



Fundação Oswaldo Cruz (Fiocruz)
Oswaldo Cruz Foundation

The use of non-invasive clinical specimens as a tool for epidemiological surveillance of Zika virus

Patricia C Sequeira, PhD



Ministério da Saúde

FIOCRUZ
Fundação Oswaldo Cruz



Oswaldo Cruz
(1872-1917)



Oswaldo Cruz Foundation FIOCRUZ

STAFF: >12,000 / 1,100 PHD

RESEARCH: >1,500 projects

PUBLICATIONS: > 1,500 papers per
year

TEACHING: >7 thousand students

PRODUCTION:

>130 million doses /vaccines

> 9 million diagnostic kits

> 4 billion medicines

> 17 million biopharmaceuticals

FIOCRUZ areas of expertise



Research

Education

Innovation and Production

Surveillance and Reference Services

Health & Medical Care

Environment and Health Promotion

Information and Communication

History of Science and Health

Planning and Management



PRESENT SITES

- Rio de Janeiro
- Belo Horizonte
- Recife
- Salvador
- Manaus
- Porto Velho
- Curitiba
- Brasília

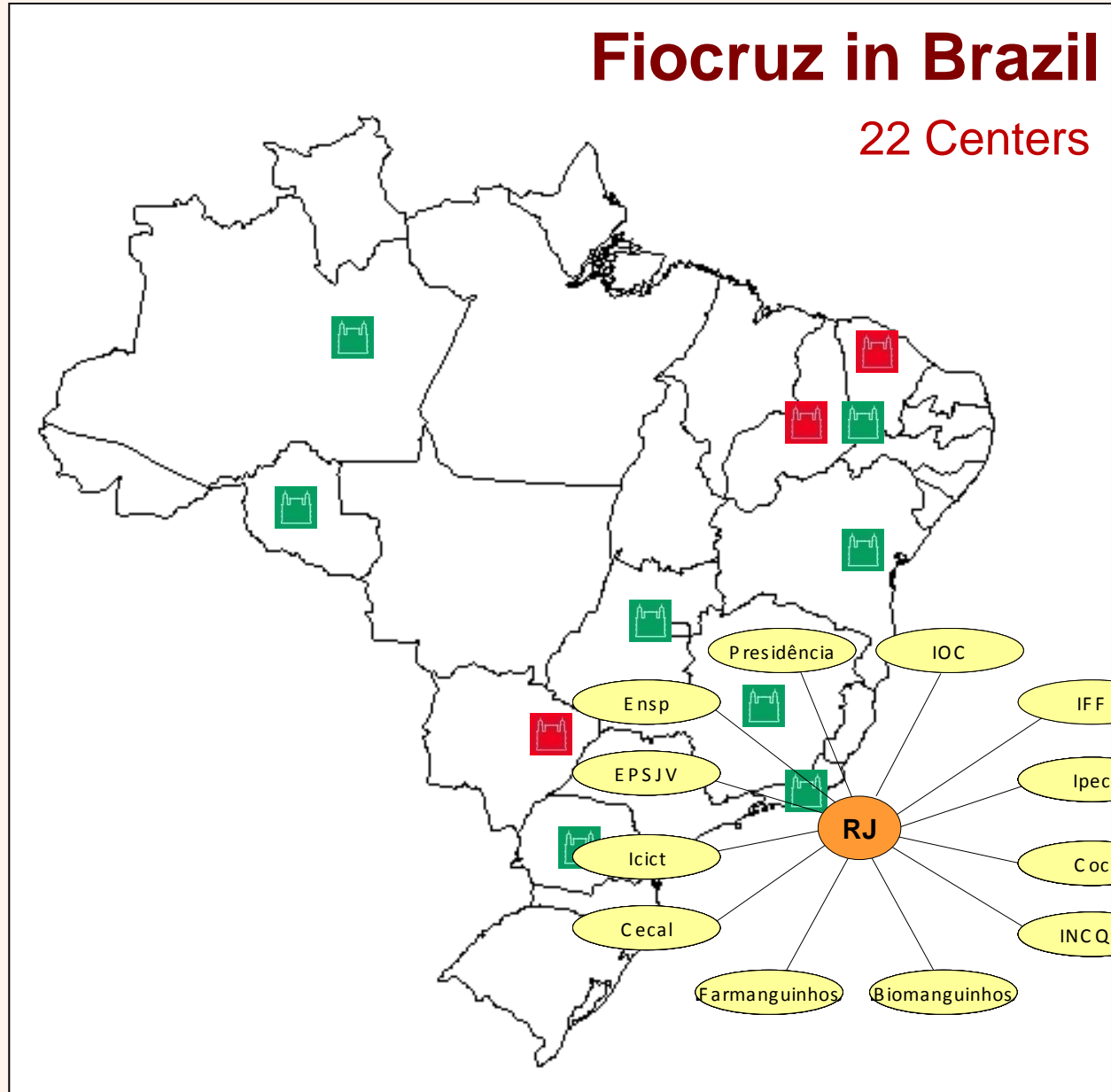


