Laboratory Testing for Chikungunya Virus

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Presentation Summary

• Technology, Testing Algorithm, & Available Tests

• CDC Experience with CHIK Testing

• Proposed Algorithm for CHIK Testing
Laboratory Diagnosis of Arboviruses

• What tools are available for human diagnosis & environmental surveillance?

• What methods are desirable in an emergency involving an arbovirus outbreak?
  – Technology is easily transferable
  – Training, gold standards & calibrated panels have been established

• What have we learned from West Nile Virus?
  – Importance of comprehensive broad-based testing algorithms
  – Importance of collaborations
Simplified Depiction of CHIK Human Viremia & Immune Response

Days Post Onset

10^6

CHIK viremia

IgM

IgG Neutralizing Ab

DAYS POST ONSET

ELISA P/N

20

2

Simplified Depiction of CHIK Human Viremia & Immune Response

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DAYS POST ONSET

ELISA P/N

20

2
Current Laboratory Testing
Strategy for Arboviruses

• **Human Infection**
  – Acute antibody (IgM) in serum and/or csf.
    • *IgM ELISA or Microsphere Immunoassay*
    • *Confirmation by PRNT*
  – Seroconversion in paired specimens
    • *IgG ELISA and/or 4-fold rise in neutralizing antibody by PRNT*
  – Detection of virus and/or viral RNA in serum and/or csf.
    • *Real time RT-PCR, Consensus RT-PCR, or virus isolation*

• **Environmental Surveillance**
  – Detection of virus and/or viral RNA in mosquito vectors or amplifying hosts.
    • *Real time RT-PCR, Consensus RT-PCR, or virus isolation*
Serological Assays
IgM Capture ELISA

1. Coat With Goat anti-Human IgM
   ➢ 4° Overnight

2. Add Patient Serum @ 1:400
   ➢ 37° 1 Hour

3. Add Antigen
   ➢ 4° Overnight

4. Add HRP anti-group McAb
   ➢ 37° 1 Hour
IgG ELISA

1. Coat with anti-group McAb
   ➢ 4° Overnight

2. Add viral antigen
   ➢ 4° Overnight

3. Add Patient serum @ 1:400
   ➢ 37° 1 hour

4. Add AP anti-human IgG McAb
   ➢ 37° 1 Hour
Interpretation of Results

- P/N > 3 = positive
- P/N < 2 = negative
- P/N 2-3 = equivocal

- Positive & Negative Controls (including negative antigen) within correct range.
Neutralization Assay for West Nile Virus

Plaque Reduction Neutralization Test (PRNT)
Patient serum dilutions + 100 pfu of virus; incubate with cells.
100 plaques = no virus antibody present
90% reduction of virus plaques = virus antibody present
Microsphere-based assay to detect IgM to WN and SLE viruses in human serum

Beadsets are coupled to 6B6C-1

One beadset is reacted with WNV antigen and the other with SLEV antigen

Add reacted beadsets to IgG-depleted serum and anti-IgM R-PE.

- The assay gives concurrent WN and SLE virus IgM values
- All samples reacted on viral and control antigens
- Time of reaction 1.5 hours
- Assay for multiplex WN, SLE, DEN 1-4, LAC, JE, POW, MVE, YF, RR, MAY, VEE, EEE, WEE, BF, CHIK, in progress.
IgM Cross-Reactivity of Human CHIK Cases With Other Alphaviruses

<table>
<thead>
<tr>
<th>CHIK</th>
<th>RR</th>
<th>ONN</th>
<th>VEE</th>
<th>MAY</th>
<th>EEE</th>
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<td>9.1</td>
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Figure 1. Phylograms examining the complete genomes of the alphaviruses. These trees were generated using Clustal X (Thompson et al., 1994) and TreeView (Page, 1997). Each of the nodes in the trees is labeled with the number of bootstrap replicates that supported the branching of those taxa at the node.
Chikungunya
Virus Detection
Assays
## Sensitivity of WN Virological Assays

