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

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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 of 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

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)
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
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:

![Graph showing the time course of virus titer and antibody response for primary and secondary infections.](image)
Evaluation of serology methods for Zika Virus Diagnosis

- 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…

ZIKV Pos Pool - PRNT
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

- **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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Um mosquito não e mais forte que um país inteiro.

Obrigada / Gracias / THANK YOU!

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

<table>
<thead>
<tr>
<th>Classification</th>
<th>WHO Regional Office</th>
<th>Country / territory</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Category 1: Countries with a reported outbreak from 2015 onwards</td>
<td>AFRO</td>
<td>Angola; Cabo Verde; Guinea-Bissau</td>
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<tr>
<td></td>
<td>AMRO/PAHO</td>
<td>Anguilla; Antigua and Barbuda; Argentina; Aruba; Bahamas; Barbados; Belize; Bolivia; Brazil; British Virgin Islands; Cayman Islands; Colombia; Costa Rica; Cuba; Curacao; Dominica; Dominican Republic; Ecuador; El Salvador; French Guiana; Grenada; Guatemala; Guyana; Haiti; Honduras; Jamaica; Martinique; Mexico; Montserrat; Nicaragua; Panama; Paraguay; Peru; Puerto Rico; Saint Barthelemy; 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)</td>
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<td>WPRO</td>
<td>American Samoa; Fiji; Marshall Islands; Micronesia (Federated States of); Palau; Samoa; Singapore; Tonga</td>
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<tr>
<td>Subtotal</td>
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<td>Category 2: Countries with possible endemic transmission or evidence of local mosquito-borne Zika infections in 2016 or 2017</td>
<td>SEARO</td>
<td>Indonesia; Maldives; Thailand</td>
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<tr>
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<td>WPRO</td>
<td>Malaysia; New Caledonia; Philippines; Viet Nam</td>
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<td>Subtotal</td>
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<td>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</td>
<td>AFRO</td>
<td>Gabon**</td>
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<td>PAHO/AMRO</td>
<td>ISLA DE PASCUA — Chile**</td>
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<td>SEARO</td>
<td>Bangladesh**</td>
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<td>WPRO</td>
<td>Cambodia**; Cook Islands**; French Polynesia**; Lao People’s Democratic Republic; Papua New Guinea; Solomon Islands; Vanuatu</td>
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<td>Subtotal</td>
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<td>Total</td>
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