



# The challenge of Zika Virus Diagnosis after Acute Infection: Anti-Zika Virus NS1 Antibody ELISAs Exhibit poor Accuracy in Brazilian Patients

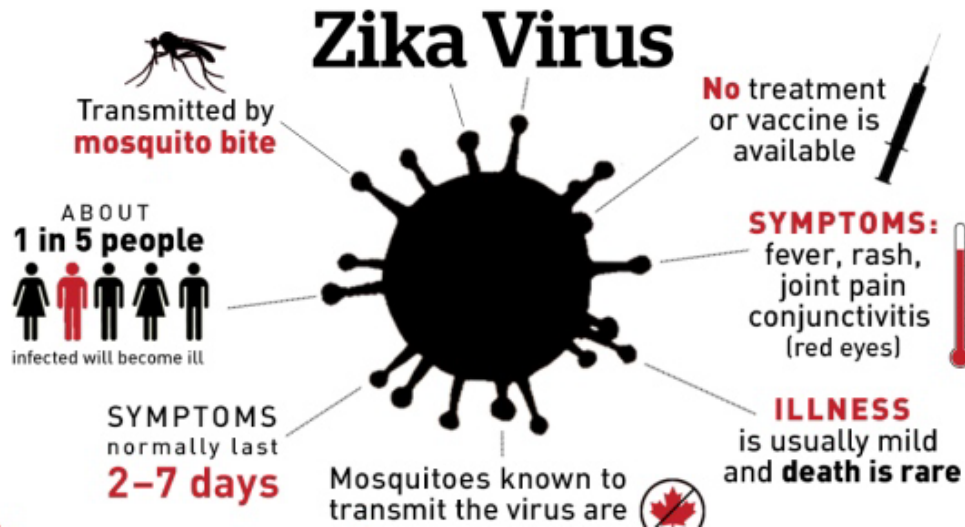
First International Zika Conference, Washington – 23<sup>rd</sup> February 2017

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# Introduction : Zika Virus



- First isolated in 1947 in **Uganda**
- First human case in Nigeria in 1952
- Outbreak in Yap Island (2007) and French Polynesia (2014)
- 2015 : WIDESPREAD EPIDEMIC in **Brazil**
- **Currently 76 countries worldwide reported mosquito-borne ZIKV transmission (WHO)**

# Introduction : Zika Virus

- Infection during pregnancy can cause ZIKV **congenital syndrome**
- Associated with **PNS** and **CNS disease** (GBS, meningoencephalitis, encephalitis, myelitis)

