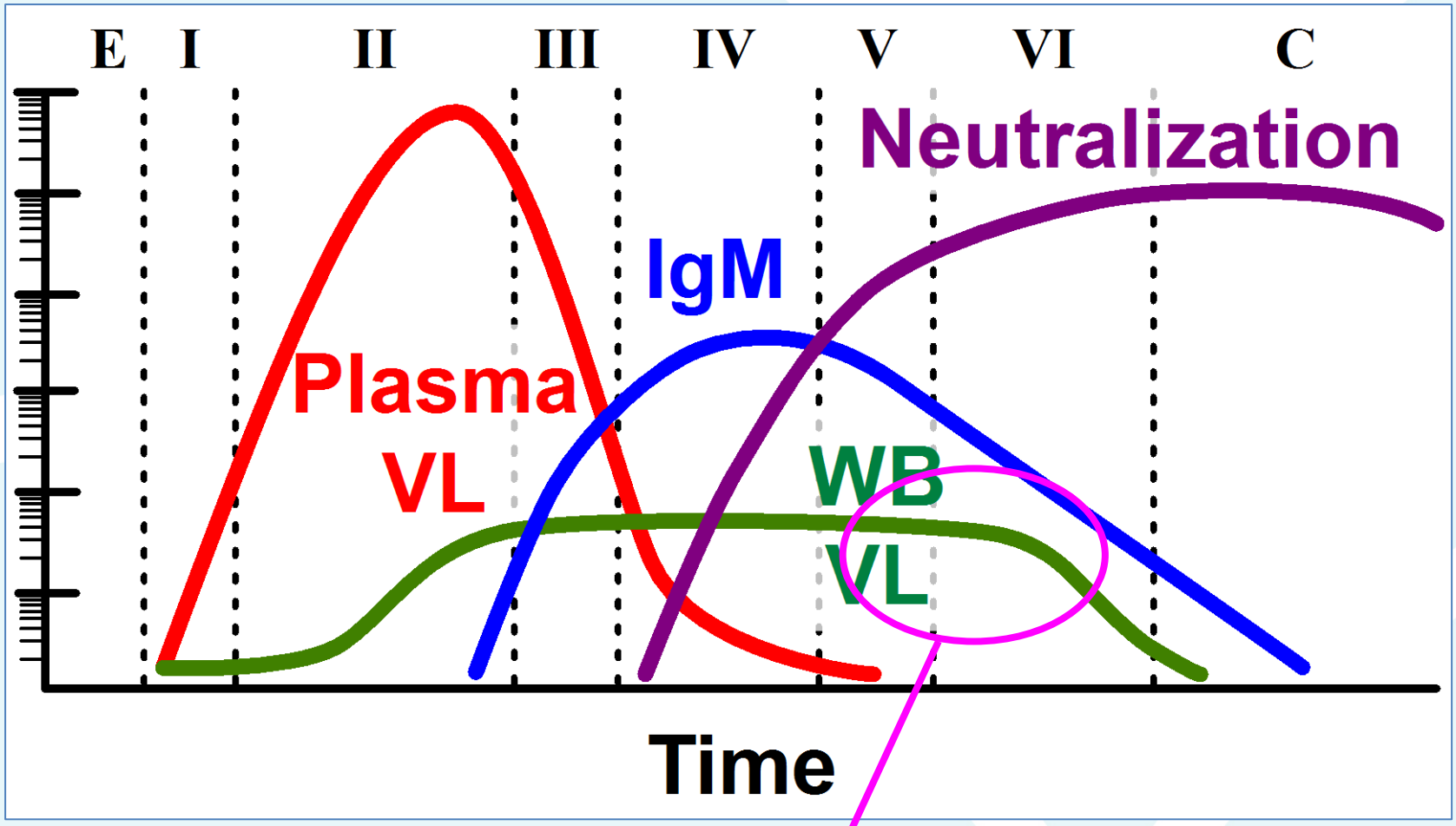
Dilution	Viral load (c/ml)	In vitro infectivity (PFU/ml)	Mouse infectivity using 100 ul
-	228500	648	Not Tested
1 in 3.33	68500	396	Not Tested
1 in 10	27900	270	Not Tested
1 in 33.3	8900	72	6/6 infected
1 in 100	3325	24	6/6 infected
1 in 333	813	4	4/6 infected
1 in 1000	423	Below LOD	6/6 infected
1 in 3333	130	Below LOD	1/6 infected
1 in 10000	49	Below LOD	0/6 infected

- **13 RNA copies = 1/6 mice infected**
- **42 RNA copies, ~0.2 PFU = 6/6 mice infected**
- **Mice more sensitive than current tissue culture**

In vitro and in vivo infectivity

Blood Systems Research Institute



Plasma RNA negative

RBC RNA positive

IgM seropositive, initial NAT reactive, repeat NAT non-reactive donor with follow-ups:

Index + five follow-ups plasma VL non-reactive, whole blood/pRBC reactive:

5860 RNA copies/ml
2450
1365
157
148
0

0.5 ml of pRBC used to challenge mice i/p

All mice negative by viral load and serology.

- **IFNAR1$^{-/-}$ and anti-IFNAR1 treated mice are exquisitely sensitive to ZIKV**
  - **Virus in plasma samples below the LOD for cell culture remain infectious**
  - **ID_{50} falls well below LOD_{50} of CDC NAT assay**

- **pRBC samples with viral loads over 3000 RNA copies were not infectious in mouse model**