PLANNED SITES

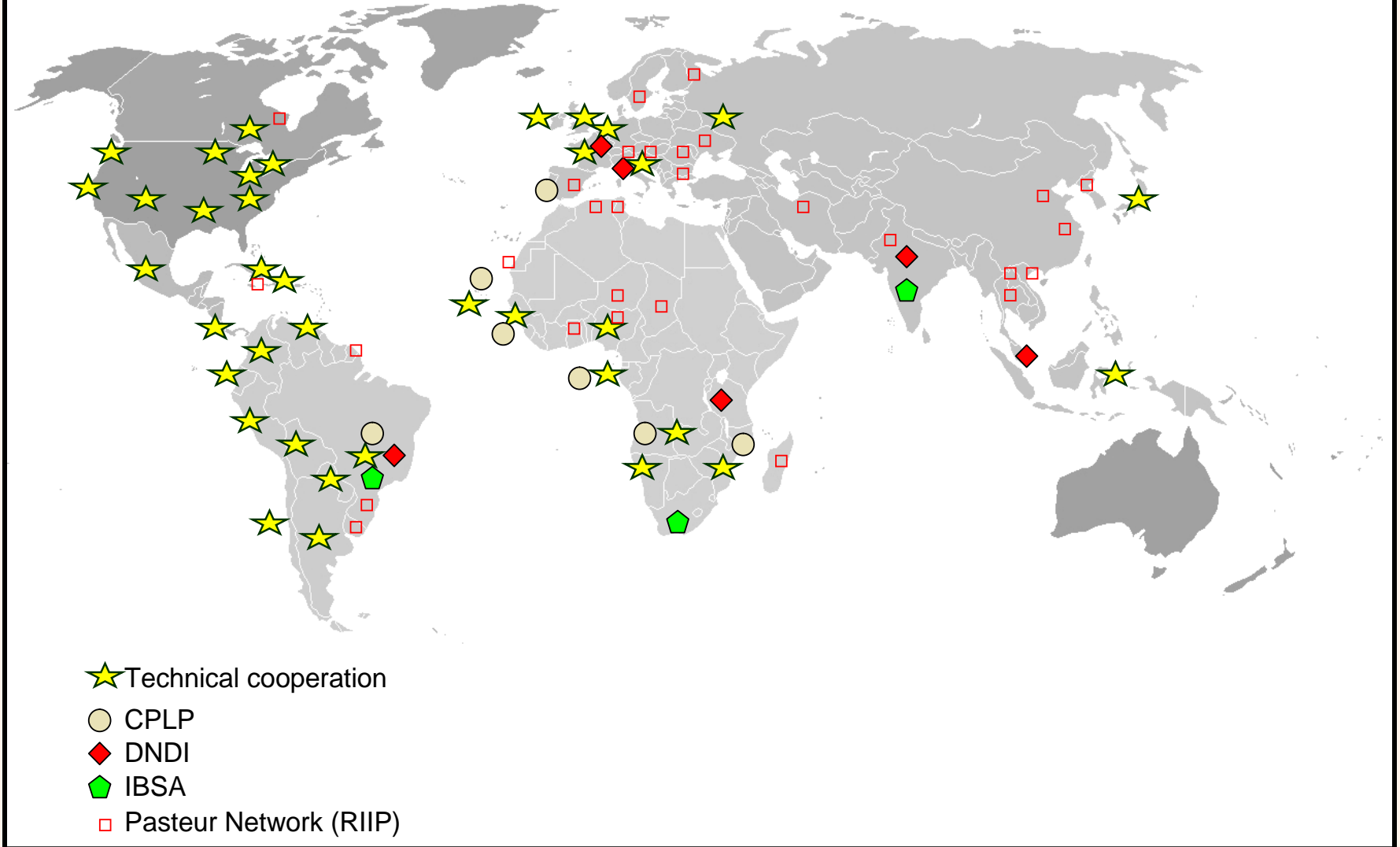
- Campo Grande
- Fortaleza
- Teresin

Fiocruz in Brazil

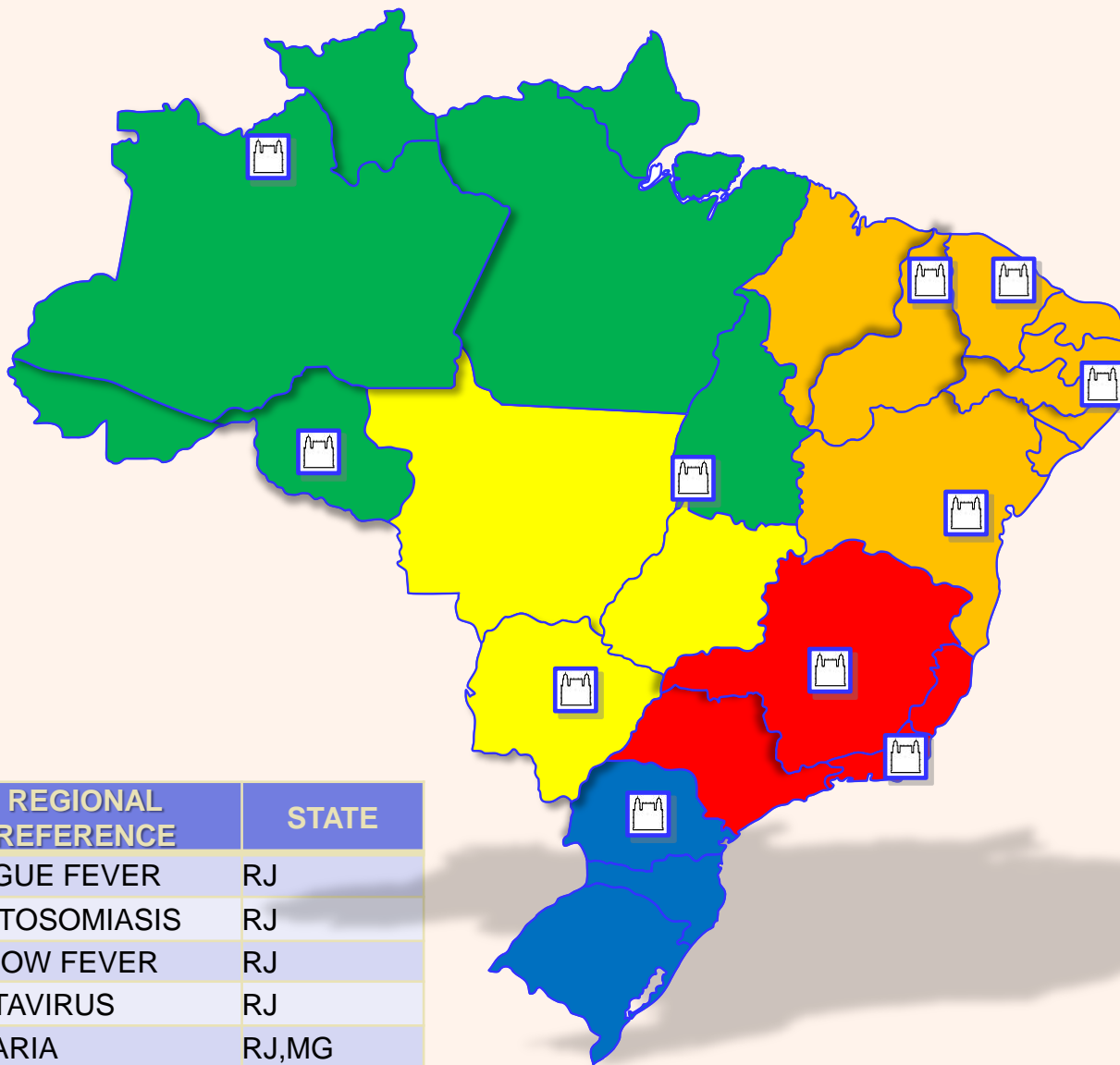
22 Centers



International Cooperation



The Reference Laboratories hosted by Fiocruz

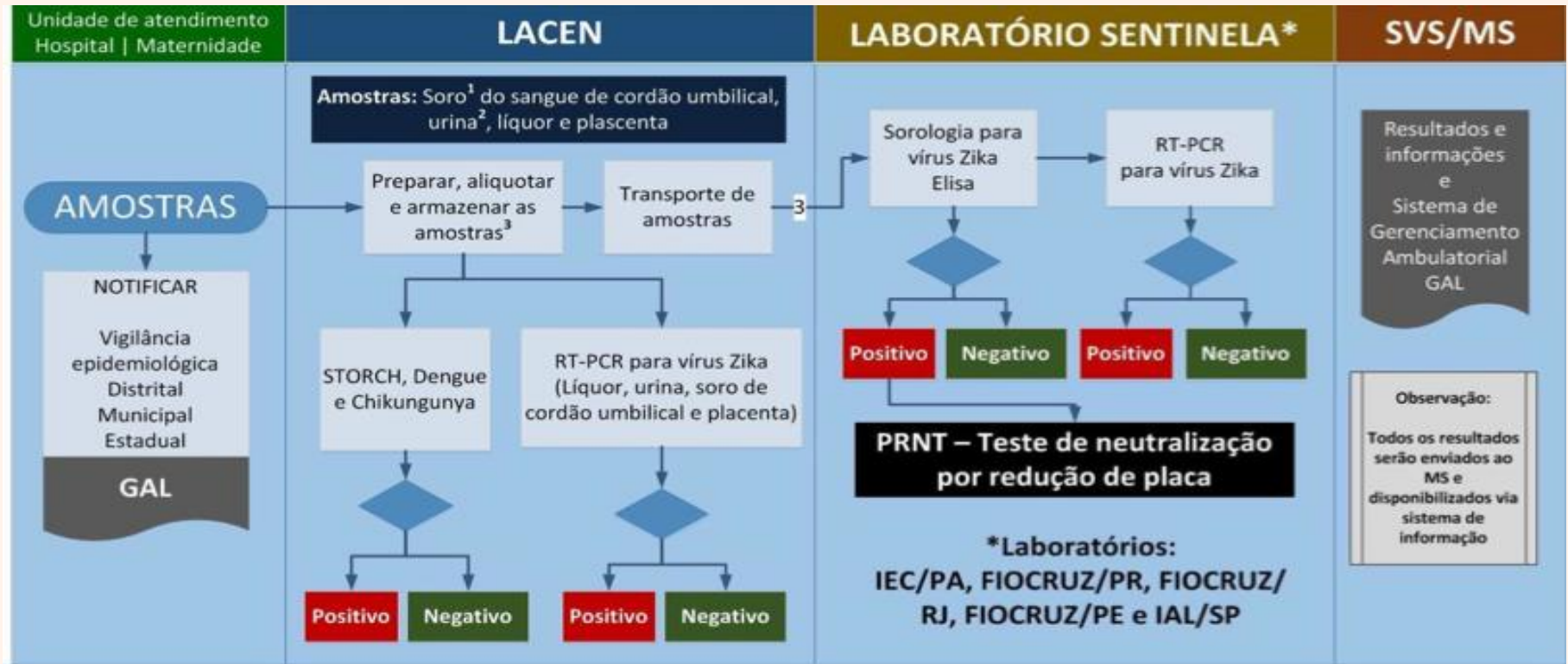


NATIONAL REFERENCE	STATE
ANTHRAX	RJ
CHAGAS DISEASE	RJ, MG, PE
BACTERIAL DIARRHOEAL DISEASES	RJ
SCHISTOSOMIASIS	RJ, MG
FILARIASIS	RJ, PE
INFLUENZA	RJ
VIRAL HEPATITIS	RJ, BA
HIDATIDOSIS	RJ
LEISHMANIASIS	RJ, MG, PE, BA
LEPTOSPIROSIS	RJ
SYSTEMIC MYCOSIS	RJ
PLAGUE	PE
POLIOMYELITIS	RJ
RICKETTSIOSIS	RJ
TUBERCULOSIS	RJ
RUBELLA/MEASLES	RJ
AIDS	RJ
LEPROSY	RJ
SARS	RJ

REGIONAL REFERENCE	STATE
DENGUE FEVER	RJ
SHISTOSOMIASIS	RJ
YELLOW FEVER	RJ
HANTAVIRUS	RJ
MALARIA	RJ, MG
ROTA VIRUS	RJ, PE

24 Diseases
 50 Reference Laboratories
 7 Outpatient clinics

Algorithm for testing of microcephaly suspected samples



Source: Ministry of Health, Brazil

Flavivirus Laboratory

Regional Reference Center for Dengue, Zika, Yellow Fever and Chikungunya diagnosis

Ana Maria Bispo de Filippis, PhD - Lab Head

Rita Maria R. Nogueira, PhD - Research Scientist

Patrícia C Sequeira, PhD - Research Scientist

Marcos C Mendonça, PhD - Research Scientist

Maria Angélica Mares-Guia, PhD - Research Scientist

Eliane M de Araújo, MSc - Research Associate

Simone A Sampaio, MSc - Research Associate

Leda M Santos, BsC - Research Associate

Jose Carlos Silva, BsC - Research Associate

Carolina Santos, BsC - Research Associate

Everton Rodrigues, BsC - Research Associate

Marcele Santos, BsC - Research Associate

Sheila Cheles, BsC - Biosafety officer

Bianca de Santis - PhD Candidate

Celeste Torres – PhD Student

Alisson Fabri - MSc Student

Cíntia Damasceno - MSc Student

Aline Santos - MSc Student

Flávia Levy - MSc Student

Mateus Felipe – Undergrad student

Maria Luiza – Undergrad student

Solange Regina - Secretary

Ronaldo Lapa – Lab support

Visiting Scholars:

Raquel Medialdea-Carrera – PhD student/

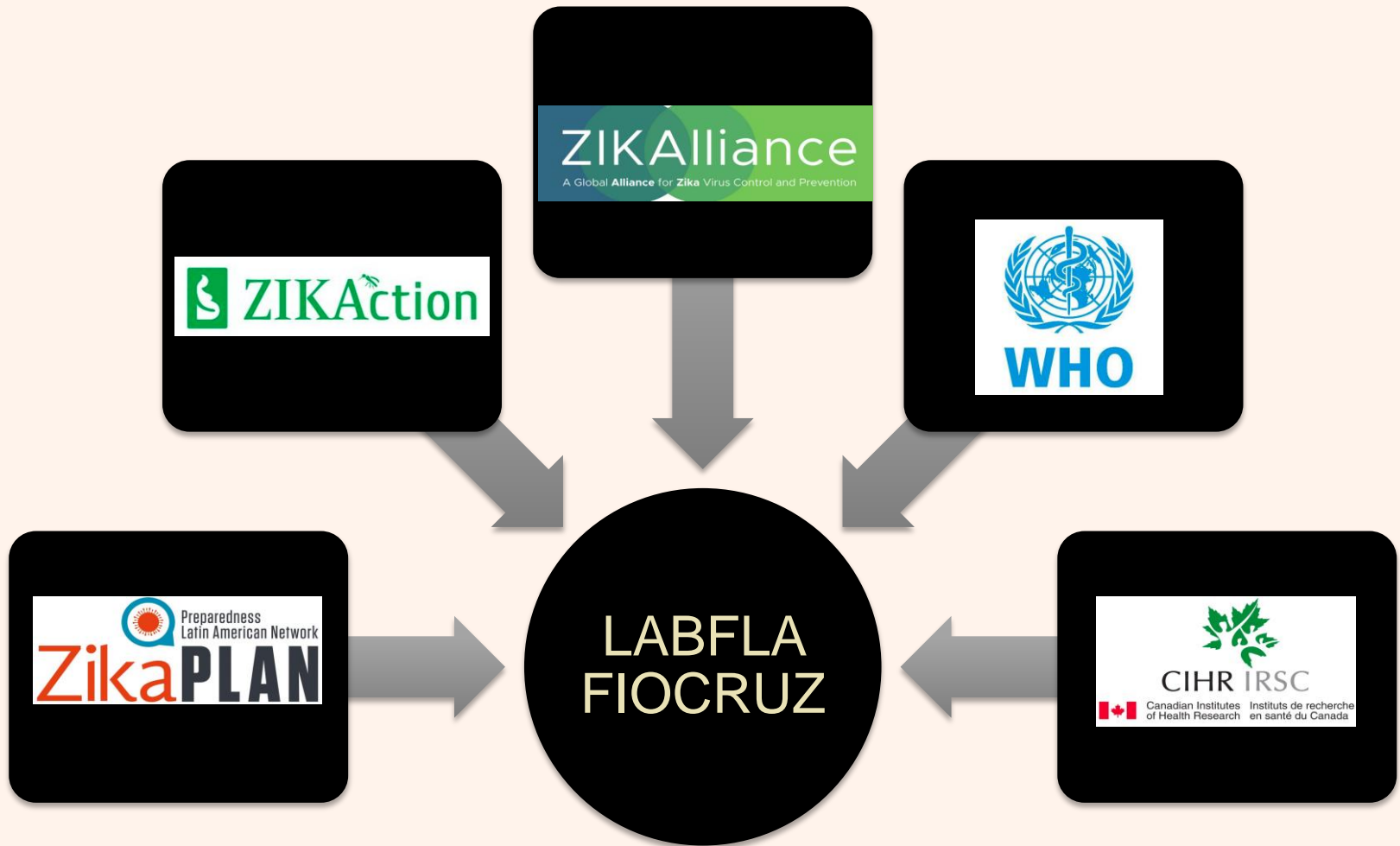
University of Liverpool

Ravi Mehta – MD/ University of Liverpool

Scientific advisor:

David Brown

Flavivirus Laboratory international collaborations:



Zika virus in Brazil

■ Whole viral genome sequencing

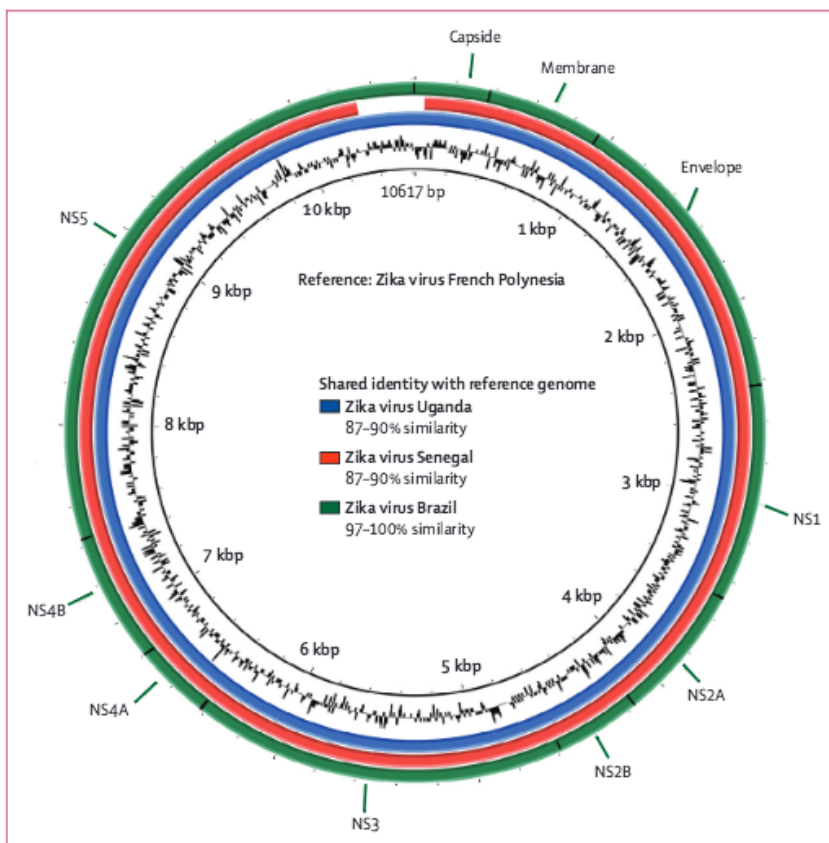


Figure 1: Comparative genome BLAST Atlas diagram of Zika virus
 The green outer circle corresponds to the complete Brazilian Zika virus genome isolated from the amniotic fluid of patient 1. 10 793 bases were sequenced. The red circle corresponds to the Senegal (KF383118.1) strain of Zika virus and the blue circle corresponds to the Uganda strain (NC_012532.1). The percentage deviation in GC content between the Brazilian Zika virus and the reference Zika virus is represented along the Zika virus genome by the varying heights of the black bars. The innermost (black) circle corresponds to the reference genome (French Polynesia, KJ776791.1). Genome shared identity between each strain and the reference genome are shown as percentages. BLAST= basic local alignment search tool.

