Zika Virus Testing for Tissue and Organ Donors

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September 22, 2016
Zika Virus

- **Family Flaviviridae, Genus Flavivirus**
- Single stranded RNA Virus
- First recognized 1947
  - monkey in Uganda
- After 1952, sporadic human cases in Africa, Southeast Asia and Pacific Islands
- 2007, outbreak on Yap Island, Micronesia
- May 2015, first confirmed cases in Brazil
# CLINICAL FEATURES

Zika virus compared to Dengue Virus and Chickungunya Virus

<table>
<thead>
<tr>
<th>Feature</th>
<th>Zika</th>
<th>Dengue</th>
<th>Chickungunya</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Rash</td>
<td>+++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Conjunctivitis</td>
<td>++</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>Arthralgia</td>
<td>++</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Myalgia</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Headache</td>
<td>+</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Hemorrhagic fever</td>
<td>-</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td>Shock</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>
Zika Virus Clinical Course and Outcomes

• Clinical illness usually mild, lasting few days to a week
• Approximately 80% infections asymptomatic
• Severe disease requiring hospitalization uncommon
• Fatalities rare
• Maternal-fetal transmission
  • associated microcephaly and other fetal/neurological effects
• Guillain-Barré syndrome following Zika virus infection
Modes of Transmission

- **Mosquito Bite**
  - Mainly by infected *Aedes* mosquito
    - *Aedes aegypti*: more efficient vector (Yellow fever mosquito)
    - *Aedes albopictus*: less efficient vector (Asian tiger mosquito)
  - Also transmit dengue and chikungunya viruses
  - Live in and around households
  - Aggressive and primarily daytime biters, but can also bite at night

- **Maternal-fetal**
  - Intrauterine
  - Perinatal

- **Other**
  - Sexual Transmission
  - Blood transfusion
  - Organ or tissue transplantation
  - Breast milk
Aedes aegypti and Aedes albopictus Mosquitoes: Geographic Distribution in the United States
Aedes albopictus: known geographic distribution in NYS

Counties with *Ae. albopictus*

Counties where additional trapping will take place to determine if northward spread has taken place
Number of confirmed and probable Zika virus disease cases reported from U.S. states and the District of Columbia — January 1–July 31, 2016

Confirmed and probable Zika virus cases reported from U.S. states, by month of illness onset and source of infection: Jan 1–Jul 31, 2016

<table>
<thead>
<tr>
<th>States</th>
<th>Travel-associated cases* No. (% of cases in states) (N=3,133)</th>
<th>Locally acquired cases† No. (% of cases in states) (N=43)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alabama</td>
<td>24 (1)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Arizona</td>
<td>29 (1)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Arkansas</td>
<td>10 (&lt;1)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>California</td>
<td>224 (7)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Colorado</td>
<td>32 (1)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Connecticut</td>
<td>58 (2)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Delaware</td>
<td>11 (&lt;1)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>District of Columbia</td>
<td>17 (1)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Florida</td>
<td>596 (19)</td>
<td>43 (100)</td>
</tr>
</tbody>
</table>
Zika Virus: Markers of Infection

First Symptoms

Infection

Incubation Period

Viral RNA

PCR Diagnosis

Serological Diagnosis

Time (days)

IgM

-14 -3 -2 -1 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16

Slide courtesy of Desiree Lebeaud and Michele Barry
Diagnostic Testing for Zika Virus

• Molecular assays, “PCR assays”
  • Most commonly real-time RT-PCR
  • Detect viral RNA

• Serology assays to detect IgM collected ≥4 days after illness onset

• Plaque reduction neutralization test (PRNT) to detect Zika virus-specific neutralizing antibodies
# Zika Virus: Diagnostic Testing at NYSDOH