Very similar clinical symptoms with Chikungunya and Dengue infections

## **IMPORTANCE OF DIAGNOSIS OF ZIKA VIRUS INFECTION**





# Introduction : Diagnosis of ZIKV



# Introduction : Diagnosis of ZIKV

Laboratory diagnosis of ZIKV is **very challenging**

**Molecular:** Low viremia in human body fluids

High antibody cross- reactivity among flaviviruses



# Introduction : Diagnosis of ZIKV

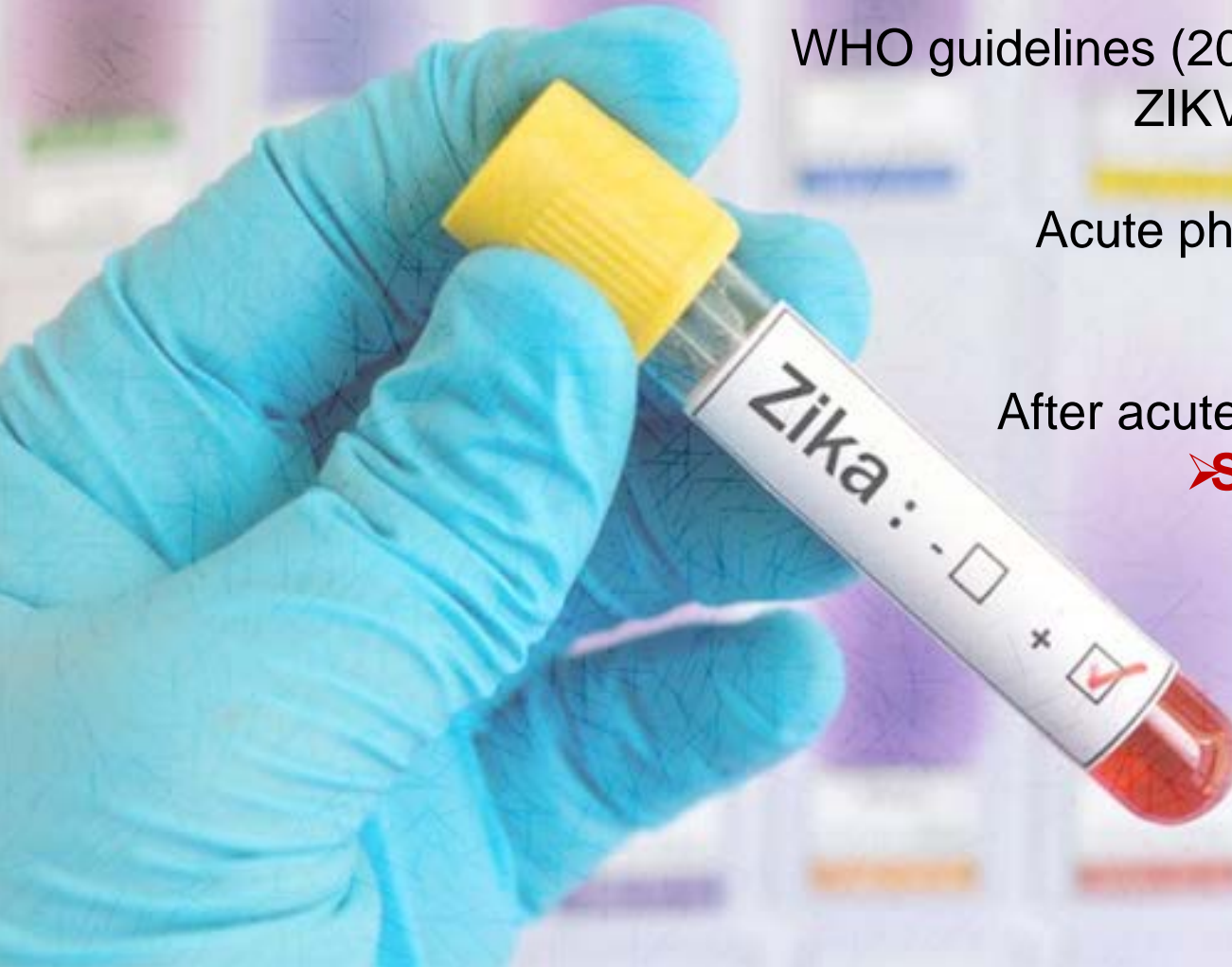
WHO guidelines (2016) for laboratory diagnosis  
ZIKV recommends:

Acute phase (up to 7 days) :

➤ **ZIKV PCR**

After acute phase (after 7 days)

➤ **Serology testing**





# Introduction : Diagnosis of ZIKV

## IMPORTANCE of **Diagnosis** after **ACUTE** Infection

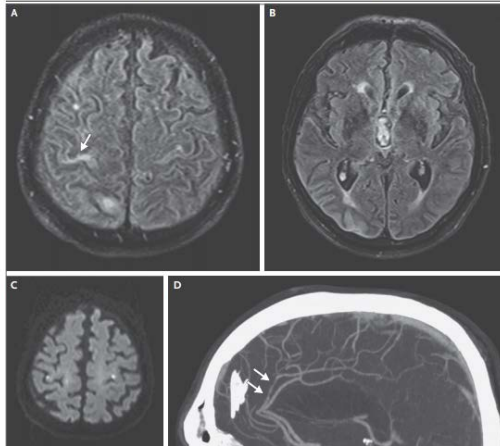
### Guillain-Barré Syndrome outbreak associated with Zika virus infection in French Polynesia: a case-control study



Van-Mai Cao-Lormeau\*, Alexandre Blake\*, Sandrine Mons, Stéphane Lastère, Claudine Roche, Jessica Vanhomwegen, Timothée Dub, Laure Baudouin, Anita Teissier, Philippe Larre, Anne-Laure Vial, Christophe Decam, Valérie Choumet, Susan K Halstead, Hugh J Willison, Lucile Musset, Jean-Claude Manuguerra, Philippe Despres, Emmanuel Fournier, Henri-Pierre Mallet, Didier Musso, Arnaud Fontanet\*, Jean Neil\*, Frédéric Ghawché\*