Summary

Blood Systems Research Institute

BSRI

Marcus Muench

Sonia Bakkour

Marion Lanteri

Tzong-Hae Lee

Kai Lu

Mars Stone

Michael Busch

UC Davis

Koen Van Rompay

Lark Coffey

JoAnn Yee

Anil Singapuri

Hologic/Grifols

Jeff Linnen

REDS-III

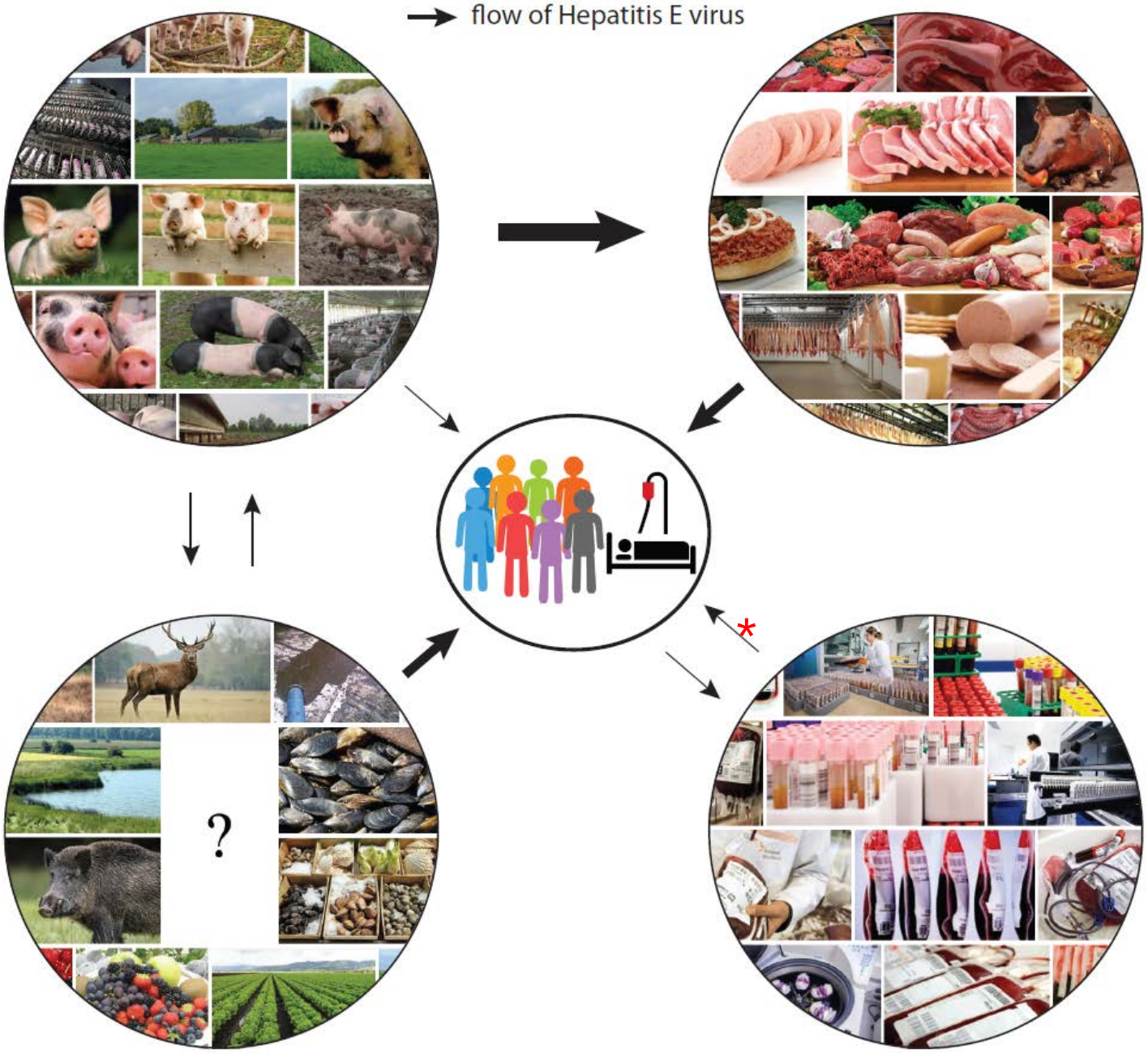
Acknowledgements

Blood Systems Research Institute

Latest findings on HEV gt3 sources and phylogeny

Boris Hogema
Sanquin Research
Sanquin Diagnostic Services

HEV sources and transmission routes



* estimate:
0.15% of HEV infections
29% of chronic HEV in transplant patients
(De Vos et al, 2017)

Sources and risk factors

- Quantitatively, surprisingly little known about HEV sources
- Evidence based on several case-control studies, surveys in blood donors, HEV RNA in meat products etc.
- It seems no actions will be taken as long as hard, direct evidence is lacking

- HEV RNA has been detected in pork, pork products, surface water, vegetables, berries, leafy greens, etc.
- Risk factors: consumption of
  - Pork meat (0.03)
  - Pork liver sausages ($p<0.001$)
  - Game meat, offal meat ($p<0.01$)
  - Oysters ($p=0.02$)
  - Wild boar meat
  - Protective effect from drinking bottled water ($p<0.02$)

    Mansuy et al, Hepatology 2016; Wichmann et al, J Infect Dis 2008
  - Pork pie, ham and sausage from “a major UK supermarket chain” ($p=0.02$)

    Said et al, Epidemiol Infect, 2013
  - Consumption of meat in general ($p=0.002$; 40% lower seroprevalence in vegetarian Dutch blood donors)

    Slot et al, Plos one, 2017
  - Several types of pork-containing meat products

    Manuscript in preparation

HEV RNA in Dutch meat products

No RNA detected in various types of dry pork sausages

HEV-RNA was tested in 54 ‘different’ types of liver sausage and paté from 15 manufacturers, sold at 14 retail chains.

Some products were tested multiple times (from different batches)

Type of meat	HEV RNA pos	HEV RNA neg	% pos
Regular	95	7	93%
Organic	6	17	26%
Totaal	101	24	81%