<table>
<thead>
<tr>
<th>Test</th>
<th>Purpose</th>
<th>Results</th>
<th>Next steps</th>
</tr>
</thead>
<tbody>
<tr>
<td>MOLECULAR: (serum or urine)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>--RT-PCR</td>
<td>Detects Zika virus genetic material</td>
<td>Detected or not detected (indeterminate, equivocal)</td>
<td>If Zika virus RNA detected, infection is confirmed. No further testing needed</td>
</tr>
<tr>
<td>SEROLOGY: (serum)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>--Zika IgM ELISA antibody</td>
<td>Screening test for IgM antibodies to flaviviruses</td>
<td>Presumptive positive, negative (inconclusive, equivocal)</td>
<td>Results interpreted in conjunction with MIA and exposure history to determine if convalescent titers and PRNT needed</td>
</tr>
<tr>
<td>--Arbovirus Total Antibody Microsphere Immunofluorescence Assay (MIA)</td>
<td>Screening test for total antibodies to flaviviruses</td>
<td>Reactive, nonreactive</td>
<td>Results interpreted in conjunction with IgM and exposure history to determine if convalescent titers and PRNT needed</td>
</tr>
<tr>
<td>--Plaque Reduction Neutralization Testing (PRNT)</td>
<td>Quantifies amount of neutralizing antibody to viruses of interest</td>
<td>Positive, negative (for each flavivirus tested)</td>
<td>Usually conducted on paired sera in follow-up of possible IgM presence and/or reactive MIA. May be done on single specimen to provide preliminary information in pregnant women</td>
</tr>
</tbody>
</table>
## Zika virus molecular assays with FDA emergency use authorization

<table>
<thead>
<tr>
<th>Company</th>
<th>Test</th>
<th>Assay type</th>
<th>Specimens</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roche Molecular Systems</td>
<td>LightMix® Zika rRT-PCR Test</td>
<td>Zika virus RNA detection</td>
<td>serum and plasma</td>
<td></td>
</tr>
<tr>
<td>Luminex Corporation</td>
<td>xMAP® MultiFLEX™ Zika RNA Assay</td>
<td>Zika virus RNA detection</td>
<td>serum, plasma, and urine*</td>
<td>*collected with matched serum or plasma specimen</td>
</tr>
<tr>
<td>Siemens Healthcare Diagnostics</td>
<td>VERSANT® Zika RNA 1.0 Assay (kPCR) Kit</td>
<td>Zika virus RNA detection</td>
<td>serum, plasma, and urine*</td>
<td>*collected with matched serum or plasma specimen</td>
</tr>
<tr>
<td>Viracor-IBT Laboratories</td>
<td>Zika Virus Real-time RT-PCR test</td>
<td>Zika virus RNA detection</td>
<td>serum, plasma, and urine*</td>
<td>*collected with matched serum or plasma specimen</td>
</tr>
<tr>
<td>Hologic</td>
<td>Aptima® Zika Virus assay</td>
<td>Zika virus RNA detection</td>
<td>serum and plasma</td>
<td></td>
</tr>
<tr>
<td>altona Diagnostics</td>
<td>RealStar® Zika Virus RT-PCR Kit U.S.</td>
<td>Zika virus RNA detection</td>
<td>serum or urine*</td>
<td>*collected with matched serum specimen</td>
</tr>
<tr>
<td>Focus Diagnostics</td>
<td>Zika Virus RNA Qualitative Real-Time RT-PCR test</td>
<td>Zika virus RNA detection</td>
<td>serum</td>
<td></td>
</tr>
<tr>
<td>Centers for Disease Control and Prevention</td>
<td>Triplex Real-time RT-PCR Assay</td>
<td>Zika, dengue and chikungunya RNA detection</td>
<td>serum or CSF*</td>
<td>*collected with matched serum specimen</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Zika virus RNA detection</td>
<td>urine and amniotic fluid</td>
<td></td>
</tr>
</tbody>
</table>
Additional specimen types for Zika molecular testing: Urine

- ZIKV RNA detectable in urine for longer and at higher titer than in serum.