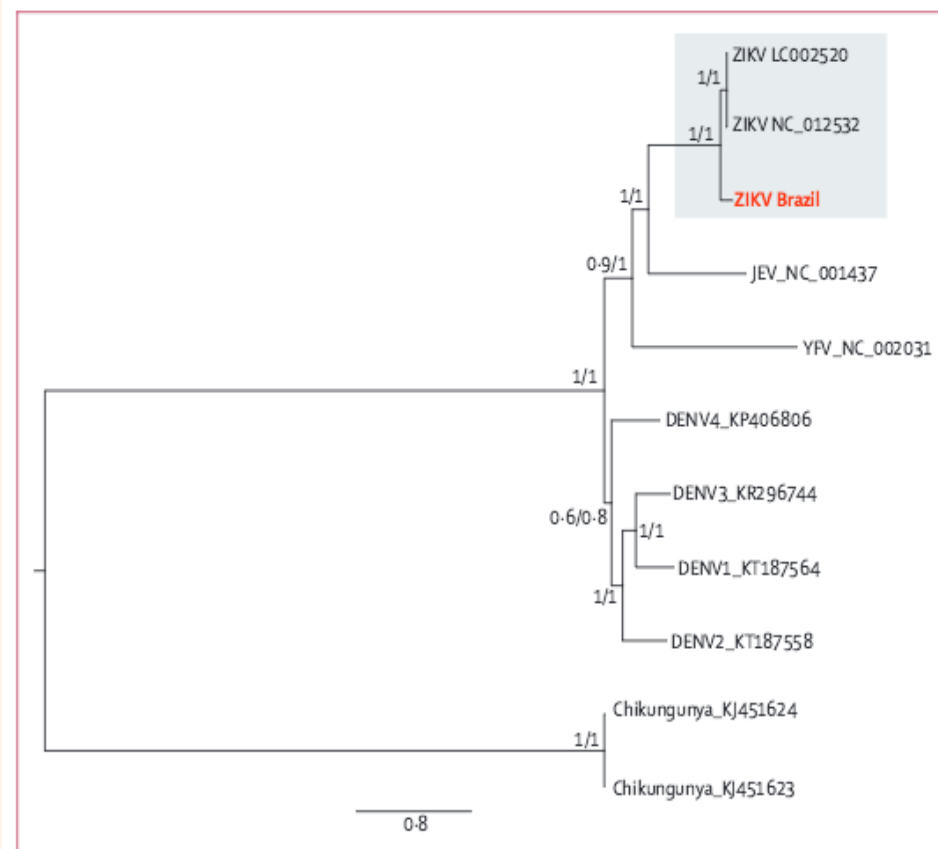


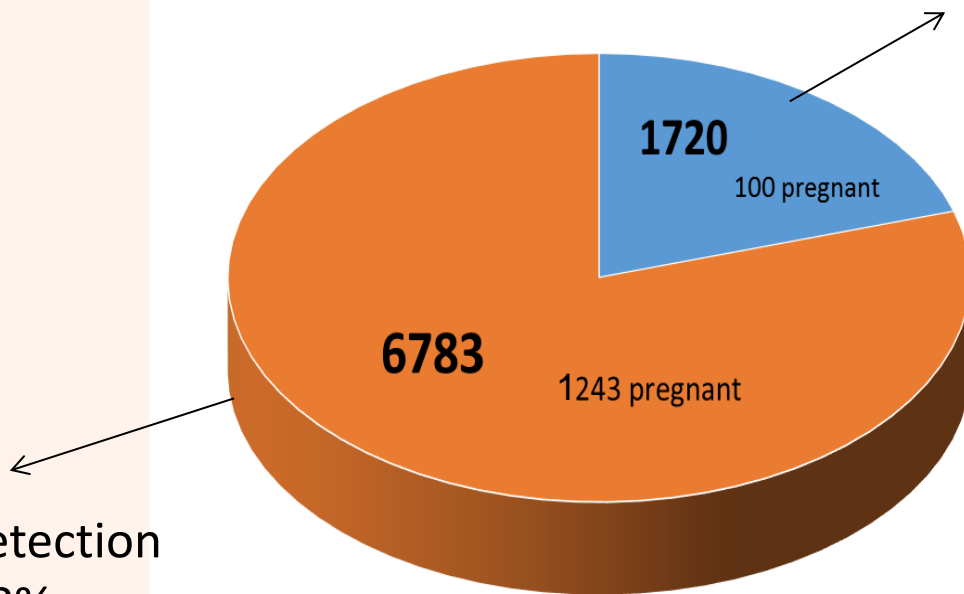
Figure 4: Maximum likelihood phylogeny of Brazilian Zika virus, other Flaviviridae genomes, and an alphavirus genome
 Brazilian Zika virus (in red) was isolated from the amniotic fluid of patient 1, whose fetus was diagnosed with microcephaly. Approximate likelihood-ratio test and Bayesian inference support values are shown at nodes. Chikungunya is an alphavirus; all other viruses are from the Flaviviridae family. DENV=dengue virus. JEV=Japanese encephalitis virus. YFV=yellow fever virus. ZIKV=Zika virus.

