Zika Virus Response in the US

Julie Villanueva, PhD

APHL Annual Meeting 2016
Albuquerque, NM
6 June 2016
Zika Virus

- Discovered in the Zika forest, Uganda 1947
- Flavivirus, single-stranded RNA
- Mosquito borne (Aedes)
- Symptoms may include fever, rash, conjunctivitis, myalgia
- Most infections are asymptomatic
- Virus is detectable in serum, urine, saliva, and semen
- Recent outbreaks include Yap Island, Federated States of Micronesia 2007 and French Polynesia 2013-2014
Zika Virus Outbreak 2015: Western Hemisphere

- May 2015, the Pan American Health Organization (PAHO) issued an alert regarding the first confirmed Zika virus infection in Brazil
- Reports of Guillain-Barré syndrome and pregnant women giving birth to babies with birth defects and poor pregnancy outcomes
Zika Pathology

- Specimens from 2 newborns with microcephaly that died within one day of birth and from 2 miscarriages from Brazil
- Specimens from all 4 cases were positive by rRT-PCR
- 2/4 were positive by immunohistochemistry; viral antigen detected in mononuclear cells
Zika Virus Linked to Birth Defects

- Utilizing criteria proposed for the assessment of teratogens: “a causal relationship exists between prenatal Zika virus infection and microcephaly and other serious brain anomalies.”
Zika Virus Epidemiology US Update: 1 June 2016

- Zika virus disease cases reported to ArboNET in the US, 2015-2016
- 618 travel-associated cases in the United States
  - 195 pregnant women, 1 GBS
- 1114 cases (~99% local transmission) in US Territories
  - 146 pregnant women, 8 GBS
- 11 cases of sexual transmission
Zika Virus Disease in the US and Territories – 1 June 2016
Zika Diagnostics in the US 2016

- FDA issued Emergency Use Authorization for two CDC assays:
  - Zika MAC-ELISA for presumptive detection of Zika IgM antibodies (serum, CSF)
  - Trioplex rRT-PCR for detection of Zika, dengue, and chikungunya viral RNA (serum, CSF for all; urine, amniotic fluid for Zika only)
- Specimens positive for Zika MAC-ELISA are further analyzed using plaque reduction neutralization tests (PRNT)

http://www.fda.gov/EmergencyPreparedness/Counterterrorism/MedicalCountermeasures/MCMLegalRegulatoryandPolicyFramework/ucm182568.htm#current
Distribution of CDC Diagnostic Assays: Domestic

- Zika MAC-ELISA
  - 52 laboratories have received reagents
  - 38 laboratories have completed the CDC verification panel: 32 States, DC, and 3 DoD laboratories

- Triplex rRT-PCR Assay
  - 102 laboratories have received reagents
  - 74 laboratories have completed the CDC verification panel: 39 States, DC, PR and 15 DoD laboratories
Distribution of CDC Diagnostic Assays: International

- 94 countries have requested the Zika MAC-ELISA and/or the Trioplex rRT-PCR Assay
  - 25 in the Americas
  - 22 in Europe
  - 22 in Africa
  - 13 in Western Pacific
  - 8 in Eastern Mediterranean
  - 4 in Southeastern Asia
Zika Cases in the US, 3 January – 5 March 2016

- 4,534 people tested
- 4,282 negative (94.4%)
- 3,335 pregnant
  - 28 (0.8%) Zika positive
  - 19 (0.6%) flavivirus positive

MMWR, April 22, 2016 / 65(15);395–399
Molecular Testing Knowledge Expanding

DISPATCHES

Detection of Zika Virus in Urine

Ann-Claire Gourinat,¹ Olivia O’Connor,¹ Elodie Calvez, Cyrille Goazant, and Myrielle Dupont-Rouzyrol

We describe the kinetics of Zika virus (ZIKV) detection in serum and urine samples of 6 patients. Urine samples were positive for ZIKV >10 days after onset of disease, which was a notably longer period than for serum samples. This finding supports the conclusion that urine samples are useful for diagnosis of ZIKV infections.

The Study

In October 2013, a ZIKV outbreak was reported in French Polynesia (9). This was the second outbreak of ZIKV infection reported in the Pacific region. In New Caledonia, where ZIKV infection had never been documented, the first cases of ZIKV infection imported from French Polynesia were confirmed by the end of November, and the first diagnostic utility of urine as a source for detection of ZIKV RNA by real-time RT-PCR.