Gourinat et al., Emerg Infect Dis 2015
Distribution of Zika virus rRT-PCR Serum and Urine Cycle Threshold Values for Positive Specimens (N=93)

Serum (N=31)
- Median: 36.55
- Average: 35.25
- Standard Deviation: 4.24

Urine (N=62)
- Median: 32.60
- Average: 32.58
- Standard Deviation: 2.92
Zika virus RNA in semen

Mansuy et al. Oct 2016 Zika virus in semen and spermatozoa

The Lancet Infectious Diseases 2016 16, 1106-1107 DOI: (10.1016/S1473-3099(16)30336-X)
# Samples types for Zika diagnostic testing

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Test of choice</th>
<th>Utility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum/plasma/whole blood*</td>
<td>rRT-PCR, ELISA, PRNT</td>
<td>Infection, surveillance</td>
</tr>
<tr>
<td>Urine</td>
<td>rRT-PCR</td>
<td>Infection, surveillance</td>
</tr>
<tr>
<td>Saliva</td>
<td>rRT-PCR</td>
<td>Infection but no added benefit</td>
</tr>
<tr>
<td>Semen</td>
<td>rRT-PCR</td>
<td>Infectivity ?</td>
</tr>
<tr>
<td>CSF</td>
<td>rRT-PCR, ELISA, PRNT</td>
<td>Neurological infection, GBS</td>
</tr>
<tr>
<td>Amniotic Fluid</td>
<td>rRT-PCR</td>
<td>Transmission to fetus</td>
</tr>
<tr>
<td>Placental tissue, umbilical cord, fetal tissues</td>
<td>rRT-PCR, pathology, IHC</td>
<td>Transmission to fetus</td>
</tr>
</tbody>
</table>
Timing of Viremia and Antibody

DETECTING ZIKA VIRUS RNA AND ANTIBODIES

Blood
Zika Virus RNA

Anti-Zika IgM Antibodies

Symptom Onset

TIME
Zika Virus Antibody Detection – IgM

• IgM detected ≥4 days after onset of illness, may persist for 2-12 weeks or longer

• Previous infection or vaccination with other related flaviviruses (e.g., DENV, JEV, YFV) may result in false positive IgM

• If **negative**, suggests no infection (unless early after infection)

• If **positive**, will need confirmatory testing (PRNT)
  – Takes an additional 10-14 days due to time it takes for virus to grow in culture
Flaviviruses with serologic cross reactivity to Zika virus

• Dengue virus types 1-4
• Yellow fever virus
• West Nile virus
• St. Louis encephalitis virus
• Japanese encephalitis virus
• Powassan virus
# Zika virus serological assays with FDA emergency use authorization

<table>
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<tr>
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<th>Assay type</th>
<th>Specimens</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>InBios International</td>
<td>ZIKV Detect™ IgM Capture ELISA</td>
<td>presumptive detection of Zika IgM antibodies</td>
<td>serum</td>
<td></td>
</tr>
<tr>
<td>Centers for Disease Control and Prevention</td>
<td>Zika IgM Antibody Capture ELISA (Zika MAC-ELISA)</td>
<td>presumptive detection of Zika IgM antibodies</td>
<td>serum or CSF*</td>
<td>*collected with matched serum specimen</td>
</tr>
</tbody>
</table>
Disclaimers: CDC MAC ELISA and PRNT

MAC ELISA has very low specificity

PRNT is unable to resolve 17.2% (16/93) of Zika MAC ELISA positive specimens

Testing is limited to qualified laboratories designated by the CDC

NOTE: Given the current volume of samples, test results will not be available for at least 3 weeks after specimen receipt. Reporting times for test results may be longer during summer months or when arbovirus activity increases.

Plaque Reduction Neutralization Test (PRNT)

• More specific diagnostic assay
• Measures neutralizing antibody titer in serum
• Biological assay based on the principle of inactivation of virus by specific antibody, resulting in loss of ability to infect and replicate in cell culture
• PRNT may discriminate between cross-reacting antibodies in individuals who have never had a flavivirus infection before
• But, it may still be difficult to distinguish infecting virus in people with these prior exposures
• Commonly need a convalescent specimen as well as acute, with demonstration of >= 4x rise in PRN titer
Plaque Reduction Neutralization Test