#### Zika Virus Associated with Meningoencephalitis

**TO THE EDITOR:** Zika virus (ZIKV) is currently spreading widely, while its clinical spectrum remains a matter of investigation. Evidence of a relationship between ZIKV infection and cerebral birth abnormalities<sup>1,2</sup> is growing.<sup>1</sup> An increased incidence of some peripheral nervous syndromes



**Figure 1.** Imaging of the Brain. MRI with the use of fluid-attenuated inversion recovery (FLAIR) imaging revealed subcortical white-matter hyperintensities in the right frontal region, the right parietal region (Panel A), the right temporo-occipital region (Panel B), and bilateral Rolandic regions (Panel A). The slight hyperintensity of the right Rolandic fissure (Panel A, arrow) is suggestive of meningitis. The multiple punctuated hyperintensities on diffusion-weighted sequences are suggestive of ischemic foci (Panel C). The MRI with FLAIR imaging and diffusion-weighted sequences were performed with the use of a 3T MRI unit (Magnetom Verio, Siemens). The computed tomographic angiogram shows an irregular narrowing of the right callosomarginal artery (Panel D, arrows). Angiography was performed with the use of a Discovery CT750 HD scanning system (GE Medical Systems).



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#### CORRESPONDENCE

### Zika Virus Associated with Meningoencephalitis

N Engl J Med 2016; 374:1595-1596 | April 21, 2016 | DOI: 10.1056/NEJMc1602964

#### Case Report

## Acute myelitis due to Zika virus infection

Sylvie Mécharles, MD, Cécile Herrmann, MD, Pascale Poullain, MD, Tuan-Huy Tran, MB, Nathalie Deschamps, MB, Grégory Mathon, MD, Anne Landais, MD, Sébastien Breurec, MD, Prof Annie Lannuzel, PhD

# Introduction : Diagnosis of ZIKV

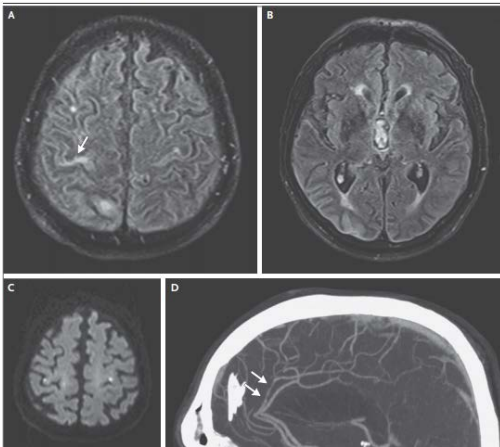
## IMPORTANCE of **Diagnosis** after **ACUTE** Infection

Estimated time between infection and neurology symptoms:

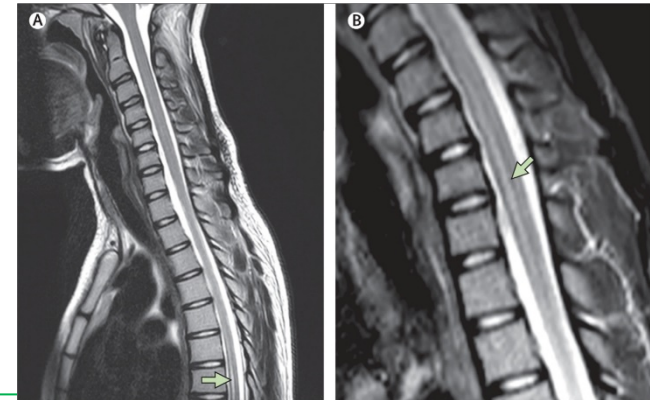
- Brasil: average 9,1 days ( 0 – 49 days)
- Colombia : Average 8,8 days (0 – 36 days)

### Zika Virus Associated with Meningoencephalitis

**TO THE EDITOR:** Zika virus (ZIKV) is currently spreading widely, while its clinical spectrum remains a matter of investigation. Evidence of a relationship between ZIKV infection and cerebral birth abnormalities<sup>1,2</sup> is growing.<sup>3</sup> An increased incidence of some peripheral nervous syndromes