In many sausages the HEV RNA is resistant to RNAse incubation

Phylogenetic analysis

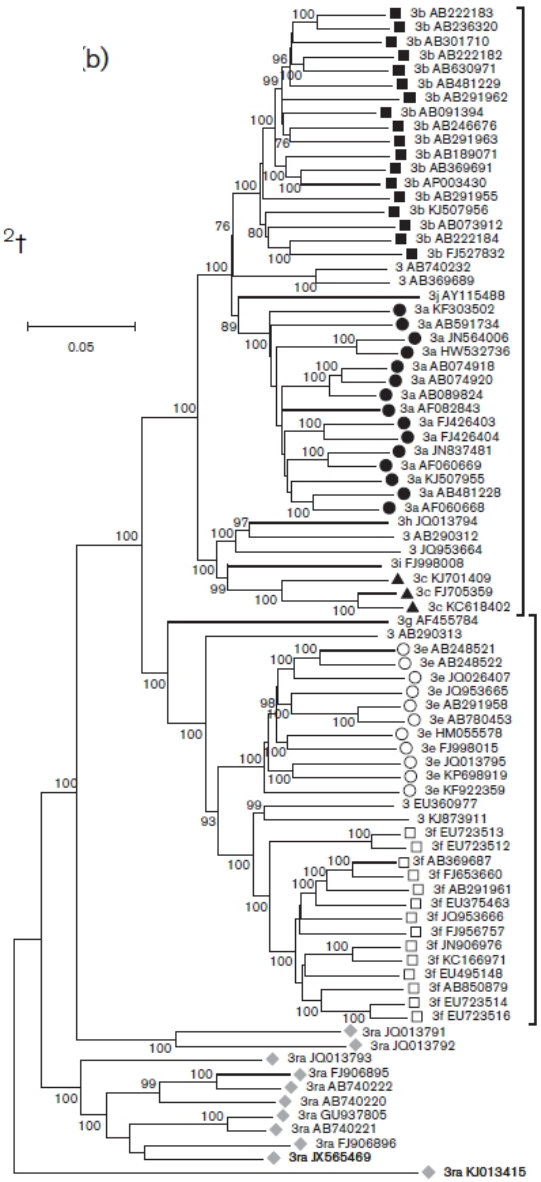
Journal of General Virology (2016), **97**, 537–542 DOI 10.1099/jgv.0.000393

Insight Review

Proposed reference sequences for hepatitis E virus subtypes

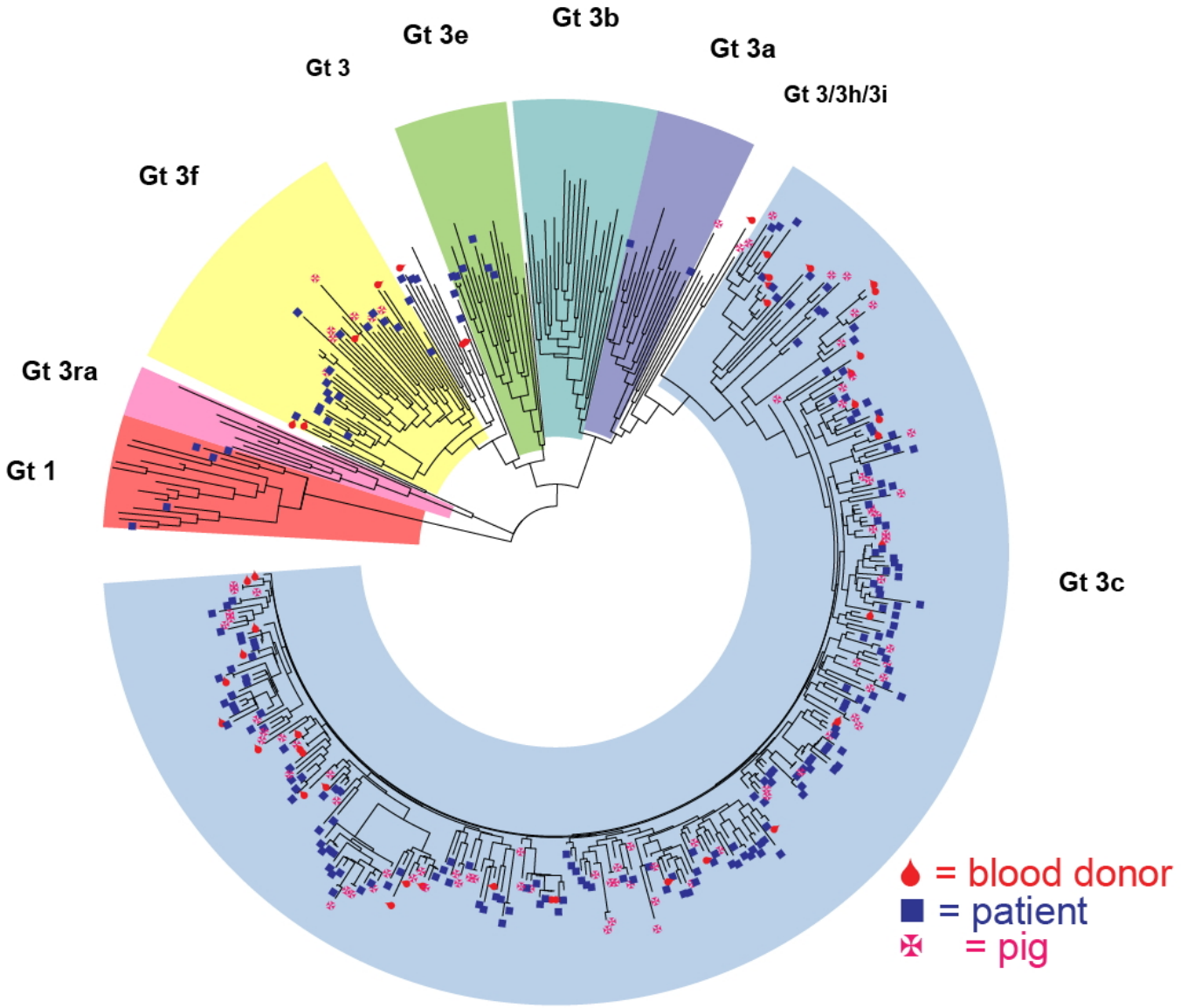
Donald B. Smith,[1] Peter Simmonds,[1] Jacques Izopet,[2] Edmilson F. Oliveira-Filho,[3] Rainer G. Ulrich,[4,5] Reimar Johne,[6] Matthias Koenig,[7] Shahid Jameel,[8]† Tim J. Harrison,[9]† Xiang-Jin Meng,[10]† Hiroaki Okamoto,[11]† Wim H. M. Van der Poel[12]† and Michael A. Purdy[13]†

- 7 known genotypes
- Genotype 3:
  - Two groups (3efg = clade 1, 3abchij = clade 2) and 3ra (rabbit)