Zika virus surveillance in the state of Rio de Janeiro

Flavivirus Laboratory – FIOCRUZ

Samples from suspected ZIKV cases, 2015-2016

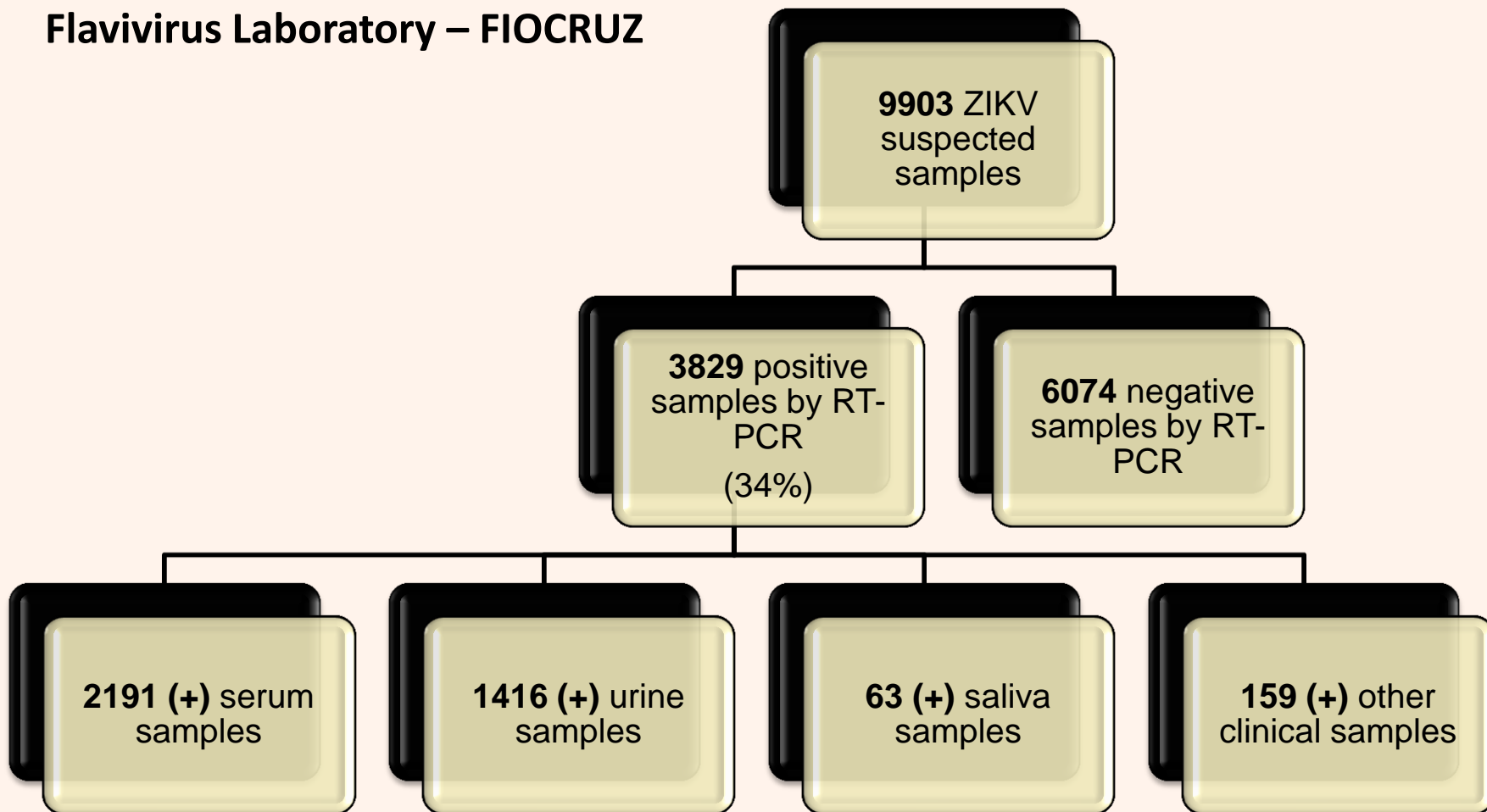
ZIKV detection rate: 29%



■ 2015 ■ 2016*

Zika virus surveillance in the state of Rio de Janeiro

Flavivirus Laboratory – FIOCRUZ



Zika virus surveillance in the state of Rio de Janeiro

ZIKV Detection in Alternative Samples

Specimen	Days after the first symptoms	Result RT-qPCR
Urine	0-18 days	Positive
Saliva	4 - 9 days	Positive
Amniotic fluid	20 weeks	Positive
Breast milk	3- 20 days	Positive
Semen	4 - 16 days	Positive
Vaginal secretion	3- 23 days	Positive
Cerebrospinal fluid (CSF)	4-9 days	Positive

Flavivirus Laboratory – FIOCRUZ

Zika virus surveillance in the state of Rio de Janeiro

Molecular diagnosis

Genetic and Serologic Properties of Zika Virus Associated with an Epidemic, Yap State, Micronesia, 2007

Robert S. Lanciotti,* Olga L. Kosoy,* Janeen J. Laven,* Jason O. Velez,* Amy J. Lambert,* Alison J. Johnson,* Stephanie M. Stanfield,* and Mark R. Duffy*

Table 3. Description and performance characteristics of Zika virus real-time RT-PCR primer/probe sets*

Primer	Genome position†	Sequence (5' → 3')	Sensitivity, no. copies	Specificity‡
ZIKV 1086	1086–1102	CCGCTGCCCCAACACAAG	25	ZIKV
ZIKV 1162c	1162–1139	CCACTAACGTTCTTTGCAGACAT		
ZIKV 1107-FAM	1107–1137	AGCCTACCTTGACAAGCAGTCAGACACTCAA		

*RT-PCR, reverse transcription-PCR; ZIKV, Zika virus.
 †Based on ZIKV MR 796 GenBank accession no. AY832536.
 ‡ZIKV specificity indicates a positive result with ZIKV only and no reactivity with dengue virus-1 (DENV-1), DENV-2, DENV-3, DENV-4, West Nile virus, St. Louis encephalitis virus, yellow fever virus, Powassan virus, Semliki Forest virus, o'nyong-nyong virus, chikungunya virus, and Spondweni virus.

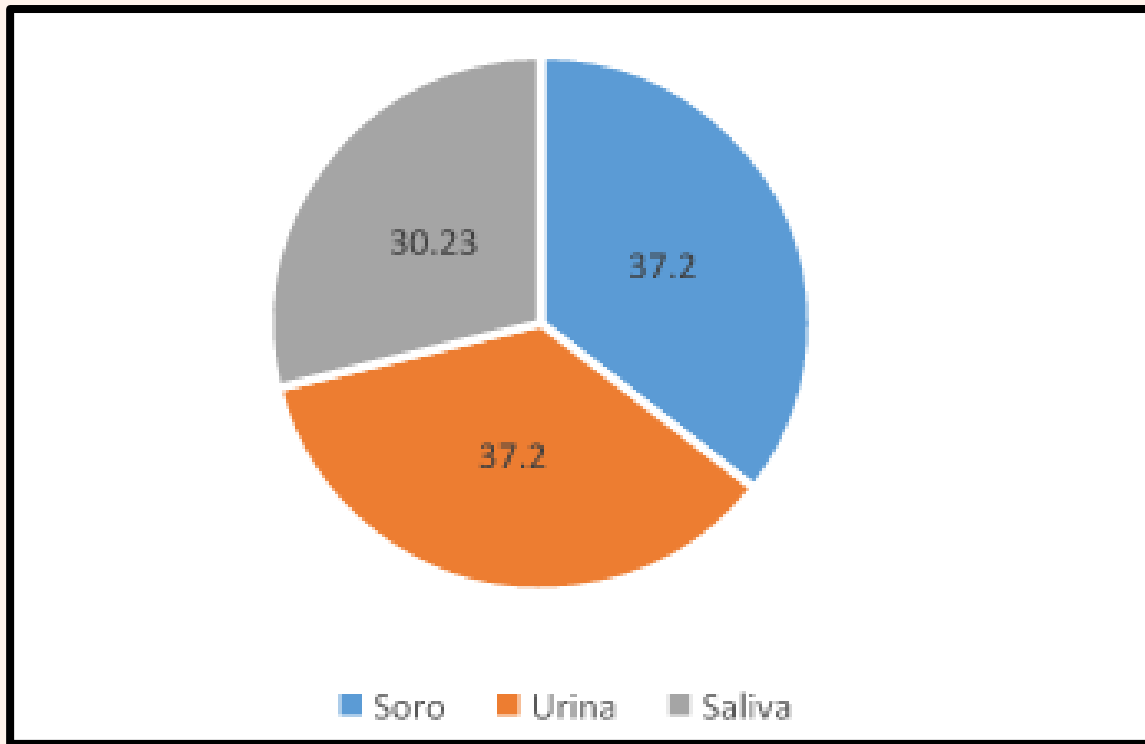
Gold standard for molecular diagnosis of ZIKV

