Zika Diagnosis: Challenges and Opportunities

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Zika, dengue and chikungunya

- Three similar diseases
- Transmitted by the same mosquitoes
- Similar geographical distribution (approx. 100 countries)
- 2 billion people at risk of infection every year
- Currently, there are no vaccines or therapeutics commercially available for Zika, dengue and chikungunya virus infections
Zika, dengue and chikungunya

Common Symptoms
- Febrile Illness
- Rash
- Body aches

Specific symptoms/risks
- Dengue: hemorrhagic disease
- Chikungunya: severe arthritis
- Zika: Guillain Barre’ syndrome and birth defects
Integrated diagnosis and care of dengue, chikungunya and Zika cases
The changing landscape of arbovirus diagnostics

Before Chik or Zika
- Good understanding of dengue test sensitivity and specificity

After Chikungunya
- Limited chikungunya test options compared to dengue
- Unaltered dengue test sensitivity or specificity

After Zika
- Unspecific dengue and Zika immunodiagnostics
- Increased need for screening asymptomatic individuals for recent and previous infections
Diagnostic markers in serum during symptomatic infections

Virus

Days of illness

Diagnostic Window

-7 0 7 14

IgM antibodies
Sensitivity of Dengue Diagnostic Tests

Hunsperger et al, 2016
New Diagnostic Challenges

- **Flavivirus serological cross-reaction**
  IgM and IgG cross reactivity, particularly in secondary flavivirus infections and in co-endemic areas

- **Large proportion of asymptomatic infections**
  Need for detection of IgG antibodies to determine immune status (previous exposure) due to ZIKV and DENV infections.
  - Screening of pregnant women or women of reproductive age
  - Screening of potential dengue vaccine recipients
Traditional MAC-ELISAs Cannot Differentiate ZIKV vs DENV Infections

ZIKV IgM test

DENV IgM test

103/103 (100%)
78/134 (58%)

35/103 (34%)
134/134 (100%)
Percent confirmation of Zika IgM positive results by PRNT(90)

<table>
<thead>
<tr>
<th>PRNT(90) Interpretation</th>
<th>US States (n=759)</th>
<th>USVI (n=52)</th>
<th>American Samoa (n=103)</th>
<th>Puerto Rico (n=123)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZIKV infection</td>
<td>27</td>
<td>21</td>
<td>15</td>
<td>11</td>
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<tr>
<td>Unspecified flavivirus infection</td>
<td>48</td>
<td>62</td>
<td>83</td>
<td>84</td>
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<tr>
<td>DENV infection</td>
<td>9</td>
<td>8</td>
<td>1</td>
<td>6</td>
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<td>Negative</td>
<td>19</td>
<td>10</td>
<td>1</td>
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</table>

Lindsey et al, 2018

Study of symptomatic and asymptomatic infections, including pregnant women.
Potential of PRNT\(_{(50)}\) to confirm Zika infections in people with or without previous dengue

Longitudinal neutralizing antibody responses against dengue virus serotypes 1–4 (DENV1–4) and Zika virus (ZIKV) in samples obtained after ZIKV infection. (Montoya et al, 2018)
The ZIKV/DENV Duo MAC-ELISA can Discriminate ZIKV vs DENV Infections

ZIKV/DENV Duo for ZIKV

ZIKV/DENV ratio

0
10
20
30

ZIKV+ DENV+ NEG (AFI)

103/103 (100%)

0/133 (0%)

* One DENV+ specimen was equivocal

ZIKV/DENV Duo for DENV

ZIKV/DENV ratio

0
10
20
30
40

DENV+ NEG (AFI)

133/133 (100%)

0/133 (0%)
Recent lessons on Immunoassay Development

- Dengue vs Zika ratios increase specificity of IgM tests without reducing sensitivity:
  - E.g. InBios Zika Detect test (Granger et al. 2017)
  - CDC Tri-ELISA (under development)

- NS1 antibody detection tests are more specific than E antibody detection tests, but with some reduction in sensitivity
  - E.g. Eurolimmune (Lustig et al. 2017)
  - BOB ELISA, Balmaseda et al, 2017

- Differential avidity of antibodies for dengue and Zika E and NS1/NS5 antigens (Wong et al, 2017)
Increased reliance on molecular testing

- Highly sensitive during acute illness
- Approx. 60-75% of cases (Zika, dengue or chik) can be diagnosed during the first 6 days of illness
- Useful as confirmatory test for dengue or Zika IgM positive cases
- PRNT less useful in areas of flavivirus co-endemicity
- Long Zika viremias in pregnant
- Recommendation to test in 3 trimesters of pregnancy
- Able to detect pathogens in all fluids and tissues
Characteristics of the CDC-Trioplex RT-PCR assay

- Detects DENV (no subtyping), CHIKV and ZIKV
- Recommended for serum collected 0-7 DPO
- Recommended for urine, amniotic fluid and CSF (paired with serum)
- Contains internal control
- Adapted and validated for widely available equipment
Comparative analytical sensitivity of Zika virus NATs

Relative analytical sensitivity of donor nucleic acid amplification technology screening and diagnostic real-time polymerase chain reaction assays for detection of Zika virus RNA

Stone et al, Transfusion (2016)

* Donor screening NAT includes the RMS and Hologic NAT assays; CDC Puerto Rico (PR) low-input (LI) assay results were combined for Singleplex and Trioplex versions of the Trioplex assay (Trioplex-LI).

<table>
<thead>
<tr>
<th>Supernatent</th>
<th>Donor screening NAT, %*</th>
<th>CDC PR Trioplex-LI, %</th>
<th>CDC PR Trioplex-HI, %</th>
<th>CDC FC 1087-LI, %</th>
<th>CDC FC 108-HI, %</th>
<th>BSRI/UC Devis, %</th>
<th>FDA, %</th>
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<tbody>
<tr>
<td>cp/mL</td>
<td>PFU/mL</td>
<td>N = 7</td>
<td>N = 6</td>
<td>N = 4</td>
<td>N = 3</td>
<td>N = 20</td>
<td>N = 12</td>
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</table>

95% LOD: 17.9 (5.6, 38.1) 1529 (362, 3829) 28.8 (17.3, 37.9) 205 (80, 337) 20.3 (8, 33.6) 1102 (466, 2053) 4918 (1596, 10,660)

50% LOD: 2.5 (1.3, 4.9) 123 (55.4, 273) 24.8 (17, 36.1) 152 (77.2, 201) 15.1 (7.7, 20.2) 81.7 (52.3, 127) 321 (179, 576)
Utility of the CDC Trioplex RT-PCR by sample types

Santiago et al, 2018
Persistence of Zika virus in body fluids

Paz-Bailey et al, 2017
Possible Routs for Test Development

1- Symptomatic Patients:
   • Increase sensitivity of multiplexed (dengue/chik/Zika) antigen- or nucleic acid-based tests
   • Increase specificity of IgM tests (E.g. Duo ELISA for Zika/dengue detection and differentiation)

2- Asymptomatic or post-symptomatic:
   • Specific dengue and Zika IgG detection
     – Screening for asymptomatic pregnant women
     – Applications for vaccine evaluations and effectiveness
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