1. Dilute test serum in serial dilutions
2. Neutralize virus by combining 100 ul of each serum dilution with 200 PFU of each virus to be tested (eg. Zika, Dengue 1, 2, 3, 4)
3. Incubate at 4ºC overnight
4. Infect Vero monolayers and overlay with agar
5. When plaques develop (4-7 days), stain cells with neutral red and count plaques
6. The Neutralizing Titer is the highest dilution of serum that neutralizes 90% of the virus
Plaque Reduction Neutralization Test (PRNT)

In each well: inoculate diluted patient serum + 100 pfu of virus
After incubation:
100 plaques = no plaque reduction = no virus antibody present
90% reduction of virus plaques = virus neutralizing antibody present
Interim Guidance for Interpretation of Zika Virus Antibody Test Results: MMWR June 3, 2016

<table>
<thead>
<tr>
<th>ZIKV &amp; Dengue IgM ELISA</th>
<th>ZIKV PRNT</th>
<th>Dengue PRNT</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive or Equivocal (either)</td>
<td>&gt;10</td>
<td>&lt; 10</td>
<td>Recent ZIKV infection</td>
</tr>
<tr>
<td>Positive or Equivocal (either)</td>
<td>&lt; 10</td>
<td>&gt;10</td>
<td>Recent Dengue infection</td>
</tr>
<tr>
<td>Positive or Equivocal (either)</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>Recent flavivirus infection; cannot determine specific virus</td>
</tr>
<tr>
<td>Inconclusive in at least one</td>
<td>&gt;10</td>
<td>&lt;10</td>
<td>Evidence of ZIKV but cannot tell timing</td>
</tr>
<tr>
<td>Inconclusive in at least one</td>
<td>&lt;10</td>
<td>&gt;10</td>
<td>Evidence of Dengue but cannot tell timing</td>
</tr>
<tr>
<td>Any result</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>No evidence for either virus</td>
</tr>
</tbody>
</table>
Issues, challenges and unknowns ...

• Test method – molecular vs serology
• Sample type
• Viral load
• Brief viremic period
• Antigenic cross-reactivity
• Tissue/organ distribution of virus
• Test availability
• FDA EUA
• Biosafety concerns
Commercial Laboratories and Zika Virus Testing

- Commercially available testing varies by laboratory
  - Serum PCR only
  - Serum PCR and urine PCR
  - Serum/urine PCR and Zika IgM

- Negative predictive value is highly dependent on time since exposure

- Zika IgM titers are affected by cross-reactivity with other flaviviruses (eg. Dengue virus)

- Comprehensive serologic evaluation (ie, total flavivirus antibodies, PRNT) is not available through commercial laboratories
NYS Zika Data as of 9/19/16

• Wadsworth Center has received specimens from 7,760 patients
• Total Zika cases for NYS: **788**
  – NYS (excluding NYC) 199 cases; NYC 589 cases;
• Gender/Pregnancy status:
  – 560 females
    • 89 pregnant
    • 466 NOT pregnant
    • 5 unknown pregnancy status
  – 228 males
• Method of confirmation:
  • RT-PCR on Serum ONLY - 187
  • RT-PCR on Urine ONLY - 422
  • RT-PCR on Serum & Urine - 126
  • RT-PCR on Placenta – 1
  • RT-PCR on Serum and PRN - 4
  • RT-PCR on Urine and PRN - 11
  • PRNT - 37
Zika Virus Laboratory Testing
Options and Limitations

• Molecular assays
  – IND for blood donor (currently 2 – Roche and Hologic)
  – Confirms presence of infection but non-detection does not exclude
  – Infectious ZIKV might persist in organs after resolution of viremia/viruria

• Serology
  – IgM capture ELISA:
    • Cross-reactivity with other flaviviruses
    • Duration variable - could exclude donors with resolved infection
  – Plaque Reduction Neutralization Test (PRNT)
    • Less cross reactivity
    • Extremely labor intensive, long turnaround times, very few labs

• Practical issues: rapid access and result availability for deceased donors

9/22/2016
Acknowledgements

• NYC DOHMH
• NYSDOH Wadsworth Center
• NYSDOH
• Centers for Disease Control and Prevention