<table>
<thead>
<tr>
<th>TEST</th>
<th>DETECTION</th>
<th>DETECTION</th>
<th>ASSAY TIME</th>
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<tr>
<td>VecTest</td>
<td>100,000 pfu/ml</td>
<td>NT</td>
<td>15 min</td>
</tr>
<tr>
<td>CDC Ag-cap EIA</td>
<td>10,000 pfu/ml</td>
<td>NT</td>
<td>24 hour</td>
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<tr>
<td>RAMP</td>
<td>1,500 pfu/ml</td>
<td>NT</td>
<td>90 min</td>
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<tr>
<td>Isolation in SMB</td>
<td>100 pfu/ml</td>
<td>NT</td>
<td>5-10 days</td>
</tr>
<tr>
<td>Isolation in Vero</td>
<td>100 pfu/ml</td>
<td>NT</td>
<td>5-10 days</td>
</tr>
<tr>
<td>RT-PCR-standard</td>
<td>5 pfu/ml</td>
<td>2,500 copies/ml</td>
<td>8 hours</td>
</tr>
<tr>
<td>NASBA</td>
<td>0.1 pfu/ml</td>
<td>50 copies/ml</td>
<td>4 hours</td>
</tr>
<tr>
<td>RT-PCR-probe</td>
<td>0.1 pfu/ml</td>
<td>50 copies/ml</td>
<td>4 hours</td>
</tr>
<tr>
<td>GenProbe TMA</td>
<td>0.02 pfu/ml</td>
<td>10 copies/ml</td>
<td>4 hours</td>
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</table>
NAAT Assay Platform Options

- Standard RT-PCR
  - Agarose gels, nested

- Real time RT-PCR
  - Single/multiplex format
  - SYBR Green

- NASBA (nucleic acid sequence based amplification)
  - ECL, molecular beacons

- LAMP (Loop-mediated isothermal amplification)
# Comparison of CHIK NAAT Assays:

<table>
<thead>
<tr>
<th>Assay</th>
<th>Sensitivity</th>
<th>Contamination</th>
<th>Start-up cost(^1)</th>
<th>Reagent cost(^1)</th>
<th>Thru-put/automate?</th>
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<td>RT-PCR (several)</td>
<td>&gt; 100 copies</td>
<td>Yes</td>
<td>minimal</td>
<td>$4.80</td>
<td>Low/no</td>
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<tr>
<td>SYBR (several)</td>
<td>&gt; 100 copies</td>
<td>No</td>
<td>$25,000</td>
<td>$3.73</td>
<td>High/yes</td>
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<tr>
<td>Real-Time (several)</td>
<td>5-100 copies</td>
<td>No</td>
<td>$25,000</td>
<td>$2.80</td>
<td>High/yes</td>
</tr>
<tr>
<td>NASBA RT (1 published)</td>
<td>200 copies</td>
<td>No</td>
<td>$25,000</td>
<td>$8.00</td>
<td>Low/no</td>
</tr>
<tr>
<td>LAMP (1 published)</td>
<td>20 copies</td>
<td>No</td>
<td>$17,000</td>
<td>$5.00</td>
<td>Low/no</td>
</tr>
</tbody>
</table>

\(^1\) Start-up cost for iCycler; reagent cost per reaction all QIAGEN kits except for LAMP
Genetic Lineages of Chikungunya Virus

- Chikungunya virus is within the Semliki Forest Virus complex.
- Chikungunya viruses are genetically classified into 3 clusters;
  - Asian, West African, and Central/South/East African.
  - 2006 Indian Ocean epidemic caused by CHIK virus from the CSEA cluster.
CDC CHIK Real-Time RT-PCR Assay

Chikungunya Virus in US Travelers Returning from India, 2006

Robert S. Lanciotti, Olga L. Kosoy, Janeen J. Laven, Amanda J. Panella, Jason O. Velez, Amy J. Lambert, and Grant L. Campbell

- Developed 4 primer/probe sets
- Detects all CHIK genotypes (WA, SCEA, & Asia)
- Analytical sensitivity; 25 copies/reaction
Controls for RT-PCR Assays

• **Negative Controls**
  – Negative Extraction Controls
    • Extract RNA from several negative specimens
  – No Template Controls (NTC)
    • Add water to the amplification reaction

• **Positive Controls**
  – Positive Extraction Controls
    • Extract RNA from a quantitative range of positive controls
  – Positive RNA Control
    • Add template RNA to amplification reaction
  – Internal RNA Control
    • Non-template RNA added to monitor amplification conditions in every sample
Real Time RT-PCR
Interpretation of Results

- All positive controls yield predicted result, including appropriate Ct values
- All negative controls negative
- Ct value < 38 *presumptive positive*
- Ct value > 38 < 45 *equivocal*
- Ct value > 45 negative
- All presumptive positive and equivocal specimens are re-tested (including RNA extraction) with a second set of primer pairs
Virus Isolation in Shell Vials

1. Inoculate virus
2. Centrifuge ≈ 500g, 45 minutes, 37°C
3. Assay for virus by RT-PCR

**2-10 fold increase in sensitivity for alphaviruses, flaviviruses, bunyaviruses with CPE observed 2-3 days earlier compared to flask isolation**
### Serological & RT-PCR Test Results of CHIK Infected Returning Travelers

