A Selective Immunoassay for the Detection of Zika Virus in Human Bodily Fluids

Sylvia Daunert
Professor and Lucille P. Markey Chair
Department of Biochemistry and Molecular Biology
Associate Director
Dr. JT Macdonald Biomedical Nanotechnology Institute

Zika Research Symposium, Florida Department of Health
Boca Ratón, FL. October 9, 2017
Zika Virus (ZIKV)

Belongs to the family of Flaviridae (Dengue Fever, Yellow Fever, West Nile Virus, Japanese Encephalitis, Chikungunya Virus)

Transmitted by Yellow Fever Mosquito (Aedes aegypti)

Global Yellow Fever Mosquito (Aedes aegypti) distribution (predicted)

Maculopapular Rash

Microcephaly

Patient with Guillain-Barré Syndrome

Economic development distribution
Zika Virus (ZIKV) in Florida

Florida  Sep. 25, 2017

<table>
<thead>
<tr>
<th>LOCAL + TRAVEL</th>
<th>LOCAL CASES</th>
<th>TRAVEL CASES</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Local + Travel 1551</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Local Cases 287</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Travel Cases 1264</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pregnant Women 402</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Unknown Origin (Local or Travel) 80</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Unknown County (Local Cases) 9</td>
</tr>
</tbody>
</table>

In the news
As Zika season looms, Senate panel approves $100 million in mosquito control funding
April 26, 2017

Miami Herald as of September 25, 2017.
Point of Care Testing

Point-of-care testing, also known as bedside testing, is medical diagnostic testing at or near the point of care; that is, at the time and place of the patient care.

1. Test ordered
2. Test Sample Collected
3. Sample is analyzed
4. Clinician acts on the results

**Advantages**

- Decreased turnaround time
- Evidence-based medical decisions in “real time”
- Elimination of specimen transport and processing time
- Reduction in duplicate test orders
- Reduction in unnecessary medications
- Reduced consumption of other expensive services/products (lab tests, pharmaceuticals)
Current Methods for ZIKV Detection

RNA NAT (RT-PCR)
Trioplex RT-PCR Assay
Concurrently detects ZIKV, DENV, CHIKV

- Gives information about active infection
- Detects low copies of viral RNA
- Sensitive and selective
- Requires trained personnel and expensive equipment

ZIKV MAC-ELISA
- Detects the IgM antibodies developed against ZIKV) in serum or CSF)
- Due-to cross reactivity with other flaviviruses the results may be difficult to interpret. Inconclusive results must be confirmed with plaque-reduction neutralization test

Plaque Reduction Neutralization Test (PRNT)
- Considered to be the gold standard for detecting and measuring antibodies that can neutralize the virus. It has higher sensitivity than other tests
- Relatively cumbersome and time intensive.
Development of two Assays:

- Clinical laboratory based assay for the direct detection of virus in bodily fluids of a patient with an active infection.

- A rapid lateral-flow type point-of-care assay.

Novelty: use of highly specific monoclonal antibodies against ZIKV that was developed by Watkins’ Group.

Assay Scheme

1. Microtiter plate
2. Coat with capture antibody (P3E11)
3. Add Zika Virus Sample
4. Add detection antibody (P1F12, P1H6B, and P1B04)
5. Add biotinylated secondary antibody
6. Add Poly-SA HRP, substrate and measure signal

Capture antibody  Biotinylated secondary antibody  Detection antibody  Zika Virus  Poly-Streptavidin-Horseradish Peroxidase (poly-SA-HRP)
Optimization of the ZIKV Assay

Selectivity

Response of the Immunoassay to the same concentrations (2.0 x 10⁵ pfu/mL) of ZIKV and different DENV variants

Optimization of the antibody concentrations for the primary and secondary antibody pair.

Optimization of the immunoassay incubation times
**ZIKV Assay Validation**

- Data obtained by spiking clean bodily fluids with ZIKV
- Representative calibration curves with individual L.O.D.
- Data in table are an average of at least three separate experiments

