Diagnostics for CHIKV Vaccine Evaluation

Ann M. Powers, PhD
CDC
Division of Vector-Borne Diseases, Arboviral Diseases Branch
Infection

Incubation Period

First Symptoms (low grade fever, rash, conjunctivitis, myalgia)

Viral RNA

Time (days)

Molecular Diagnosis

Serological Diagnosis

Diagnostic Windows
Important Lab Findings…

• 80% of specimens collection days 1-8 post onset are qRT-PCR and/or virus isolation positive

• 45% of specimens collected days 1-8 post onset are IgM and/or PRNT positive

• Viremia range: 3.9 – 6.8 pfu/ml
Summary

- Day 1-3: RT-PCR / Isolation positive  IgM / PRNT negative
- Day 4-8: RT-PCR / Isolation pos/neg  IgM / PRNT pos/neg
- > Day 8: RT-PCR / Isolation negative  IgM / PRNT positive
Optimal Traits of Diagnostic Tests

• Sensitive
• Definitive
• Specific
• Rapid
• Inexpensive
• Uses range of samples (non-invasive)
• Easy to use
• No requirement for specialized equipment
For detection of immunogenicity:

(ie – phase I/II studies), collect samples at appropriate times post immunization and perform serological testing.
Standardization of Immunoglobulin M Capture Enzyme-Linked Immunosorbent Assays for Routine Diagnosis of Arboviral Infections

DENISE A. MARTIN,* DAVID A. MUTH, TERESA BROWN, ALISON J. JOHNSON, NICK KARABATSOS, AND JOHN T. ROEFLIG

Division of Vector-Borne Infectious Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Public Health Service, U.S. Department of Health and Human Services, Fort Collins, Colorado 80522
## 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>
</tr>
</thead>
<tbody>
<tr>
<td>12.3</td>
<td>1.5</td>
<td>15.3</td>
<td>0.89</td>
<td>1.9</td>
<td>1.2</td>
</tr>
<tr>
<td>10.6</td>
<td>1.2</td>
<td>13.9</td>
<td>1.1</td>
<td>1.9</td>
<td>1.3</td>
</tr>
<tr>
<td>14.5</td>
<td>1.7</td>
<td>17.3</td>
<td>1.1</td>
<td>3.1</td>
<td>0.89</td>
</tr>
<tr>
<td>20.4</td>
<td>0.92</td>
<td>20.7</td>
<td>1.5</td>
<td>7.1</td>
<td>1.8</td>
</tr>
<tr>
<td>26.6</td>
<td>4.9</td>
<td>27.4</td>
<td>2.2</td>
<td>1.7</td>
<td>1.5</td>
</tr>
<tr>
<td>21.2</td>
<td>1.5</td>
<td>24.7</td>
<td>2.2</td>
<td>1.2</td>
<td>1.6</td>
</tr>
<tr>
<td>15.3</td>
<td>1.5</td>
<td>8</td>
<td>1.6</td>
<td>1.9</td>
<td>2.2</td>
</tr>
<tr>
<td>31.3</td>
<td>1.5</td>
<td>24.6</td>
<td>1.7</td>
<td>2.9</td>
<td>1.8</td>
</tr>
<tr>
<td>22</td>
<td>1.6</td>
<td>15.9</td>
<td>1.8</td>
<td>1.4</td>
<td>1.4</td>
</tr>
<tr>
<td>18.8</td>
<td>0.59</td>
<td>13.1</td>
<td>1.2</td>
<td>1.5</td>
<td>1.1</td>
</tr>
<tr>
<td>34.3</td>
<td>3.7</td>
<td>22.6</td>
<td>1.6</td>
<td>2.8</td>
<td>9.1</td>
</tr>
</tbody>
</table>
Antibody Neutralization Assay

- Diagnostic and Vaccine evaluation: Most important functional serology assay (correlates with protection and more specific than ELISA)
Plaque Reduction Neutralization Test (PRNT)

- Detects neutralizing antibodies present in specimens
  - If anti-CHIKV antibody is present in the specimen:
    - virus cannot attach to cells (virus is neutralized)
    - infectivity is blocked (reduced/reduction in plaques)

Preferred method for antibody detection for vaccine clinical studies.
Advantages/Disadvantages of PRNT

- PRNT (plaque reduction neutralization assay)
  - Long standardized (Gold Standard), much data to compare with
  - No reagents (antigens, antibodies, etc.) required – just live virus
  - Functional assay
  - Time to completion can be up to 6-8 days (depending on virus used)
  - Labor intensive
  - Not high throughput
  - Work with live virus → safety issues
  - Cross reactivity may still be a problem
Ways to further improve PRNT
automated using fluorescent reporter chimeric molecules