HEV in the Netherlands

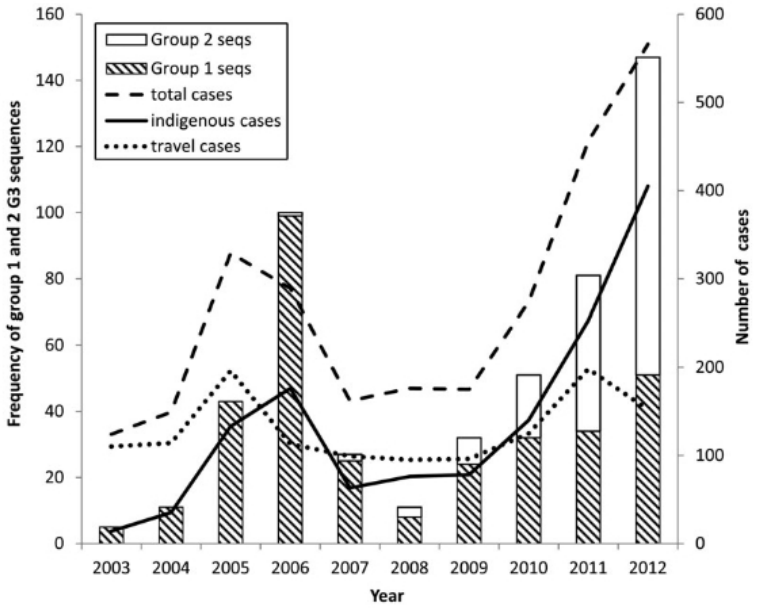
- Comparison of HEV ORF2 RNA sequences in Dutch blood donors (n=56), patients (n=225) and pigs (n=104)
- Samples collected between 2010 and 2016



HEV in England and Wales

Indigenous Hepatitis E in England and Wales From 2003 to 2012: Evidence of an Emerging Novel Phylotype of Viruses

Samreen Ijaz,[1] Bengü Said,[2] Elizabeth Boxall,[3] Erasmus Smit,[3] Dilys Morgan,[2] and Richard S. Tedder[1,4,5]



- Between 2009 and 2012 shift from clade 1 to clade 2 HEV infections
- Comparison of Dutch and UK sequences: no clustering

HEV in the UK

- Clade 2 HEV is rare in pigs in the UK. Grierson et al, 2015

Hepatitis danger from Full English breakfast... caused by EU meat: Sausage bug has been dubbed 'the Brexit virus'

- **60,000 Britons a year falling ill after eating European meat riddled with virus**
- **New strain of deadly hepatitis E found linked to pig farms on the continent**
- **Surge in severe cases of 'Brexit virus' reported with numbers trebling since 2010**
- **Have you fallen ill with the virus? Email katie.french@mailonline.co.uk**

By KATIE FRENCH FOR MAILONLINE and ELEANOR HAYWARD FOR THE DAILY MAIL
PUBLISHED: 16:00 BST, 21 May 2017 | **UPDATED:** 01:16 BST, 22 May 2017

Tens of thousands of people are being infected with a potentially deadly illness after eating imported meat riddled with what has been described as '**Brexit** virus'.

The strain of hepatitis E, which used to be mainly a tropical disease but has now mutated to infect livestock, causes a flu-like illness and in severe circumstances, could cause death.

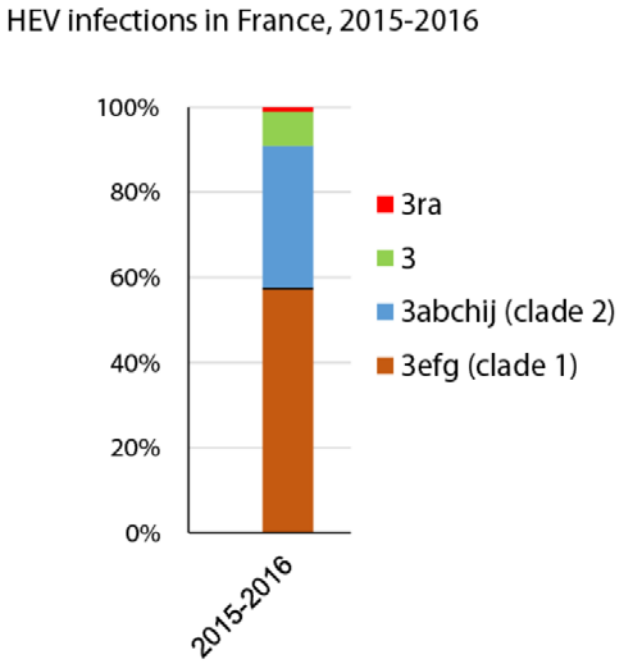
Experts predict 10 per cent of pork imported from Europe has the strain and they warn pregnant women and transplant patients to avoid the meat altogether.

The strain is linked to pig farms in France, Holland, Germany and Denmark is infecting more than 60,000 in Britain annually.

Scientists announced a new strain of hepatitis E (HEV) has spread to the UK through imported meat. Pregnant women and transplant patients are being warned not to eat pork because of the spread of 'Brexit virus'

HEV infections in France

- Five out of 919 HEV-infected patients in France were infected with 3ra variant (Abravanel et al, Emerg Inf Dis, in press)