**Table: Assay Validation Results**

<table>
<thead>
<tr>
<th></th>
<th>Buffer</th>
<th>Urine Neat</th>
<th>Saliva 1:10</th>
<th>Serum 1:10</th>
<th>Whole Blood 1:10</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>LOD (copies/mL)</strong></td>
<td>$1.11 \pm 1.38 \times 10^5$</td>
<td>$8.51 \pm 5.55 \times 10^4$</td>
<td>$7.39 \pm 2.35 \times 10^4$</td>
<td>$5.81 \pm 0.39 \times 10^4$</td>
<td>$2.05 \pm 2.5 \times 10^5$</td>
</tr>
<tr>
<td><strong>LOQ (copies/mL)</strong></td>
<td>$2.99 \pm 3.72 \times 10^5$</td>
<td>$1.70 \pm 1.53 \times 10^6$</td>
<td>$1.68 \pm 0.98 \times 10^5$</td>
<td>$1.11 \pm 0.20 \times 10^6$</td>
<td>$4.14 \pm 5.2 \times 10^5$</td>
</tr>
<tr>
<td><strong>CV Min (%)</strong></td>
<td>$5.48 \pm 3.86$</td>
<td>$3.23 \pm 1.47$</td>
<td>$8.66 \pm 6.66$</td>
<td>$2.14 \pm 2.74$</td>
<td>$3.33 \pm 2.13$</td>
</tr>
<tr>
<td><strong>CV Med (%)</strong></td>
<td>$6.07 \pm 2.40$</td>
<td>$7.44 \pm 7.99$</td>
<td>$4.08 \pm 0.90$</td>
<td>$3.96 \pm 2.90$</td>
<td>$5.80 \pm 1.74$</td>
</tr>
<tr>
<td><strong>CV Max (%)</strong></td>
<td>$2.10 \pm 0.46$</td>
<td>$2.48 \pm 1.75$</td>
<td>$1.30 \pm 1.08$</td>
<td>$1.84 \pm 0.46$</td>
<td>$4.70 \pm 3.43$</td>
</tr>
<tr>
<td><strong>Recovery Min (%)</strong></td>
<td>$90.99 \pm 14.04$</td>
<td>$94.92 \pm 2.15$</td>
<td>$75.89 \pm 34.83$</td>
<td>$87.41 \pm 6.46$</td>
<td>$92.41 \pm 11.10$</td>
</tr>
<tr>
<td><strong>Recovery Med (%)</strong></td>
<td>$86.23 \pm 24.88$</td>
<td>$78.70 \pm 8.84$</td>
<td>$102.42 \pm 4.57$</td>
<td>$105.35 \pm 3.71$</td>
<td>$92.20 \pm 2.51$</td>
</tr>
<tr>
<td><strong>Recovery Max (%)</strong></td>
<td>$92.20 \pm 25.85$</td>
<td>$95.10 \pm 20.38$</td>
<td>$112.83 \pm 11.18$</td>
<td>$105.33 \pm 6.44$</td>
<td>$96.71 \pm 3.48$</td>
</tr>
<tr>
<td><strong>LOD (copies/mL)</strong></td>
<td>$3.81 \pm 0.72 \times 10^4$</td>
<td>$4.40 \pm 6.03 \times 10^4$</td>
<td>$5.50 \pm 5.99 \times 10^4$</td>
<td>$5.55 \pm 6.55 \times 10^4$</td>
<td>$5.50 \pm 8.1 \times 10^4$</td>
</tr>
<tr>
<td><strong>LOQ (copies/mL)</strong></td>
<td>$5.08 \pm 1.48 \times 10^4$</td>
<td>$4.71 \pm 0.56 \times 10^4$</td>
<td>$7.11 \pm 0.06 \times 10^4$</td>
<td>$7.62 \pm 1.53 \times 10^4$</td>
<td>$7.62 \pm 1.0 \times 10^5$</td>
</tr>
<tr>
<td><strong>CV Min (%)</strong></td>
<td>$5.74 \pm 7.59$</td>
<td>$4.18 \pm 2.67$</td>
<td>$5.94 \pm 7.15$</td>
<td>$3.61 \pm 2.31$</td>
<td>$4.65 \pm 2.04$</td>
</tr>
<tr>
<td><strong>CV Med (%)</strong></td>
<td>$2.53 \pm 1.14$</td>
<td>$13.32 \pm 9.86$</td>
<td>$4.31 \pm 1.98$</td>
<td>$5.13 \pm 3.20$</td>
<td>$3.64 \pm 1.05$</td>
</tr>
<tr>
<td><strong>CV Max (%)</strong></td>
<td>$1.10 \pm 0.75$</td>
<td>$1.66 \pm 0.56$</td>
<td>$1.03 \pm 0.71$</td>
<td>$2.90 \pm 3.45$</td>
<td>$0.85 \pm 0.44$</td>
</tr>
<tr>
<td><strong>Recovery Min (%)</strong></td>
<td>$95.25 \pm 14.16$</td>
<td>$98.90 \pm 19.16$</td>
<td>$81.08 \pm 31.99$</td>
<td>$75.7 \pm 4.61$</td>
<td>$101.71 \pm 2.60$</td>
</tr>
<tr>
<td><strong>Recovery Med (%)</strong></td>
<td>$102.53 \pm 29.31$</td>
<td>$97.29 \pm 29.36$</td>
<td>$98.76 \pm 26.24$</td>
<td>$88.57 \pm 15.16$</td>
<td>$92.96 \pm 4.52$</td>
</tr>
<tr>
<td><strong>Recovery Max (%)</strong></td>
<td>$105.87 \pm 12.48$</td>
<td>$109.90 \pm 3.72$</td>
<td>$118.45 \pm 8.33$</td>
<td>$103.87 \pm 11.93$</td>
<td>$94.18 \pm 0.57$</td>
</tr>
</tbody>
</table>

**Graphs:**
- L.O.D: $2.55 \times 10^6$ copies/mL
- L.O.D: $1.58 \times 10^6$ copies/mL
- L.O.D: $1.46 \times 10^5$ copies/mL
Preparation of the Conjugate

1. Add the reaction mixture to blocking buffer, Incubate 2 h while mixing at 40 °C
2. Centrifuge 20 min at 14,400 xg at 20 °C
3. Wash with wash buffer
4. Vortex and Sonicate to resuspend in wash buffer
Point-of-Care ZIKV Test

Detection of ZIKV in Buffer

Detection of ZIKV in Urine
Florida Department of Health (FLDOH) Zika Initiative

Team

Dr. David Watkins
Dr. Sapna K. Deo
Dr. Esper Kallas
Devon Pawley
Dr. Emre Dikici
Michael Ricciardi