Laboratory diagnosis of Chikungunya virus infections:
Comparative evaluation and performance of chikungunya laboratory tests in the Americas

Consultation and Partners’ Forum on Chikungunya in the Caribbean:
Meeting Today’s Challenge and Preparing for the Future
CARPHA, March 3 – 5, 2015

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Introduction to CDC Arbovirus Diagnostic and Reference laboratory

Laboratory diagnosis of CHIKV infections at CDC

Evaluation of commercial CHIKV IgM detection assays
DVBD Arboviral Diseases Branch

- Japanese encephalitis virus
- West Nile virus
- Saint Louis encephalitis virus
- Tick-borne encephalitis viruses
- Yellow fever virus
- Dengue viruses (Dengue Branch San Juan, Puerto Rico)
- Zika virus
- Equine encephalitis viruses (EEEV, WEEV, VEEV)
- Chikungunya, O'nyong-nyong, and other alphaviruses
- LaCrosse and other bunyaviruses
- Emerging and re-emerging arboviruses

WHO Collaborating Center for Arbovirus Reference and Research
CDC Arboviral Diseases Diagnostic Laboratory provides reference and confirmatory diagnostic testing for suspected arboviruses to:

- Travelers/physicians
- US State public health laboratories
- International public health laboratories
- National and international outbreak investigations
- Serosurveys, following epidemic or after vaccination campaigns
- Other CDC divisions
Factors that determine the CDC/DVBD arbovirus diagnostic testing algorithm:

• Geographical origin of specimen

• Clinical symptoms

• Specimen type and timing of collection

• Age of patient

• Time of patient in endemic area (resident vs traveler)

• Characteristics of the virus infection and immune response

• Volume and condition of sample
Geographical Origin of Specimen
Specimens are tested for arboviruses from geographical region, based on clinical information and volume of sample.
Geographical Origin of Specimen
Update 2013!

Antigen Panels for Arboviral Testing

- LAC
- SLE
- WEE
- CTF (VEE)
- DEN1-4
- WN

- CHIK

- EEE
- VEE
- WEE
- MAY
- DEN1-4
- WN
- SLE

- CHIK

- CHIK

- EEE
- WEE
- VEE
- MAY
- DEN1-4
- YF
- WN
- SLE

- CHIK
- SIN
- TBE (POW)
- WN
- (TAH, INK)

- CHIK
- SIN
- WEE
- EEE
- EVE
- DEN1-4
- WN

- CHIK
- SIN
- YF
- DEN1-4
- TAH
- WN

- CHIK
- SIN
- RR
- SIN
- MVE
- DEN1-4

- BF

- SSH
- JE
Geographical Origin of Specimen
2015?

Antigen Panels for Arboviral Testing
First priority testing method based on characteristics of the virus infection and immune response and timing of specimen collection.
Clinical symptoms: similar between

- Dengue fever
- Chikungunya fever
- Zika infection
- Other febrile illnesses
CDC differential diagnostics: serology

First test: IgM antibody capture ELISA

P/N: O.D. patient serum on viral antigen/O.D. negative control serum on viral antigen
- P/N > 3 = positive
- P/N < 2 = negative
- P/N 2-3 = equivocal

Ref = pos control serum
N = normal control serum

Test validity criteria: OD for the test specimen must be $\geq$ twice the mean OD of the test specimen reacted on normal antigen. If this requirement is not met, non-specific background is being generated, and the result MUST be reported as uninterpretable.

Each lot of reagents must be standardized for each assay
1st priority testing method depends on type and volume of sample

Serological Assays
- IgM ELISA
- Microsphere immunoassay
- IgG ELISA
- PRNT

Serum, CSF

Virus Detection Assays
- Viral RNA detection
- Nucleotide sequencing
- Virus isolation
- Immunofluorescence assays
- Antigen detection ELISA

Mosquito pools, tissues, serum, CSF
Interpretations of test results for a single acute specimen

Acute Specimen

- IgM ELISA
  - NEG
    - No Interpretation
  - POS
    - PRNT
      - 4-fold
        - ID Virus
      - No 4-fold
        - No Interpretation

- IgG ELISA
  - NEG
    - No Interpretation
  - POS
    - Possible 2° Infection

- Consensus RT-PCR
  - POS
    - Nucleic acid sequencing
      - ID Virus
    - No Interpretation

- Real-Time RT-PCR
  - POS
    - ID Virus

- Virus Isolation
  - POS
    - ID Virus

- RT-PCR or IFA
  - POS
    - ID Virus
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
Example:
CDC Testing algorithm for West Nile Virus