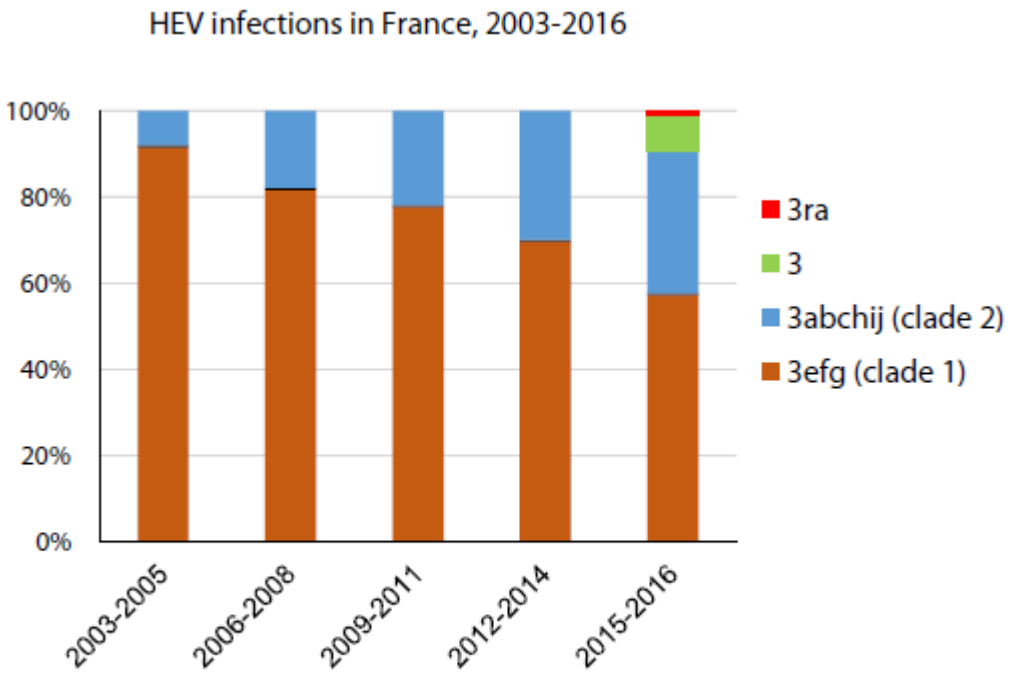


Data reconstructed from:

Abravanel et al, Emerg Inf Dis, in press (2015-2016)

HEV infections in France

- Five out of 919 HEV-infected patients in France were infected with 3ra variant (Abravanel et al, Emerg Inf Dis, in press)
- Slow transition of Clade 1 to Clade 2 strains seems to continue



Data reconstructed from:
L'Homme et al, 2015, Inf Genet Evol (2003-2014)
Abravanel et al, Emerg Inf Dis, in press (2015-2016)

Conclusions

- Pigs are the most likely source of human gt3 infections, but the exact soucres and route(s) of infection are uncertain
- More direct evidence needed: from HEV RNA to animal infectivity studies
- Even more needed: reduce HEV in the food chain
- Until the source of HEV is reduced, countries with high HEV incidence should consider screening

Acknowledgements

Sanquin
Michel Molier
Peter van Swieten
Anneke de Vos
Mart Janssen
Hans Zaaijer

Central Veterinary Institute NL
Wim van der Poel
Renate Hakze

Dutch Institute for Public Health
Sofie Mooij
Winfrid van Pelt

Public Health England
Richard Tedder
Samreen Ijaz

Irish Blood Transfusion Service
Joan O'Riordan
Niamh O'Flaherty

Frequency of HEV-viremic blood donors in West Pacific region

Ai Leen ANG (Health Sciences Authority, Singapore)

Faddy Helen (Australian Red Cross)

Masahiro Satake (Japanese Red Cross)

HEV NAT trial in Tokyo metropolitan area using ID-NAT format

Testing period

- Week days in April ~ June 2016 (totally 34 days)

Samples

- Randomly selected samples in Tokyo metropolitan area

NAT

- Device: PANTHER System by Grifols
- Reagent: Procleix HEV Assay, sensitivity 7.1 IU/mL

Screening	Confirmatory test			Determination
NAT sample	NAT sample	Plasma bag		
Positive	Positive	-	-	Positive
	Negative	Positive	Positive	
		Positive	Negative	
	Negative	Negative	Negative	Negative

Results of ID-NAT trial for HEV

	Tokyo	Hokkaido
Period	Apr – Jun, 2016	Aug, 2014 – Apr, 2016
No. tested	15,039	444,892
Initial reactive	12	
Repeat reactive	**11**	**158**
Invalid;	128 (0.84%)	
Internal control low;	9 (0.06%)	
Positivity rate	0.073 %	0.036 %
	1 / 1,367	1 / 2,816
genotypes	All G3	G3;123 G4;13
IgG prevalence	8.6 %	3.9 %

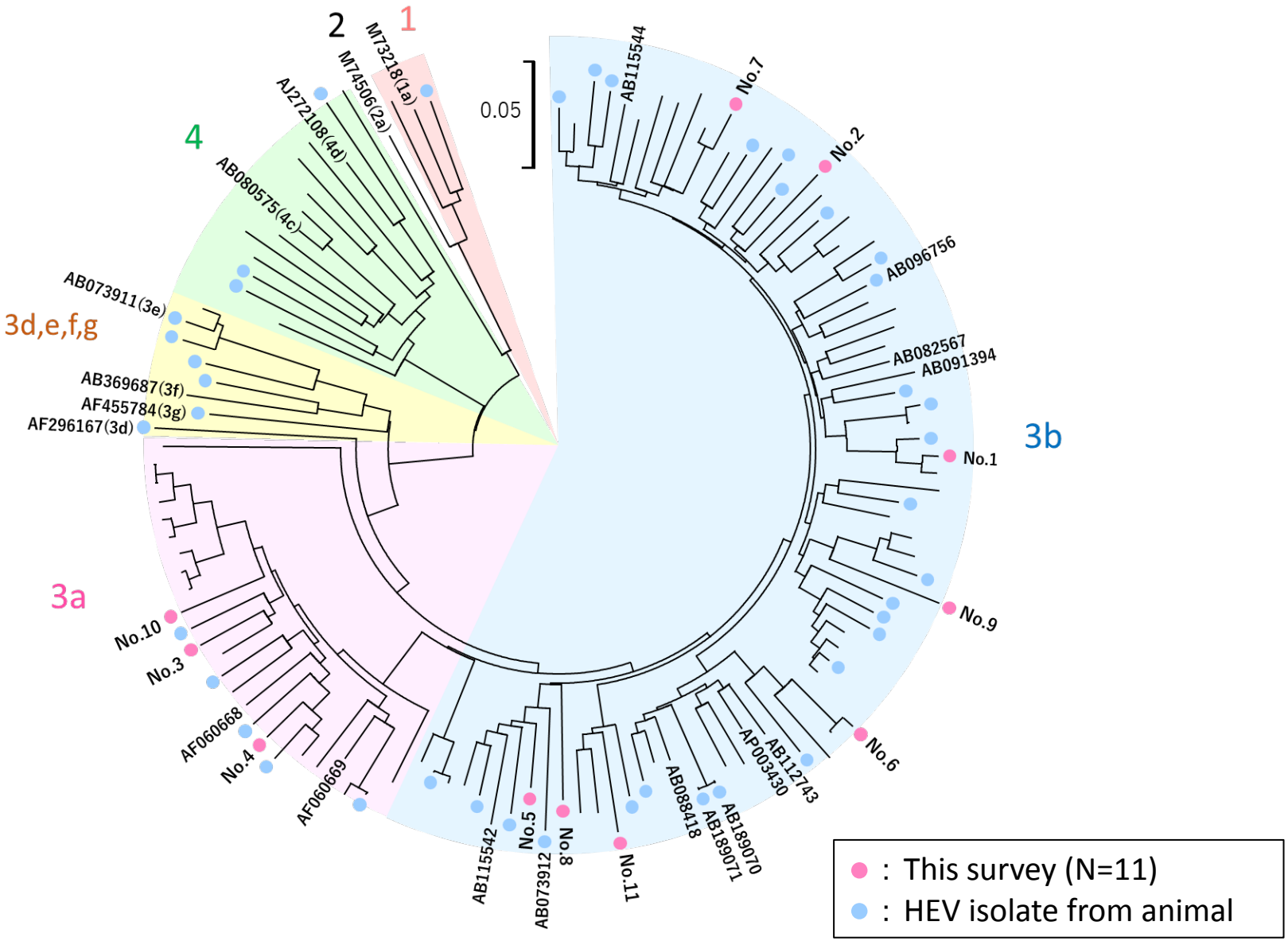
HEV-RNA prevalence in the previous study in Japan