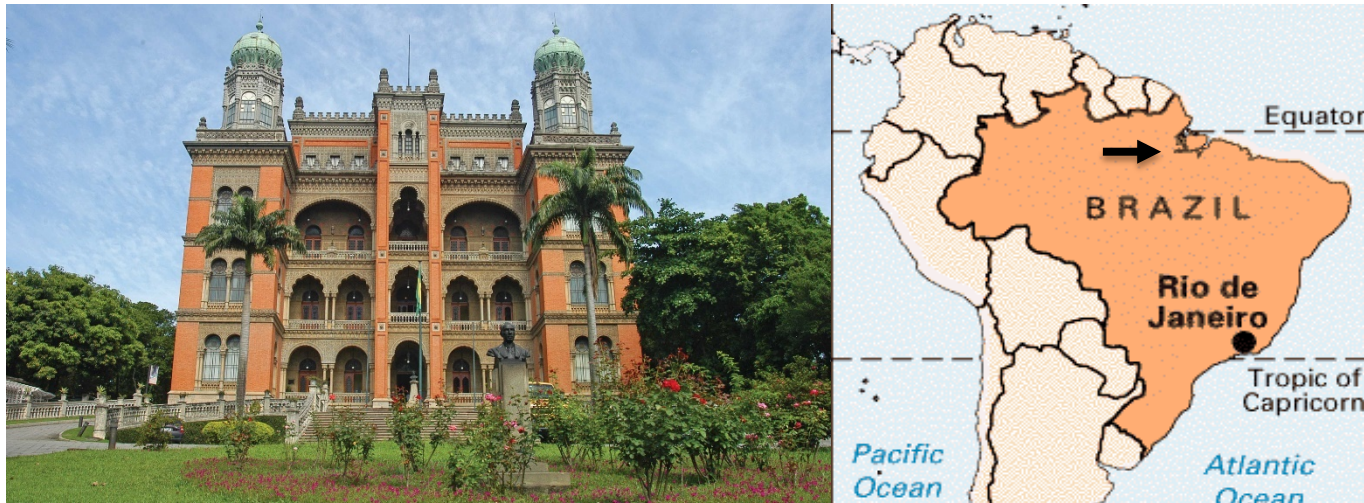
**Figure 1. Imaging of the Brain.** MRI with the use of fluid-attenuated inversion recovery (FLAIR) imaging revealed subcortical white-matter hyperintensities in the right frontal region, the right parietal region (Panel A), the right temporo-occipital region (Panel B), and bilateral Rolandic regions (Panel A). The slight hyperintensity of the right Rolandic fissure (Panel A, arrow) is suggestive of meningitis. The multiple punctuated hyperintensities on diffusion-weighted sequences are suggestive of ischemic foci (Panel C). The MRI with FLAIR imaging and diffusion-weighted sequences were performed with the use of a 3T MRI unit (Magnetom Verio, Siemens). The computed tomographic angiogram shows an irregular narrowing of the right callosomarginal artery (Panel D, arrows). Angiography was performed with the use of a Discovery CT750 HD scanning system (GE Medical Systems).





# Research work in Brazil

- **Evaluation of various Diagnosis methods of ZIKV with Brazilian Patients**  
Zika, Dengue, Yellow Fever and Chikungunya Reference Laboratory- Fiocruz



# Evaluation of serology methods for Zika Virus Diagnosis

## Commercial IgM and IgG Anti-ZIKV ELISA

- Recombinant ZIKV NS1 antigen
- Euroimmun ELISA

## METHODS


### Specificity:

More than 200 serum samples from **before 2014** from Brazilian confirmed patients with:

- **PCR Pos for Dengue (DENV 1 , DENV 2, DENV 3 and DENV4), Yellow Fever (Vaccinated and non-vaccinated), Hepatitis (A, C and E),**
- **Antibody pos for: Measles, Rubella, Chagas, Leptospirosis and CMV and General Population.**