<table>
<thead>
<tr>
<th>Specimen</th>
<th>1st Choice</th>
<th>Other</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human serum and CSF</td>
<td>Serology: WN and SLE ELISA + PRNT</td>
<td>WN-specific qRT-PCR, flavivirus RT-PCR + sequencing, virus isolation</td>
<td>WN qRT-PCR sensitivity: 57% acute CSF, &lt;10% serum</td>
</tr>
<tr>
<td>Human tissue</td>
<td>WN-specific qRT-PCR</td>
<td>Virus isolation, IHC</td>
<td>Fatal WN cases: WN qRT-PCR sensitivity ~ 100%</td>
</tr>
<tr>
<td>Non-Human 1st Choice</td>
<td></td>
<td>2nd Choice</td>
<td></td>
</tr>
<tr>
<td>Avian tissue</td>
<td>WN-specific qRT-PCR, Virus isolation</td>
<td>VecTest/ antigen capture ELISA, flaviviruses RT-PCR</td>
<td>Ag.-based tests require 1000 PFU</td>
</tr>
<tr>
<td>Mosquito pool</td>
<td>WN qRT-PCR, flavivirus RT-PCR, virus isolation</td>
<td>VecTest/Ag. Cap. ELISA/RT-PCR</td>
<td></td>
</tr>
</tbody>
</table>
### CDC/DVBID Diagnostic Testing Algorithm for Medically Important Arthropod-Borne Viral Diseases in North America *

<table>
<thead>
<tr>
<th>AGENT OR DISEASE</th>
<th>SPECIMEN(S) TO COLLECT</th>
<th>METHOD OF CONFIRMATION OR IDENTIFICATION**</th>
<th>COMMENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>California encephalitis/ La Crosse encephalitis</td>
<td>Sera</td>
<td>IgM ELISA, NT</td>
<td>Except where noted freeze specimens for virus isolation at –65°C (dry ice)</td>
</tr>
<tr>
<td></td>
<td>Brain</td>
<td>Real-time RT-PCR, virus isolation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CSF</td>
<td>IgM ELISA, NT, Real-time RT-PCR,† Virus isolation†</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mosquitoes</td>
<td>Real-time RT-PCR, Virus isolation</td>
<td></td>
</tr>
<tr>
<td>Colorado tick fever</td>
<td>Whole blood/clot</td>
<td>Real-time RT-PCR, Virus isolation</td>
<td>Do not freeze samples for CTF virus isolation.</td>
</tr>
<tr>
<td></td>
<td>Sera</td>
<td>Real-time RT-PCR, Virus isolation, paired NT</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ticks</td>
<td>Real-time RTPCR, Virus isolation</td>
<td></td>
</tr>
<tr>
<td>Dengue 1-4</td>
<td>Sera</td>
<td>IgM ELISA, NT, Real-time RT-PCR,† † virus isolation††</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Liver, lung, lymph nodes</td>
<td>Real-time RT-PCR, Virus isolation</td>
<td></td>
</tr>
<tr>
<td>Eastern equine encephalitis/ Venezuelan equine encephalitis/ Western equine encephalitis</td>
<td>Sera</td>
<td>IgM ELISA, NT, Real-time RT-PCR,† virus isolation†</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Brain</td>
<td>Real-time RT-PCR, Virus isolation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CSF</td>
<td>IgM ELISA, NT, Real-time RT-PCR, † Virus isolation†</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mosquitoes</td>
<td>Real-time RT-PCR, Virus isolation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Horse sera</td>
<td>IgM ELISA, NT, Real-time RT-PCR, Virus isolation</td>
<td></td>
</tr>
<tr>
<td>St. Louis encephalitis</td>
<td>Sera</td>
<td>MIA/IgM ELISA, NT, Real-time RT-PCR†</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Brain</td>
<td>Real-time RT-PCR, virus isolation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CSF</td>
<td>IgM ELISA, NT, Real-time RT-PCR/virus isolation</td>
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</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>AGENT OR DISEASE</th>
<th>SPECIMEN(S) TO COLLECT</th>
<th>METHOD OF CONFIRMATION OR IDENTIFICATION</th>
<th>COMMENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>West Nile virus</td>
<td>Sera</td>
<td>MIA/IgM ELISA, NT, Real-time RT-PCR†</td>
<td>Isolation requires biosafety level 3 containment</td>
</tr>
<tr>
<td></td>
<td>Brain, brain stem, spinal cord</td>
<td>Real-time RT-PCR, Virus isolation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CSF</td>
<td>IgM ELISA, NT, Real-time RT-PCR,† Virus isolation†</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mosquitoes</td>
<td>Real-time RT-PCR, Virus isolation,Dipstick, RAMP</td>
<td></td>
</tr>
<tr>
<td>Yellow fever§</td>
<td>Sera</td>
<td>IgM ELISA, NT, Real-time RT-PCR,† Virus isolation†</td>
<td>Isolation requires biosafety level 3 containment with HEPA filtered exhaust air flow; Yellow fever immunization required</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>Real-time RT-PCR, Virus isolation, histopathology</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mosquitoes</td>
<td>Real-time RT-PCR, Virus isolation</td>
<td></td>
</tr>
</tbody>
</table>