		Tokyo	Hokkaido
20 pool-NAT Sensitivity ; 1,020 IU/mL		2006	2005 - 2010
	No. screened	44,332	1,628,908
	Positives	3 G3 ; 3	194 G3 ; 180 G4 ; 11
	Rate	0.0068% 1／14,800	0.012 % 1/8,400
IgG positivity rate		8.6 %	3.9 %

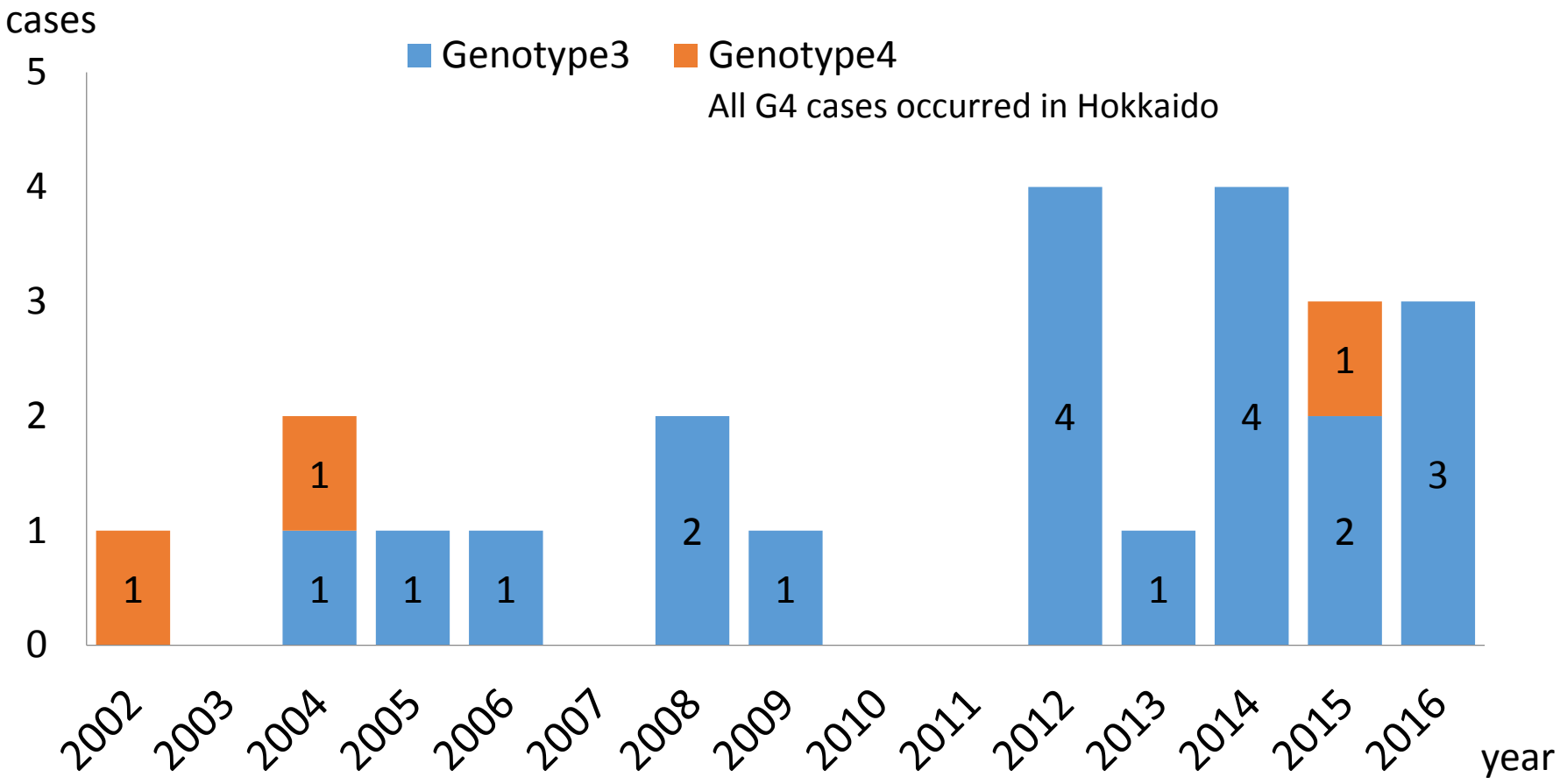
HEV NAT-positive donors

	Age/ gender	Geno-type	HEV RNA log IU /mL	IgM	IgA	IgG	ALT IU/L	Nearest strain (similarity %)					
								Human HEV			Swine HEV		
1	20s M	3b	3.6	0.20	0.07	0.42	17	LC086313	Japan	(98.5)	AB578963	Japan	(97.3)
2	20s F	3a	2.0	0.02	0.02	0.01	14	AB525054	Japan	(93.2)	AB194486	Japan	(93.0)
3	20s F	3a	**4.0**	0.08	0.02	0.09	8	AB671041	Japan	(94.9)	AB094214	Japan	(93.2)
4	30s F	3b	3.9	0.94	**1.25**	**2.94**	32	KT718043	USA	(96.6)	KU130391	China	(95.1)
5	30s F	3b	2.5	0.05	0.02	0.21	16	AB288364	Japan	(95.4)	AB177357	Japan	(95.6)
6	30s M	3b	ND	**4.56**	**1.50**	**11.43**	19	LC055639	Japan	(99.0)	AB471978	Japan	(92.2)
7	50s M	3b	2.8	0.07	0.02	0.11	25	AB288357	Japan	(98.1)	AB194514	Japan	(92.9)
8	50s M	3b	1.6	**3.25**	0.71	**4.56**	33	AB671025	Japan	(93.4)	AB668382	Japan	(92.9)
9	50s M	3b	3.1	0.02	0.02	0.03	51	AB671028	Japan	(92.7)	AB605227	Japan	(92.0)
10	50s M	3a	1.8	0.30	0.76	5.99	28	AB115541	Japan	(93.9)	AB094242	Japan	(92.9)
11	60s M	3b	2.0	**3.33**	**4.23**	**10.01**	46	AB671041	Japan	(94.9)	KJ507956	Canada	(93.0)

Phylogenetic tree of HEV strain based on 412nt of ORF2



Annual changes of TT-HEV cases



Health insurance reimbursement for IgA-HEVAb testing

HEV viraemia among blood donors (Singapore)

- Study period: 20 Feb to 26 Mar 2017
- Total number of donations tested: 12,541
- Testing system: Procleix assay (Procleix Panther system), individual donation testing

HEV viraemia among blood donors (Singapore)

28 positive samples

- 17 repeat reactive:

  all positive by HEV NAT confirmatory test at Sanquin

- 11 initial reactive
  - 2 weak positive (low level viraemia) on HEV NAT confirmatory test at Sanquin (95% LOD of 10 IU/mL)

    HEV IgM/IgG +ve (ELISA -Mikrogen and Wantai)

  - 8 negative on HEV NAT confirmatory test at Sanquin

    HEV IgM/IgG –ve

    7 donors with ≥ 4 weeks follow up; HEV NAT –ve, HEV IgM/IgG –ve

  - 1 negative on HEV NAT confirmatory test at Sanquin

    HEV IgM –ve and HEV IgG +ve

    Follow up 4 weeks later: HEV NAT –ve, HEV IgM –ve, HEV IgG +ve

HEV viraemia among blood donors (Singapore)

28 positive samples

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    HEV IgM –ve and HEV IgG +ve

    Follow up 4 weeks later: HEV NAT –ve, HEV IgM –ve, HEV IgG +ve

HEV viraemia among blood donors (Singapore)

- HEV viraemia rate of 1:660

  (19 true positive, 9 false positive)

  Viraemia load for true positives:

  median 33 (range 3 to $3.19X10^5$) IU/mL (only 1 sample was $> 10^4$ IU/mL

  Genotyping pending

- Ongoing evaluation using Risk-based Decision Making Framework for Blood Safety

HEV: Australian perspective

- HEV seroprevalence in blood donors ~6%[1]
  - HEV IgG in donors with no overseas travel
- Majority of overseas-acquired HEV infections were in travelers to South Asian countries, which are subject to donation-related travel restrictions (malaria)[2]
- Rates of HEV viremia:
  - 1 in 14,799 donations (all donation types)[3]
  - 1 in 74,131 donations (whole blood only)[4]
- Transfusion-transmission recently documented[5]