### Sensitivity

57 paired serum samples (114) from PCR Positive confirmed patients



Results section removed – awaiting for publication



# Evaluation of serology methods for Zika Virus Diagnosis

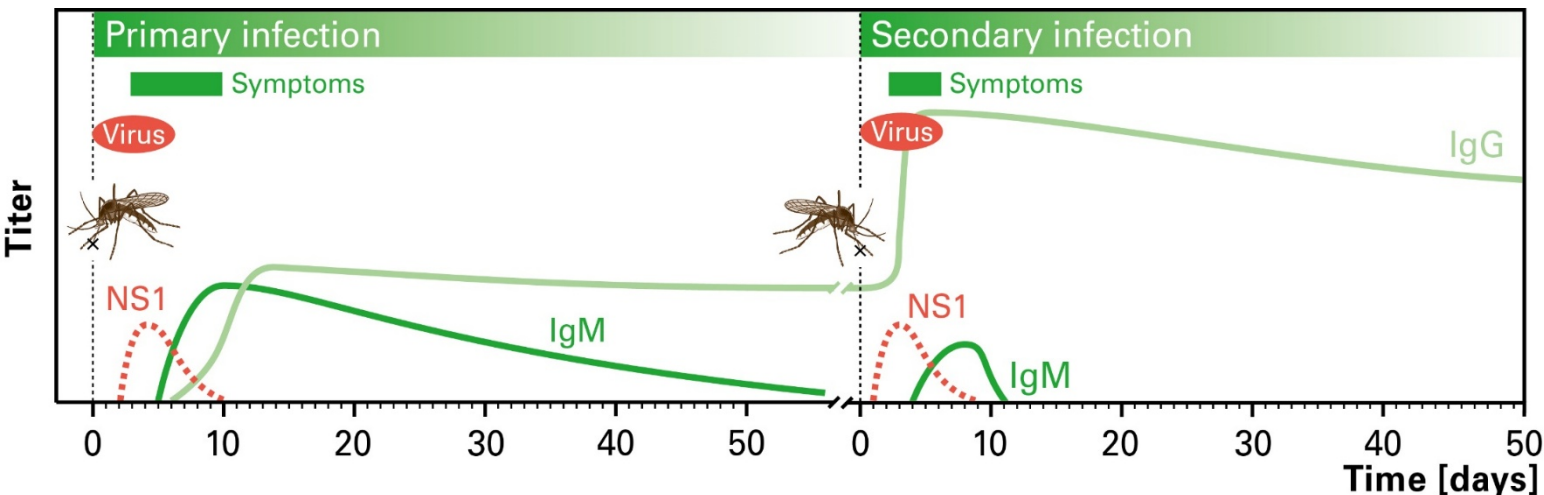
## Commercial IgM and IgG Anti-ZIKV ELISA

### Low accuracy of ZIKV IgM and IgG ELISA Tests

➤ This may be due to:

- Long-term exposure of the Brazilian population to flavivirus infections
- Low levels of specific ZIKV IgM

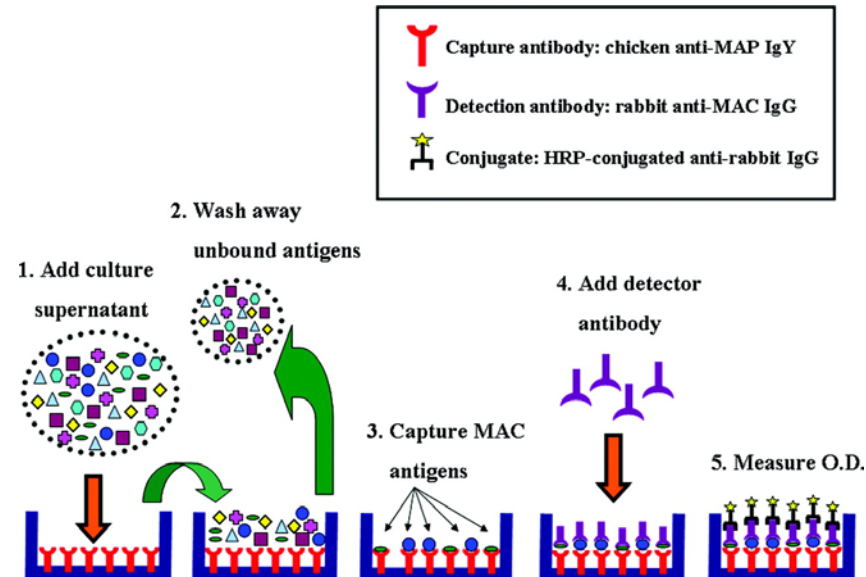
✓ Primary and Secondary Dengue Infections:




# Evaluation of serology methods for Zika Virus Diagnosis

## Evaluation of ZIKA- MAC ELISA

- FDA: **Emergency Use Authorization** (EUA) for diagnosis of Zika virus
- Capture ELISA - Detects IgM Antibodies
- Evaluation of specificity and sensitivity