Preliminary results

	No of positive samples (real-time RT-PCR)	Average day post infection	Ct value range (real-time RT-PCR)
Serum	16	3 rd	26 - 37
Saliva	13	5 th	28 - 38
Urine	16	8 th	28 - 38
TOTAL	45		

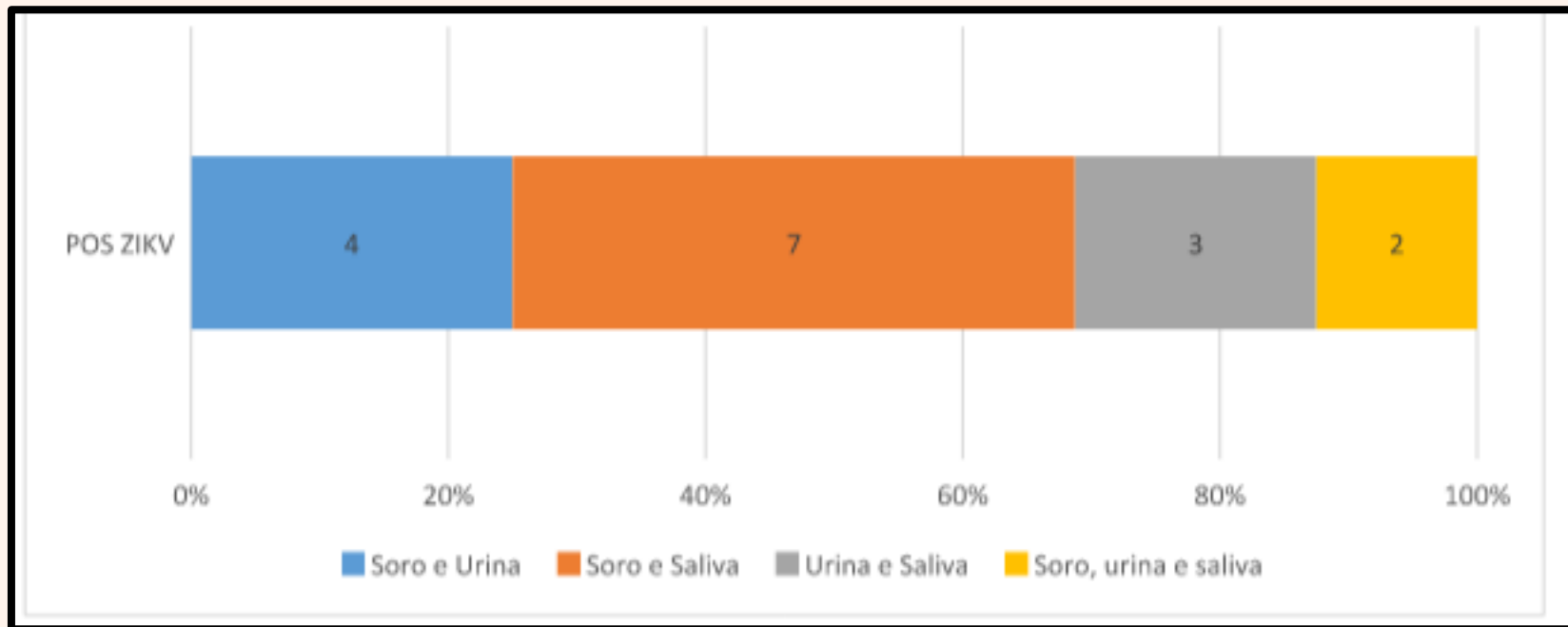
Preliminary results

Percentage of ZIKV RNA detection in serum, urine and saliva



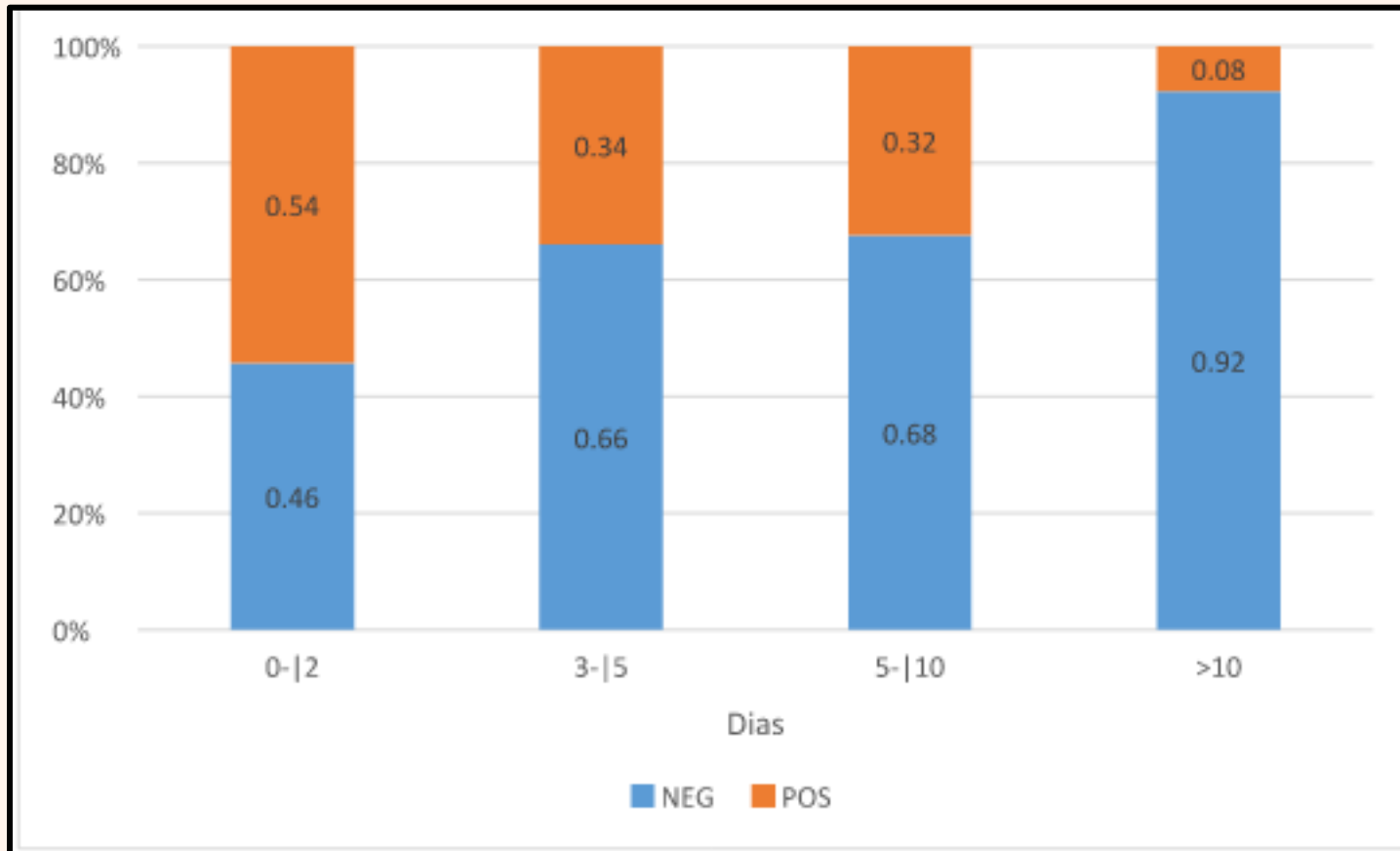
Preliminary results

Correlation between the type of clinical specimens and ZIKV detection



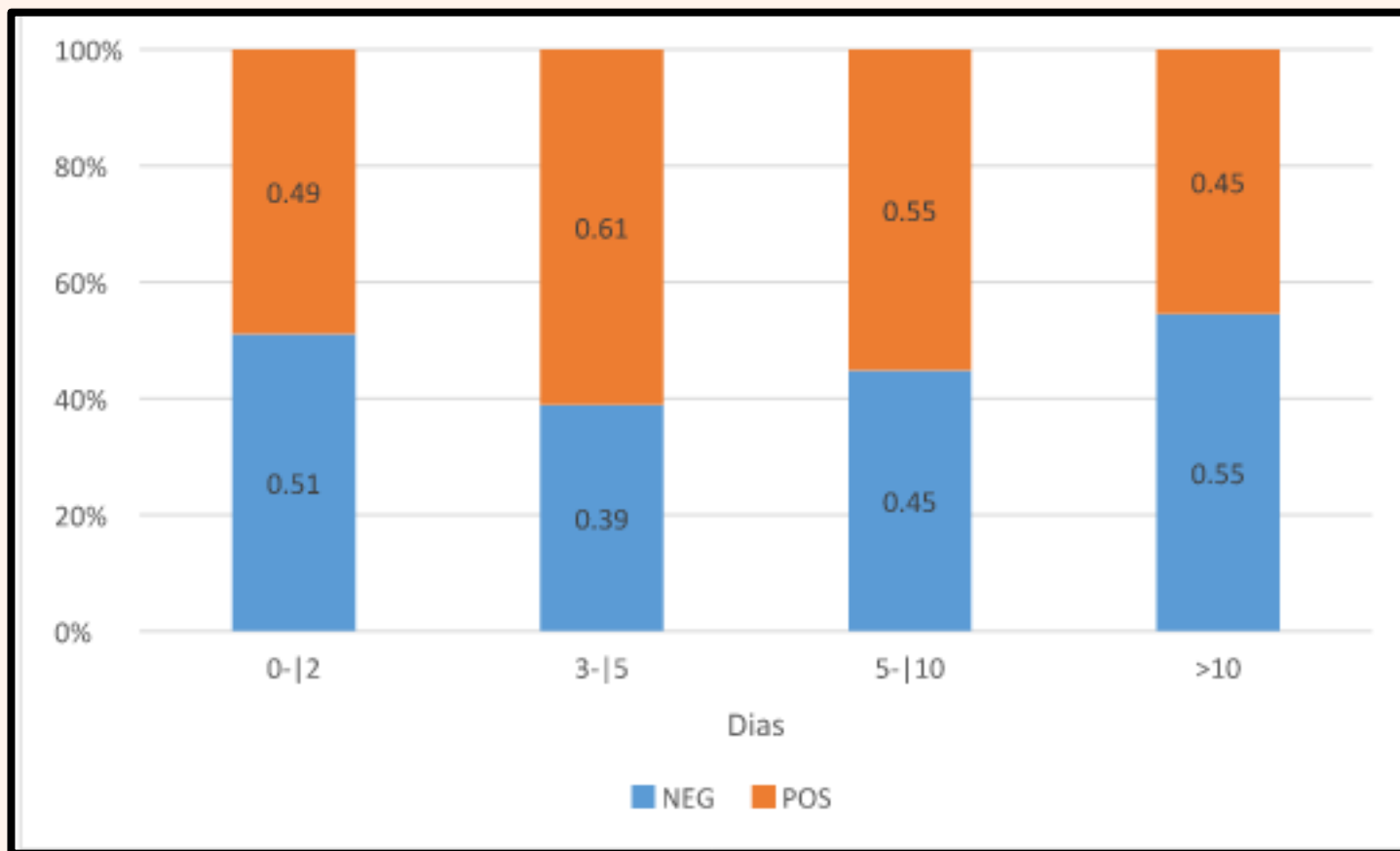
Preliminary results

Correlation between days after initial onset of symptoms and ZIKV RNA detection in serum



Preliminary results

Correlation between days after initial onset of symptoms and ZIKV RNA detection in urine



THE NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Persistence of Zika Virus in Body Fluids — Preliminary Report

Gabriela Paz-Bailey, M.D., Ph.D., Eli S. Rosenberg, Ph.D., Kate Doyle, M.P.H.,
Jorge Munoz-Jordan, Ph.D., Gilberto A. Santiago, Ph.D., Liore Klein, M.S.P.H.,
Janice Perez-Padilla, M.P.H., Freddy A. Medina, Ph.D.,
Stephen H. Waterman, M.D., M.P.H., Carlos Garcia Gubern, M.D.,
Luisa I. Alvarado, M.D., and Tyler M. Sharp, Ph.D.

Clinical specimen	Range of days of ZIKV RNA detection
Serum	14-54
Urine	8-39
Semen	34-81

Final considerations:

- ✓ Urine and saliva are useful non-invasive clinical specimens for ZIKV RNA detection;
- ✓ Prolonged ZIKV RNA detection in urine and saliva samples are important features for diagnosis and prevention of ZIKV;
- ✓ ZIKV RNA detection rates range from 30 to 37% in serum, urine and saliva samples;
- ✓ ZIKV RNA has been detected in serum, urine, saliva, semen, breast milk, amniotic fluid, vaginal secretion and CSF;
- ✓ The use of non-invasive clinical specimens collection for ZIKV diagnosis means an extra tool for epidemiological surveillance.
- ✓ Whole genome sequencing of viral RNA from different clinical specimen of one single patient will shed light into viral compartmentalization.

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Thank you



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