In-country development and evaluation of new molecular and serological methods for Zika diagnosis and surveillance and their applications

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Presentation overview

- Epidemiology of arboviruses in Nicaragua
- Study design
- Methods: 2 rRT-PCR, 4 serological assays
- Evaluation: ZIKV+ and DENV+ cases
- Applications
  - CZS/PRNT study, Salvador, Brazil
  - Index cluster study, Managua, Nicaragua
  - Cohort and seroprevalence studies, Nicaragua
Arboviral Dx & surveillance assays in Nicaragua

• Dengue
  – DENV1-4 RT-PCR, realtime RT-PCR
  – IgM MAC-ELISA (in-house) – decentralized nationally
  – Inhibition ELISA
  – NS1 ELISA

• Chikungunya
  – RT-PCR, rRT-PCR: CHIK-pan-DENV; ZCD
  – 2 IgM ELISAs: MAb and polyclonal – decentralized nationally
  – 2 Inhibition ELISAs: MAb and polyclonal – age-stratified seroprevalence, national seroprevalence

• Zika
  – rRT-PCR: ZCD, Trioplex, ZIKV monoplex + pan-DENV-CHIKV
  – IgM MAC-ELISA – for national decentralization
  – NS1 BOB ELISA
  – Inhibition ELISA

• Mayaro
  – rRT-PCR
DENV, CHIKV, and ZIKV circulation in the Nicaraguan cohort study, 2004-present
DENV, CHIKV, and ZIKV circulation in the Nicaraguan cohort study, 2004-present
DENV, CHIKV, and ZIKV circulation in the Nicaraguan cohort study, 2004-present
The Pediatric Dengue Cohort Study (3,500 children 2-14 y/o) (Dengue 2004-2020; Chikungunya 2014+; Zika 2016+)

- Yearly Healthy Samples
- Acute Sample
- Convalescent Sample
- Year 1
- DOS
- Year 2
- Year 3
- Year 4

The Hospital-based Study (Dengue 1998/2005-2022; Chikungunya 2014+, Zika 2016+)

- Acute Samples
- Convalescent Sample
- Longitudinal Samples
- DOS
- 2 wks
- 3 months
- 6 months
- 12 months
- 18 months

Enrolled at presentation to National Pediatric Reference Hospital
Monthly cases of dengue, chikungunya and Zika in cohort, Aug 2014-Sep 2016
Numbers of paired samples by group

<table>
<thead>
<tr>
<th>Category</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>DENV1 (12 1°, 12 2°)</td>
<td>24</td>
</tr>
<tr>
<td>DENV2 (17 1°, 17 2°)</td>
<td>34</td>
</tr>
<tr>
<td>DENV3 (19 1°, 20 2°)</td>
<td>39</td>
</tr>
<tr>
<td>ZIKV-positive, DENV-immune</td>
<td>65</td>
</tr>
<tr>
<td>ZIKV-positive, DENV-naïve</td>
<td>65</td>
</tr>
<tr>
<td>ZIKV-negative, DENV-negative</td>
<td>74</td>
</tr>
<tr>
<td>Total</td>
<td>301</td>
</tr>
</tbody>
</table>
Methods evaluated

<table>
<thead>
<tr>
<th>Method</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>BEI IgM ELISA</td>
<td>296</td>
</tr>
<tr>
<td>CNDR IgM ELISA</td>
<td>298</td>
</tr>
<tr>
<td>Inhibition ELISA</td>
<td>286</td>
</tr>
<tr>
<td>NS1 BOB ELISA</td>
<td>298</td>
</tr>
<tr>
<td>TRIOPLEX serum</td>
<td>273</td>
</tr>
<tr>
<td>ZCD serum</td>
<td>273</td>
</tr>
</tbody>
</table>
CNDR IgM Capture ELISA

ZIKA

TMB substrate

ZIKV MAb ZKA64 conjugated to HRP

ZIKV Mouse brain antigen

Sample

Human anti-IgM

3-4 hours
90 samples/plate

Donated by Dr. Davide Corti
CDC-BEI IgM Capture ELISA

ZIKA

Substrate

Anti-flavivirus Mab conjugated to HRP

Cell culture antigen (VeroE6)

Sample

Human anti-IgM

3 days
23 samples/plate
NS1 Blockade-of binding (BOB) assay

- Nicaragua samples:
  - National surveillance ZIKV RT-PCR-pos
  - Cohort study ZIKV RT-PCR-pos
  - Cohort study DENV RT-PCR-pos
- Samples from FIOCRUZ, Rio de Janeiro, Brazil
- Samples from Italy (returning travelers, etc.)