Results section removed – awaiting for publication



# Evaluation of serology methods for Zika Virus Diagnosis

## Hand-held Recombinase Polymerase Amplification PCR System

### - Genie III Real Time Equipment

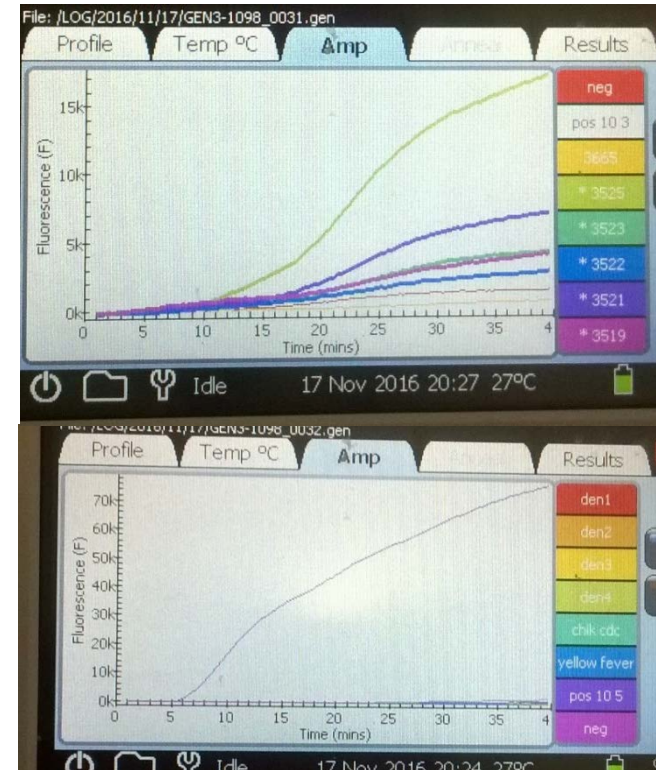
#### - Tested:

- ZIKV Controls
- ZIKV Positive Samples
- Negative Samples for arboviral infections
- Other Flavivirus samples: Yellow fever, Dengue (1 to 4)
- Chikungunya

### Results:

High Specificity (100%)

Low Sensitivity (less than 50%)



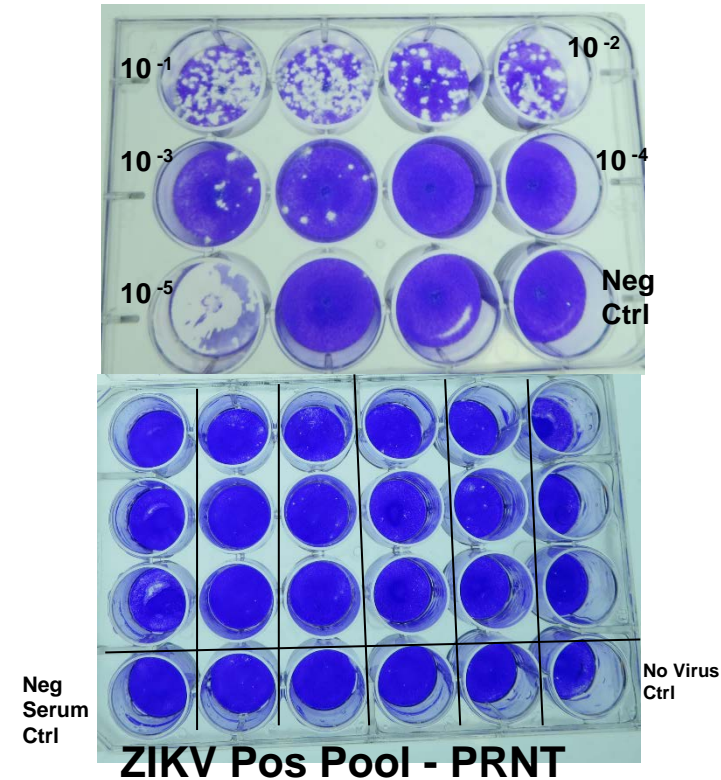
# Evaluation of methods for Zika Virus laboratory diagnosis