• High-throughput, computer generated output, does not rely on technician
• Time to completion can be as little as 24 hours (depending on assay and virus used)
• Results comparable to standard PRNT
• Work with live virus → safety issues – reduced if use of chimeric virus (attenuated) or VRP (non-infectious)
• Limited number of labs may have necessary equipment ( imagers, flow cytometers, etc.)
• Commercial options expensive (VRP)
Features of CHIKV NT assays used for clinical trials

<table>
<thead>
<tr>
<th>Features</th>
<th>EC80 SFV/CHIK OPY1 with Reporter (GFP)</th>
<th>PRNT80 181/25 Vaccine Strain</th>
<th>NT80 181/25 Vaccine Strain with Reporter (Luc)</th>
<th>PRNT50 181/25 vaccine strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Source</td>
<td>NIH/NIAID</td>
<td>WRAIR/AFRIMS</td>
<td>PaxVax</td>
<td>Themis</td>
</tr>
<tr>
<td>Study</td>
<td>VRC311 Phase 1, VRC704 Phase 2</td>
<td>Observational Study (Philippines)</td>
<td>VRC311 Phase 1, VRC704 Phase 2</td>
<td>Phase 1, Phase 2</td>
</tr>
<tr>
<td>Assay type</td>
<td>Flow cytometry</td>
<td>Plaque reduction</td>
<td>Micro-neutralization</td>
<td>Plaque reduction</td>
</tr>
<tr>
<td>Containment</td>
<td>BSL3</td>
<td>BSL2</td>
<td>BSL2</td>
<td>BSL2</td>
</tr>
</tbody>
</table>
CHIKV PRNT50 titers using 181/25 CHIKV
CHIKV EC50 NT titers using chimeric CHIKV with GFP reporter with flow cytometry detection

CHIKV OPY1 strain

Neutralization titer (IC$_{50}$)

100000
10000
1000
100
10

10 mcg
20 mcg
40 mcg

CHIKV VLP Dose

Week 0
Week 4
Week 8
Week 20
Week 24
Week 44

NIH
CHIKV NT using CHIKV with LUC reporter

Establish Vero cell monolayers
1.5 x 10^4/well

Neutralization Step
Mix serum with virus
1.5 hours @ 37 °C

Serum
+ CHIKV-Luc

Luciferase Detection
Add Steady Glo reagent
Measure RLU Luminescence

Day 1
overnight @ 37 °C

Day 2
add

Day 3
18 hours @ 37 °C
Comparison of results from NT assays
(PRNT = WRAIR; CHIKV-Luc = PaxVax; SVF/CHIKV = NIH)

<table>
<thead>
<tr>
<th>Assay Comparison</th>
<th>n of Samples Quantifiable by Each Method</th>
<th>Correlation Coefficient</th>
<th>Median Bias (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRNT vs. SFV/CHIKV</td>
<td>113</td>
<td>0.88</td>
<td>-3% (-10%, 4%)</td>
</tr>
<tr>
<td>CHIKV-Luc vs. SFV/CHIKV</td>
<td>113</td>
<td>0.94</td>
<td>15% (1%, 24%)</td>
</tr>
<tr>
<td>PRNT vs. CHIKV-Luc</td>
<td>115</td>
<td>0.89</td>
<td>8% (-6%, 30%)</td>
</tr>
</tbody>
</table>
For laboratory detection of CHIKV infection (symptomatic cases during clinical trials):

collect sample within 1 week of illness onset and perform molecular testing.
## 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>
</tr>
</thead>
<tbody>
<tr>
<td>RT-PCR (several)</td>
<td>&gt; 100 copies</td>
<td>Yes</td>
<td>minimal</td>
<td>$4.80</td>
<td>Low/no</td>
</tr>
<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>
</tr>
<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
Thank you & Questions?

For more information, contact CDC
1-800-CDC-INFO (232-4636)

The findings and conclusions in this report are those of the author(s) and do not necessarily represent the official position of the Centers for Disease Control and Prevention
A Virus-Like Particle Vaccine Elicits Broad Neutralizing Antibody Responses in Humans to All Chikungunya Virus Genotypes


J Infect Dis | Published by Oxford University Press for the Infectious Diseases Society of America 2016. This work is written by (a) US Government employee(s) and is in the public domain in the US.
From: A Virus-Like Particle Vaccine Elicits Broad Neutralizing Antibody Responses in Humans to All Chikungunya Virus Genotypes


J Infect Dis | Published by Oxford University Press for the Infectious Diseases Society of America 2016. This work is written by (a) US Government employee(s) and is in the public domain in the US.