![Diagram of the NS1 Blockade-of binding (BOB) assay](image)

- Plasma is added to ZIKV NS1 coated wells
- Probe Ab is added without washing
- Binding of the plasma polyclonal antibodies to multiple sites of the coated ZIKV NS1
- Binding of ZKA35 is not blocked
- Wash + STV-AP
- No signal
- Binding of ZKA35 is blocked
- Wash + substrate (pNPP)
- Signal
- Reading with a spectrophotometer

Balmaseda et al. In revision
Inhibition ELISA

Substrate

Conjugated MAb ZKA64

Sample

Mouse brain ZIKV antigen

MAb ZKA64 Anti-ZIKV

Image of ELISA plate with yellow spots.
ZCD rRT-PCR assay

- Single-reaction rRT-PCR assay for:
  - Detection of all DENV serotypes/genotypes
  - Detection and quantitation of CHIKV and all deposited strains of ZIKV (including recent addition for Suriname)
  - Currently in use at Stanford; Managua, Nicaragua; Guayaquil and Quito, Ecuador; and coming soon to Port of Spain, Trinidad, and Colombo, Sri Lanka

Single-Reaction Multiplex Reverse Transcription PCR for Detection of Zika, Chikungunya, and Dengue Viruses

Jesse J. Waggoner, Lionel Gresh, Alisha Mohamed-Hadley, Gabriela Ballesteros, Maria Jose Vargas Davila, Yolanda Tellez, Malaya K. Sahoo, Angel Balmaseda, Eva Harris, Benjamin A. Pinsky

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Trioplex rRT-PCR assay

Trioplex Real-time RT-PCR Assay

Centers for Disease Control and Prevention

For use under an Emergency Use Authorization only
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Zika Household Transmission Index Cluster Study

~7 contacts

day 1, 4, 5, 10, 21

Entomology (d10):
- mosquitoes
- pupal indices

blood saliva urine

- Real-time qRT-PCR
- Serology (d1-21)

Cohort study
Index Cluster Study of Zika Virus Infection in Managua, Nicaragua

Study Design

Time frame: Aug. 31 to Oct. 21, 2016, tail-end of the epidemic

• Suspected Zika cases from the HCSFV (District II) tested by RT-PCR
• Positives selected as index cases
• Next day, home visit and all present household members are enrolled
• Follow up - 4 time-points: Days 3-4, 6-7, 9-10, and 21
• Clinical data collected by study personnel on all household members

• 33 Index cases/Households
• 109 Contacts
### Sample Collection Plan

**Table 1.** Sample collection plan for index cases (n=33).

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Visit 0 Enrollment(^1)</th>
<th>Visit 1 Day 1(^2)</th>
<th>Visit 2 Days 3/4</th>
<th>Visit 3 Days 6/7</th>
<th>Visit 4 Days 9/10</th>
<th>Visit 5 Day 21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>X</td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Urine</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Saliva</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

\(^1\)Enrollment day for index cases: 28 from the PDCS and 5 from national surveillance (n=33). Surveillance cases only have blood and no urine or saliva samples at Visit 0.

**Table 2.** Sample collection plan for contacts (n=109).

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Visit 1 Day 1(^2)</th>
<th>Visit 2 Days 3/4</th>
<th>Visit 3 Days 6/7</th>
<th>Visit 4 Days 9/10</th>
<th>Visit 5 Day 21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Urine</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Saliva</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

\(^1\)Enrollment day for contacts.
Zika CNDR IgM ELISA consistently detected RT-PCR-positive cases

- Detected **100%** of the PCR positive Index Cases.
  *One of the 33 index cases missing convalescent sample - no serology was performed.

- Detected **90%** of the PCR positive Contacts.
  *One of the 11 ZIKV RT-PCR positive missing convalescent sample - no serology was performed.

- 25 contacts were IgM positive but RT-PCR negative. Likely recent not current ZIKV infections.
Cohort study: sero-incidence and effect of prior DENV infection

- Paired annual samples: 2016-2017 (March-April); age-stratified seroprevalence study (n=1400)
  - DENV Inhibition ELISA
  - CHIKV Inhibition ELISA
  - ZIKV Inhibition ELISA
  - NS1 BOB ELISA

- Calculation of the Symptomatic to Inapparent (S:I) ZIKV infection ratio when combined with rates of symptomatic illnesses recorded during transmission season

- Estimation of the force of infection and $R_o$ of ZIKV
Cohort study: sero-incidence and effect of prior DENV infection

• Effect of prior DENV exposure on ZIKV infection and disease incidence, S:I ratio, and disease severity:
  • documented prior DENV exposure in the cohort
  • number of DENV infections
  • pre-existing cross-reactive anti-ZIKV antibody titers
  • pre-existing cross-reactive anti-DENV antibody titers

• Evaluation of potential immune correlates of protection against and risk of ZIKV infection and disease
Conclusions

- Development and/or evaluation of 2 molecular and 4 serological assays in a Zika- and dengue-endemic country
- Promising new serological assays for sensitive and specific diagnosis of Zika cases (IgM MAC-ELISA)
- Promising new serological assays for sensitive and specific Zika surveillance (NS1 BOB ELISA)
- Perform well in numerous distinct applications
- Enable seroprevalence studies and analysis of the effect of pre-existing antibodies from prior DENV infection(s)
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