On May 10, 2016, this report was posted as an MMWR Early Release on the MMWR website (http://www.cdc.gov/mmwr).

In May 2015, Zika virus was reported to be circulating in Brazil. This was the first identified introduction of the virus in the Region of the Americas. Since that time, Zika virus has rapidly spread in the region, and serologic testing was performed on all serum specimens included in this analysis. The probable case definition criteria for Zika virus disease, based on serology, required Zika virus–specific IgM antibodies and no dengue virus–specific IgM antibodies detected in serum or cerebrospinal fluid.

“ZIKV RNA is detectable in urine at a higher load and with a longer duration than in serum.”
Prolonged maternal viremia: Zika virus RNA detected 10 weeks post symptom onset
## Zika Serology Results-Yap 2007

Lanciotti et al., EID 2008; 14(8), 1232-1239

### Primary flavivirus ZIKV

<table>
<thead>
<tr>
<th>Patient</th>
<th>Days after onset</th>
<th>ZIKV</th>
<th>DENV1</th>
<th>DENV2</th>
<th>DENV3</th>
<th>DENV4</th>
<th>JEV</th>
<th>YFV</th>
<th>WN</th>
<th>SLEV</th>
<th>MVEV</th>
</tr>
</thead>
<tbody>
<tr>
<td>822a</td>
<td>5</td>
<td>320</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>822b</td>
<td>10</td>
<td>2,560</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>822c</td>
<td>24</td>
<td>5,120</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>830a</td>
<td>2</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>NT‡</td>
<td>NT‡</td>
<td>NT‡</td>
<td>NT‡</td>
<td>NT‡</td>
<td>NT‡</td>
<td>NT‡</td>
<td>NT‡</td>
</tr>
<tr>
<td>830b</td>
<td>21</td>
<td>2,560</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>849a</td>
<td>3</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>849b</td>
<td>18</td>
<td>10,240</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>862a</td>
<td>6</td>
<td>320</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>862b</td>
<td>20</td>
<td>2,560</td>
<td>10</td>
<td>10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
</tbody>
</table>

### Secondary flavivirus ZIKV (probable)

<table>
<thead>
<tr>
<th>Patient</th>
<th>Days after onset</th>
<th>ZIKV</th>
<th>DENV1</th>
<th>DENV2</th>
<th>DENV3</th>
<th>DENV4</th>
<th>JEV</th>
<th>YFV</th>
<th>WN</th>
<th>SLEV</th>
<th>MVEV</th>
</tr>
</thead>
<tbody>
<tr>
<td>817a</td>
<td>1</td>
<td>80</td>
<td>80</td>
<td>160</td>
<td>320</td>
<td>160</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>817b</td>
<td>19</td>
<td>10,240</td>
<td>2,560</td>
<td>20,480</td>
<td>5,120</td>
<td>5,120</td>
<td>20</td>
<td>320</td>
<td>160</td>
<td>1,280</td>
<td>640</td>
</tr>
<tr>
<td>833a</td>
<td>1</td>
<td>160</td>
<td>320</td>
<td>80</td>
<td>40</td>
<td>20</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>833b</td>
<td>19</td>
<td>81,920</td>
<td>20,480</td>
<td>5,120</td>
<td>5,120</td>
<td>1,280</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>844a</td>
<td>2</td>
<td>20</td>
<td>1,280</td>
<td>640</td>
<td>320</td>
<td>160</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>5</td>
<td>20</td>
</tr>
<tr>
<td>844b</td>
<td>16</td>
<td>10,240</td>
<td>40,960</td>
<td>10,240</td>
<td>5,120</td>
<td>1,280</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>955a</td>
<td>1</td>
<td>40</td>
<td>1,280</td>
<td>640</td>
<td>160</td>
<td>320</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>955b</td>
<td>14</td>
<td>163,840</td>
<td>81,920</td>
<td>20,480</td>
<td>10,240</td>
<td>5,120</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>2,560</td>
<td>1,280</td>
</tr>
<tr>
<td>968a</td>
<td>1</td>
<td>80</td>
<td>320</td>
<td>320</td>
<td>80</td>
<td>40</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>40</td>
<td>20</td>
</tr>
<tr>
<td>968b</td>
<td>3</td>
<td>10,240</td>
<td>640</td>
<td>640</td>
<td>160</td>
<td>160</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>839a</td>
<td>3</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
</tbody>
</table>
Serology Interpretations