#### Chikungunya Virus in US Travelers

<table>
<thead>
<tr>
<th>Sample</th>
<th>IgM ELISA†</th>
<th>IgG ELISA†</th>
<th>PRNT‡</th>
<th>Virus isolation (Vero cells)</th>
<th>RT-PCR§</th>
<th>Viremia, PFU/mL¶</th>
<th>Days from onset of illness to collection</th>
<th>State of US residence</th>
<th>Return date, 2006</th>
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<tbody>
<tr>
<td>1</td>
<td>17.7</td>
<td>3.2</td>
<td>640</td>
<td>-</td>
<td>-</td>
<td>NA</td>
<td>0</td>
<td>NJ</td>
<td>10/12</td>
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<td>2</td>
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<td>1.7</td>
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<td>-</td>
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<td>3</td>
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<td>ND</td>
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<td>$10^4.1$</td>
<td>1</td>
<td>IL</td>
<td>9/29</td>
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<tr>
<td>4</td>
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<td>ND</td>
<td>+</td>
<td>+</td>
<td>$10^6.8$</td>
<td>2</td>
<td>CA</td>
<td>Before 9/16</td>
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<td>5</td>
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<td>0.76</td>
<td>ND</td>
<td>+</td>
<td>+</td>
<td>$10^5.1$</td>
<td>2</td>
<td>MA</td>
<td>9/10</td>
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<tr>
<td>6</td>
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<td>1.6</td>
<td>&lt;10</td>
<td>+</td>
<td>+</td>
<td>$10^6.0$</td>
<td>3</td>
<td>PA</td>
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<td>7</td>
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<td>ND</td>
<td>+</td>
<td>-</td>
<td>$10^5.3$</td>
<td>3</td>
<td>CA</td>
<td>10/2</td>
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<td>8</td>
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<td>+</td>
<td>-</td>
<td>$10^3.9$</td>
<td>4</td>
<td>WI</td>
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<td>9</td>
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<td>5,120</td>
<td>-</td>
<td>-</td>
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<td>4</td>
<td>CA</td>
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<td>-</td>
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<td>7</td>
<td>CA</td>
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<td>2,560</td>
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<td>IL</td>
<td>8/23</td>
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<td>-</td>
<td>NA</td>
<td>30</td>
<td>IL</td>
<td>6/25</td>
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</table>
CHIK Laboratory Testing at DVBID 2006-Present

Confirmed approximately 75 CHIKV infections from returning travelers

80% of specimens collected day 1-8 post-onset are real-time RT-PCR and/or isolation positive.

16 virus isolations
Calculated viremia 3.9-6.8 log10 PFU/ml**

**≈ 4 log10 PFU/ml sufficient to infect U.S Ae. Aegypti and Ae. Albopictus
Confirmed approximately 75 CHIKV infections from returning travelers

45% of specimens collected day 1-8 post-onset are IgM/PRNT positive.

Summary:

Day 1-3:    RT-PCR/Isolation POS  IgM PRNT NEG
Day 4-8:    RT-PCR/Isolation POS/NEG IgM PRNT POS/NEG
>Day 8:     RT-PCR/Isolation NEG   IgM PRNT POS
Serological Testing Algorithm for Chikungunya Virus Infection

single acute patient serum

IgM Capture ELISA
IgG ELISA
RT-PCR (<10day)

IgM POS

PRNT

POS

IgM NEG (<10day)

No

Interpretation

IgM NEG (>10day)

NEG

POS

RT-PCR or Isolation POS
Environmental Testing Algorithm for Chikungunya Virus Detection

Mosquito pool

- Real-time RT-PCR
- Virus isolation
- Consensus RT-PCR & Nucleic acid sequencing
CDC Response to Potential Chikungunya

Introduction to the Americas: Proposed Laboratory Response

• **Testing**
  – Confirmatory testing of human and/or mosquito specimens.
  – Virus sequencing

• **Training & Technology Transfer**
  – Training at CDC or on-site training
  – Protocol & reagent transfer
  – Proficiency assessment
CDC Arbovirus Proficiency Program
2000 - Present

• Comprehensive laboratory training in IgM ELISA, IgG ELISA, real time RT-PCR, plaque reduction neutralization test (PRNT), and microsphere immunoassay.

• 2000-2007 training provided to over 100 public health laboratorians representing all 50 U.S. states.

• >90% average agreement of test results.

• Network of laboratories prepared for new emerging diseases.

• Reagent distribution.
Special Thanks To

CDC Arbovirus Diagnostic & Reference Section

Roselyn Hochbien
Janeen Laven
Mara Miller
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Brandy Russell

Rob Lanciotti
Amy Lambert
Olga Kosoy

Jason Velez
Barbara Johnson
Jane Johnson
Amanda Panella