## Plaque Reduction Neutralization Assays (PRNTs)

PRNTs are the **gold standard** method for flavivirus diagnostics

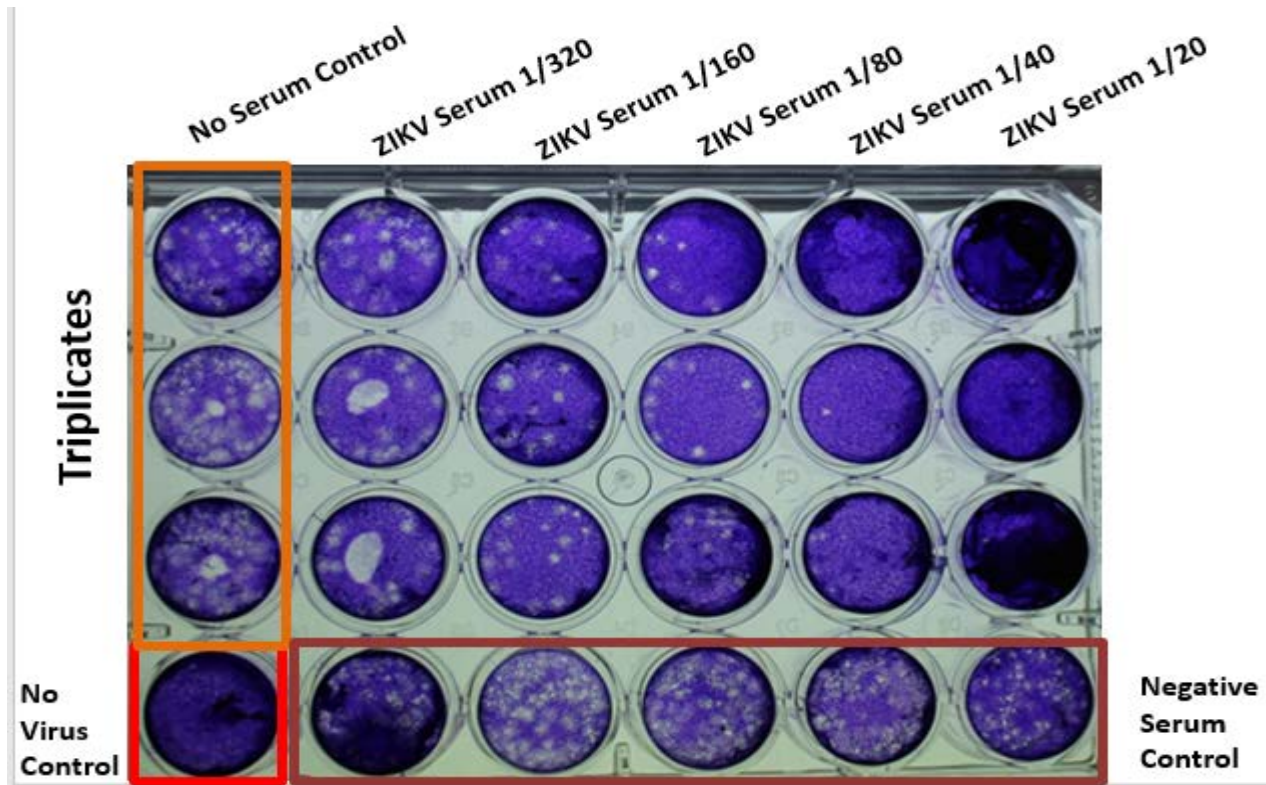
Currently established in Rio, optimised the conditions and evaluating accuracy

– Challenges of PRNTs assays :  
laborious, time-consuming, tedious...



# Evaluation of methods for Zika Virus laboratory diagnosis

## Plaque Reduction Neutralization Assays (PRNTs)





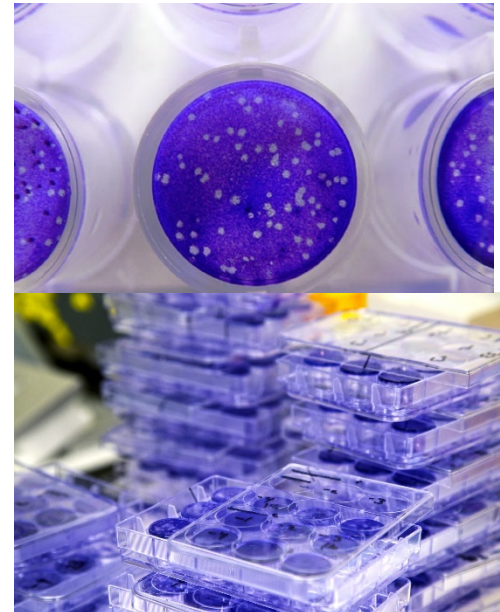
# Evaluation of methods for Zika Virus laboratory diagnosis

## Current work:

Evaluation of specificity and sensitivity of ZIKV PRNTs

Develop alternative methods to PRNTs to compare:

- Focus forming assays
- PRNT with qPCR
- Cell viability reporter assay



# Conclusions

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- **Serologic** diagnosis of ZIKV is very **challenging**
- Current methods need to be improved
  - Low IgG Specificity
  - Low IgM Sensitivity
- Antibody response corresponding to a **secondary IgM response**
- Need to develop improved accurate **IgG assays**
- Existing methods need to be evaluated in the **local population**
- **Public health importance** - provide accurate Zika diagnostic results

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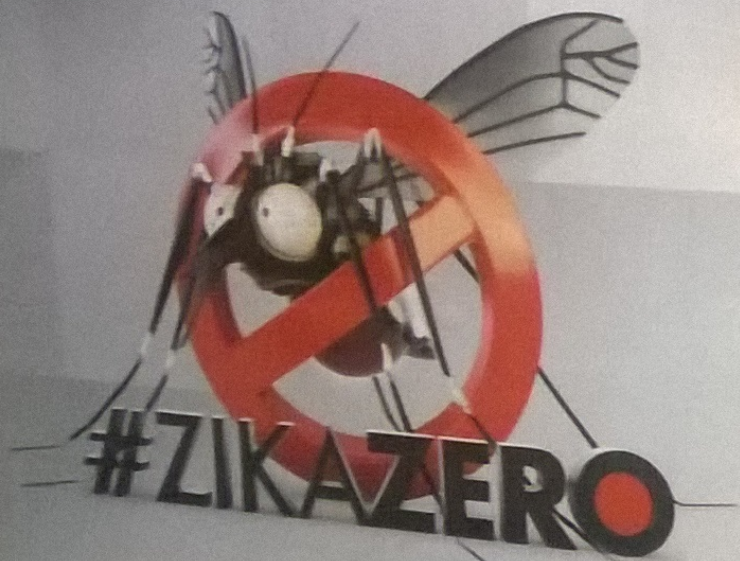


**OBRIGADA / Gracias / THANK YOU !**

**UM MOSQUITO NAO E MAIS FORTE QUE UM PAÍS INTEIRO.**

MOBILIZE SUA FAMÍLIA E ELIMINE A ÁGUA PARADA.

Participação  
de Drauzio



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**Table 1. Countries and territories that have reported mosquito-borne Zika virus transmission**

Classification	WHO Regional Office	Country / territory	Total
Category 1: Countries with a reported outbreak from 2015 onwards <sup>#</sup>	AFRO	Angola; Cabo Verde; Guinea-Bissau	3
	AMRO/PAHO	Anguilla; Antigua and Barbuda; Argentina; Aruba; Bahamas; Barbados; Belize; Bolivia (Plurinational State of); Bonaire, Sint Eustatius and Saba – Netherlands; Brazil; British Virgin Islands; Cayman Islands; Colombia; Costa Rica; Cuba; Curaçao; Dominica; Dominican Republic; Ecuador; El Salvador; French Guiana; Grenada; Guadeloupe; Guatemala; Guyana; Haiti; Honduras; Jamaica; Martinique; Mexico; Montserrat; Nicaragua; Panama; Paraguay; Peru; Puerto Rico; Saint Barthélemy; Saint Kitts and Nevis; Saint Lucia; Saint Martin; Saint Vincent and the Grenadines; Sint Maarten; Suriname; Trinidad and Tobago; Turks and Caicos; United States of America; United States Virgin Islands; Venezuela (Bolivarian Republic of)	48
	WPRO	American Samoa; Fiji; Marshall Islands; Micronesia (Federated States of); Palau; Samoa; Singapore; Tonga	8
<b>Subtotal</b>			<b>59</b>
Category 2: Countries with possible endemic transmission or evidence of local mosquito-borne Zika infections in 2016 or 2017	SEARO	Indonesia; Maldives; Thailand	3
	WPRO	Malaysia; New Caledonia; Philippines; Viet Nam	4
<b>Subtotal</b>			<b>7</b>
Category 3: Countries with evidence of local mosquito-borne Zika infections in or before 2015, but without documentation of cases in 2016 or 2017, or outbreak terminated	AFRO	Gabon**	1
	PAHO/AMRO	ISLA DE PASCUA – Chile**	1
	SEARO	Bangladesh**	1
	WPRO	Cambodia**; Cook Islands**; French Polynesia**; Lao People's Democratic Republic; Papua New Guinea; Solomon Islands; Vanuatu	7
<b>Subtotal</b>			<b>10</b>
<b>Total</b>			<b>76</b>

# Introduction : Diagnosis of ZIKV

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➤ **ZIKV PCR**

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