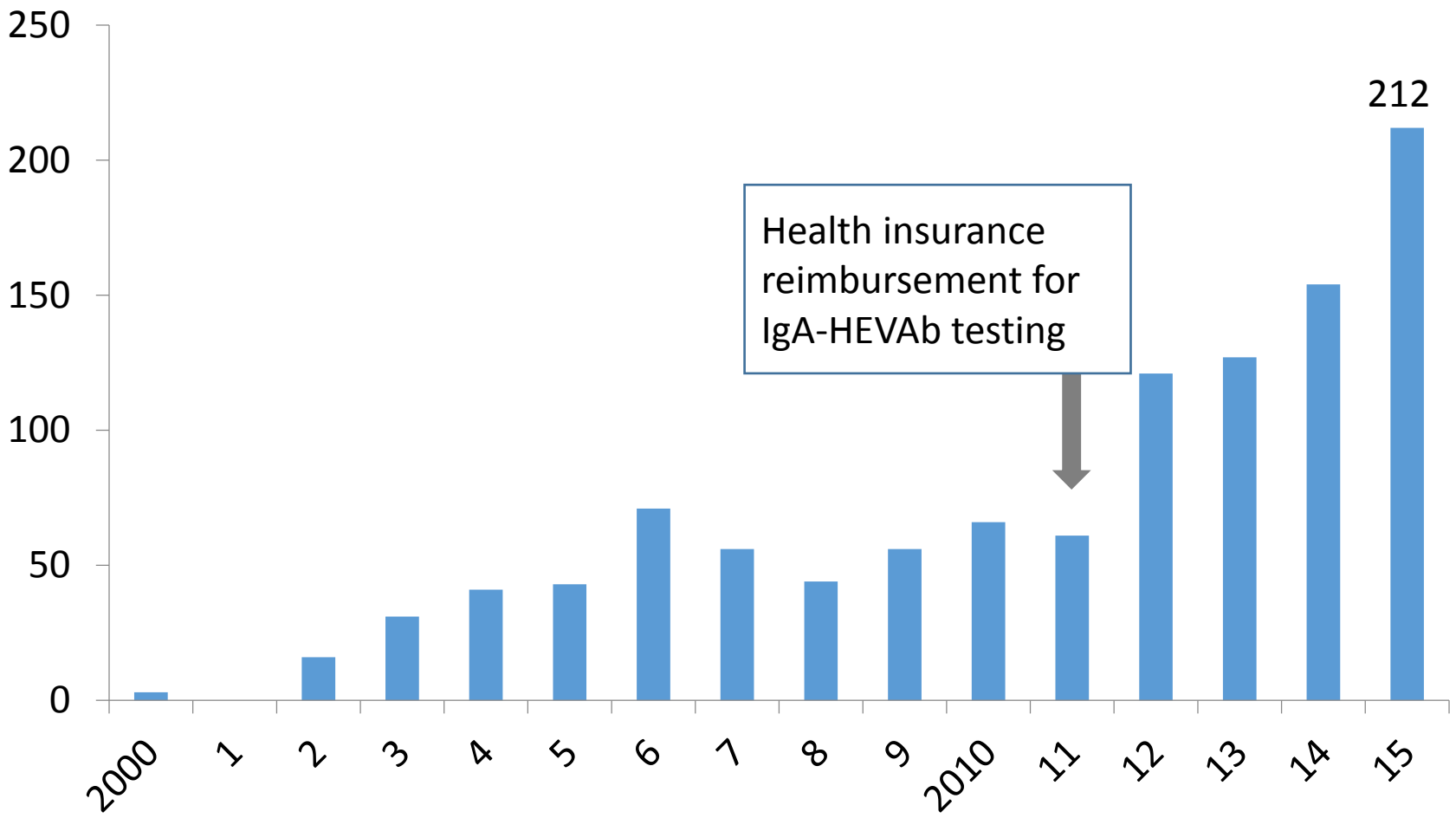
  overseas travel-related

1. Shrestha A, et al., Emerg Infect Dis 2014;20: 1940-2; 2. Shrestha Q, et al., Blood Transfus DOI 10.2450/2016.0064-16; 3. Shrestha A, et al., Transfusion. 2016 Dec;56(12):3086-3093; 4. Fryk J., et al., Pathology 49, S114-S115, 2017; 5. Hoad H, et al., Med J Aust. 2017 Apr 17;206(7):289-290

Rates of HEV viremia

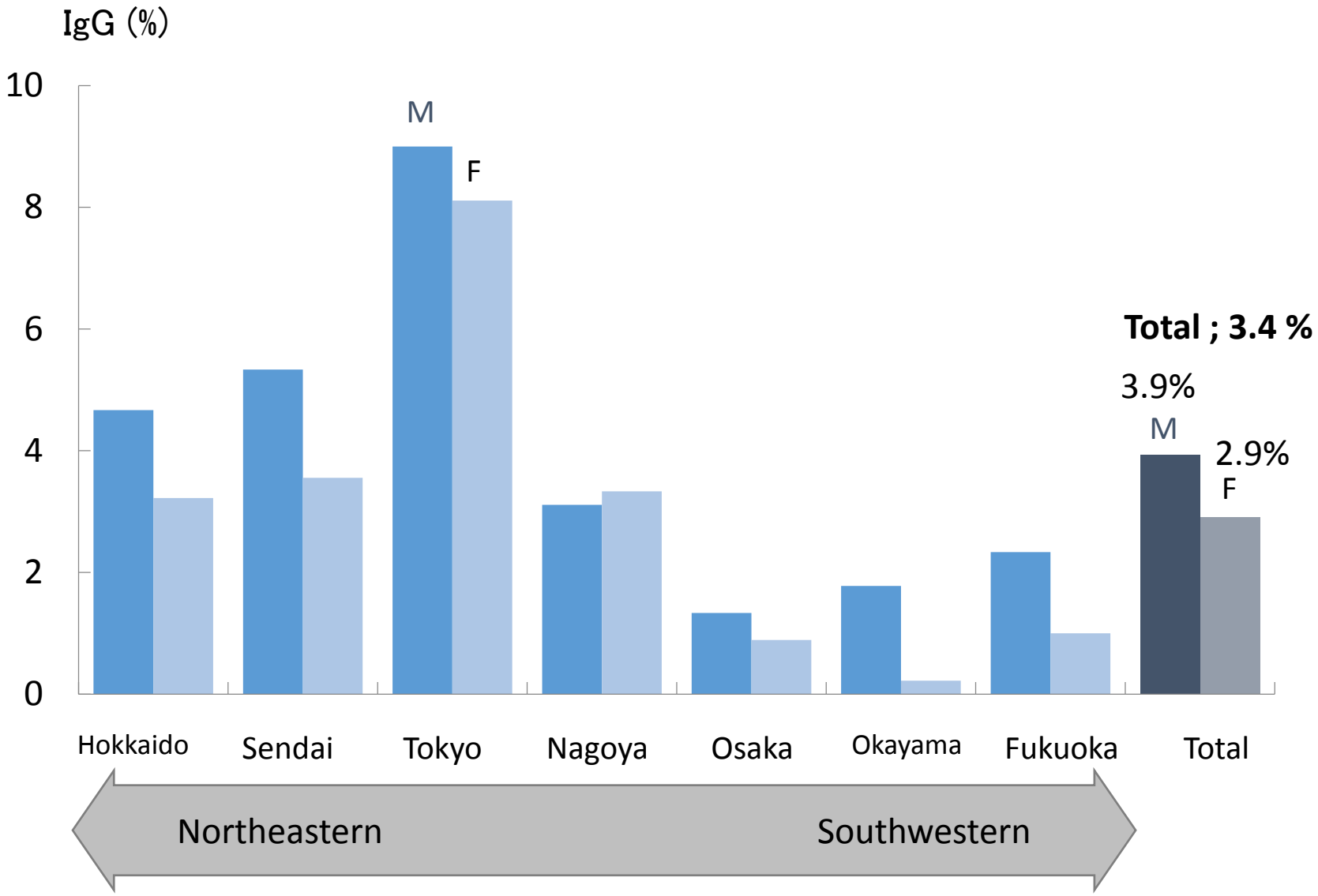
- Singapore 1:660
- Japan 1:1367 (Tokyo)
  1:2816 (Hokkaido)
- Australia 1:14,799 donations (all donation types)
  1:74,131 donations (whole blood only)

Change in the number of Hepatitis E cases in Japan



HEV-IgG positivity rate in the whole country of Japan

H. Takeda, H. Ikeda et al. Vox Sang 2010; 99: 307



Estimation of TT-HEV frequency
based on viremic donor frequency in Tokyo metropolitan area

Frequency of HEV viremic donors	0.073%
No. of blood donors in the area	1.8 million/y
Estimated no. of HEV viremic donors in the area	1,314/y
Infectivity of blood component contaminated with HEV	50 %
Estimated no. of TT-HEV	**657/y** in greater Tokyo area

No. of TT-HEV reported in the area	ca. 1 case/y