- Confirmation for Zika MAC-ELISA positive and equivocal specimens
- PRNT titer 4-fold difference may not differentiate between dengue and Zika virus neutralizing antibodies
- Pregnant women with a Zika or flavivirus infection interpretation should be evaluated and managed for adverse pregnancy outcomes and be reported to the appropriate registry
### Detection of Zika Virus: RT-PCR Results-Yap 2007

Table 4. Results of quantitative real-time RT-PCR of samples from ZIKV-positive patients, Yap State, Micronesia, 2007

<table>
<thead>
<tr>
<th>Patient</th>
<th>Days after onset</th>
<th>ZIKV real-time RT-PCR</th>
<th>Estimated copies/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ct-860†</td>
<td>Ct-1107†</td>
</tr>
<tr>
<td>824</td>
<td>1</td>
<td>34.3</td>
<td>34.7</td>
</tr>
<tr>
<td>939</td>
<td>2</td>
<td>32.0</td>
<td>32.4</td>
</tr>
<tr>
<td>947</td>
<td>2</td>
<td>34.3</td>
<td>33.9</td>
</tr>
<tr>
<td>949</td>
<td>2</td>
<td>35.1</td>
<td>35.1</td>
</tr>
<tr>
<td>969</td>
<td>1</td>
<td>29.4</td>
<td>29.3</td>
</tr>
<tr>
<td>037</td>
<td>1</td>
<td>32.1</td>
<td>32.5</td>
</tr>
<tr>
<td>830a</td>
<td>2</td>
<td>30.7</td>
<td>30.0</td>
</tr>
<tr>
<td>847a</td>
<td>5</td>
<td>34.8</td>
<td>34.7</td>
</tr>
<tr>
<td>950a</td>
<td>0</td>
<td>32.2</td>
<td>32.7</td>
</tr>
<tr>
<td>943</td>
<td>3</td>
<td>37.6</td>
<td>35.6</td>
</tr>
<tr>
<td>952</td>
<td>1</td>
<td>29.3</td>
<td>29.5</td>
</tr>
<tr>
<td>958</td>
<td>11</td>
<td>29.9</td>
<td>30.3</td>
</tr>
<tr>
<td>970</td>
<td>1</td>
<td>35.5</td>
<td>34.8</td>
</tr>
<tr>
<td>42</td>
<td>0</td>
<td>32.9</td>
<td>33.6</td>
</tr>
<tr>
<td>941</td>
<td>3</td>
<td>31.1</td>
<td>38.0</td>
</tr>
<tr>
<td>964</td>
<td>0</td>
<td>38.3</td>
<td>37.6</td>
</tr>
<tr>
<td>063a</td>
<td>2</td>
<td>37.5</td>
<td>38.0</td>
</tr>
</tbody>
</table>
CDC Zika Diagnostics Updates

- Increased specimen input volume for the Trioplex – data collection ongoing
- Study for automated extraction platforms for Trioplex – plan being finalized with FDA
- Integrate Zika VLPs into the Zika MAC-ELISA assay to speed manufacturing of antigen – data under review by FDA
- Adding automated robotic system to the Zika MAC-ELISA – initial data collected
Zika Diagnostic Landscape - Update

Available globally:

- RealStar Zika RT-PCR Kit (CE-IVD)
- genesig® Zika (RUO)
- RT-PCR (RUO)
- Zika Virus (ZIKV) Real Time RT-PCR Kit
- RT-PCR (RUO)

- Biocan Biocan Diagnostics Inc.
  - Zika Virus IgG/IgM Antibody Rapid Test
- Bioxan Biocan Diagnostics Inc.
  - Tell Me Fast Dengue/Chikungunya/Zika Virus Combo Rapid Test
- Euroimmun
  - ELISA & IFA Zika IgM/IgG
- MyBioSource.com
  - ELISA Kits Zika Virus IgG
  - Zika Virus IgM

Available in the US (EUA issued by FDA):

- Trioplex rRT-PCR Assay
- Zika MAC-ELISA
- RealStar Zika RT-PCR Kit
- Zika Virus RNA Qualitative Real-Time RT-PCR

Courtesy of R. Humes HHS/BARDA
Evaluation of Zika Diagnostic Tests

- Evaluated two commercially available tests: antibody tests
  - BioCAN Zika virus IgG/IgM rapid test
  - EuroImmun Anti-Zika Virus ELISA (IgG / IgM)
- Of 14 positive Zika MAC-ELISA serum specimens, 0/14 and 6/14 were positive for Zika, respectively
Zika Action Plan Summit – 1 April 2016

- Provide information and tools needed to improve Zika preparedness and response
- Identify possible gaps in preparedness and response at the federal, state, and local levels, and help to begin to address possible gaps
- Follow up teleconference will be held June 13

1. **Develop Capacity.** Laboratory confirmation is important for patient management and Zika action plan execution.
2. **Be prepared.** Procure necessary resources for supplies and equipment. Ensure adequate staffing levels for increased or surge-level requests for testing, potentially including cross-training laboratorians.
3. **Develop a plan.** Determine how you will prioritize testing pregnant women with possible Zika virus infection.
4. **Educate clinicians.** Reach out to healthcare providers, particularly OB/GYNs, regarding available Zika virus diagnostic testing options and how to correctly collect and transport samples for testing.
5. **Communicate.** Assess, and if necessary, modify communication plans between laboratory and epidemiology teams.
6. **Report cases.** Zika is nationally notifiable as an arboviral disease. Report positive Zika virus laboratory results to CDC (ArboNET). Reporting cases helps to inform national surveillance, guidance, and policy decisions.
Surge Capacity for Zika Diagnostic Testing

- Meeting with states with high risk of local transmission and developing capacity to meet potential testing demand
- Offering assistance to estimate testing demand
- Encouraging all labs to consider maximum capacity even if the state is not considered to be high risk
- Identifying challenges with shipping samples across state lines and reporting results
- Improving capacity by expanding Zika MAC-ELISA testing capability
- Awarding $40 million to ELC grantees in August for Zika virus surveillance, laboratory diagnostic and communication activities
Zika Virus

Pregnant Women and Women

- Interim Guidelines for Health Care Providers in the United States, 2016 (Feb 5, 2016)
- Interim Guidelines for Pregnant Women and Their Providers
- Questions and Answers for Healthcare Providers

Infants and Children

- UPDATE: Interim Guidelines for Health Care Providers in the United States, 2016 (Feb 19, 2016)
- Questions and Answers for Healthcare Providers

Sexual Transmission

- UPDATE: Interim Guidance for Preventing Zika Virus Transmission Through Sexual Contact
- Interim Guidelines for Prevention of Sexual Transmission

Zika Virus

Summary

- Zika virus challenges continue but we continue to learn more each day
- Continual improvements and access to diagnostic tests are needed for better patient care
- New diagnostic tests should be evaluated thoroughly
- Keep your seatbelt fastened at all times
### Ebola and Zika – What We Thought We Knew

<table>
<thead>
<tr>
<th>Ebola virus</th>
<th>Zika virus</th>
</tr>
</thead>
<tbody>
<tr>
<td>▪ Occasional outbreaks internationally</td>
<td>▪ Occasional outbreaks internationally</td>
</tr>
<tr>
<td>▪ Acquired from infected animals</td>
<td>▪ Mosquito borne</td>
</tr>
<tr>
<td>▪ Highly contagious</td>
<td>▪ Mild fever, rash, conjunctivitis, etc. if any symptoms</td>
</tr>
<tr>
<td>▪ Hemorrhagic fever</td>
<td></td>
</tr>
</tbody>
</table>
Ebola and Zika – Reality

Ebola virus
- Occasional outbreaks internationally
- Could be easily spread to other non-affected countries through global travel
- Highly contagious
- Sexual transmission possible for months after recovery
- Hemorrhagic fever
- Establish testing in US!

Zika virus
- Occasional outbreaks internationally
- Vector borne
- Sexual transmission possible
- Mild fever, rash, etc. if any symptoms
- Congenital birth defects possible in pregnant women; Guillain-Barré Syndrome
- Establish testing asymptomatic pregnant patients!
Acknowledgements

APHL and US public health laboratories

FDA, BARDA, HHS, PAHO, WHO

Division of Vector Borne Diseases, NCEZID; Division of High-Consequence Pathogens and Pathology, NCEZID; Division of Scientific Resources, NCEZID; Division of Bacterial Diseases, NCIRD; Division of Viral Diseases, NCIRD; Division of Healthcare Quality Promotion, NCEZID

Laboratory Preparedness and Response Branch, DPEI, NCEZID
The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.