*See Appendix 22-3 for definitions of acronyms. **Listed in order of priority. †If specimen is acute and volume allows for both serology and molecular testing. ††In acute specimens up to 7 days post-onset of fever. §Imported cases only; international travel history to yellow fever endemic areas.
Geographical Distribution of Identified Chikungunya Virus Isolates 2004

CHIKV Clades

- W. African
- Central / East African
- Asian

Locations from which CHIKV has been isolated from individuals
Countries with endemic CHIKV activity
Countries and territories where chikungunya cases have been reported* (as of February 24, 2015)

*Does not include countries or territories where only imported cases have been documented. This map is updated weekly if there are new countries or territories that report local chikungunya virus transmission.

From: http://www.cdc.gov/chikungunya/geo/index.html
Serological & RT-PCR Test Results of CHIK Infected Returning Travelers 2006*

Chikungunya Virus in US Travelers

Table 1. Diagnostic test results for 35 travelers infected with chikungunya virus (CHIKV), 2006*

<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>
</tr>
</thead>
<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>
</tr>
<tr>
<td>2</td>
<td>1.7</td>
<td>1.7</td>
<td>&lt;10</td>
<td>–</td>
<td>+</td>
<td>$10^{4.3}$</td>
<td>1</td>
<td>CA</td>
<td>Before 11/28</td>
</tr>
<tr>
<td>3</td>
<td>1.2</td>
<td>1.1</td>
<td>ND</td>
<td>+</td>
<td>+</td>
<td>$10^{4.1}$</td>
<td>1</td>
<td>IL</td>
<td>9/29</td>
</tr>
<tr>
<td>4</td>
<td>1.8</td>
<td>NS</td>
<td>ND</td>
<td>+</td>
<td>+</td>
<td>$10^{6.8}$</td>
<td>2</td>
<td>CA</td>
<td>Before 9/16</td>
</tr>
<tr>
<td>5</td>
<td>1.2</td>
<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>
</tr>
<tr>
<td>6</td>
<td>1.8</td>
<td>1.6</td>
<td>&lt;10</td>
<td>+</td>
<td>+</td>
<td>$10^{6.0}$</td>
<td>3</td>
<td>PA</td>
<td>Before 8/20</td>
</tr>
<tr>
<td>7</td>
<td>1.6</td>
<td>1.2</td>
<td>ND</td>
<td>+</td>
<td>+</td>
<td>$10^{5.3}$</td>
<td>3</td>
<td>CA</td>
<td>10/2</td>
</tr>
<tr>
<td>8</td>
<td>NS</td>
<td>1.4</td>
<td>ND</td>
<td>–</td>
<td>+</td>
<td>$10^{3.9}$</td>
<td>4</td>
<td>WI</td>
<td>10/9</td>
</tr>
<tr>
<td>9</td>
<td>22.0</td>
<td>4.3</td>
<td>5,120</td>
<td>–</td>
<td>–</td>
<td>NA</td>
<td>4</td>
<td>CA</td>
<td>Before 10/6</td>
</tr>
<tr>
<td>10</td>
<td>1.1</td>
<td>0.95</td>
<td>&lt;10</td>
<td>–</td>
<td>+</td>
<td>$10^{4.5}$</td>
<td>6</td>
<td>CA</td>
<td>Before 8/13</td>
</tr>
<tr>
<td>11</td>
<td>7.4</td>
<td>0.96</td>
<td>40</td>
<td>–</td>
<td>–</td>
<td>NA</td>
<td>7</td>
<td>CA</td>
<td>Before 9/23</td>
</tr>
<tr>
<td>12</td>
<td>15.0</td>
<td>0.60</td>
<td>320</td>
<td>–</td>
<td>–</td>
<td>NA</td>
<td>8</td>
<td>CT</td>
<td>Before 7/6</td>
</tr>
<tr>
<td>13</td>
<td>26.2</td>
<td>1.2</td>
<td>160</td>
<td>ND</td>
<td>–</td>
<td>NA</td>
<td>8</td>
<td>DC</td>
<td>Before 10/16</td>
</tr>
<tr>
<td>14</td>
<td>12.9</td>
<td>5.8</td>
<td>1,80</td>
<td>ND</td>
<td>–</td>
<td>NA</td>
<td>10</td>
<td>CA</td>
<td>Before 9/22</td>
</tr>
<tr>
<td>15</td>
<td>38.8</td>
<td>2.3</td>
<td>2,560</td>
<td>ND</td>
<td>ND</td>
<td>NA</td>
<td>19</td>
<td>CT</td>
<td>Before 7/6</td>
</tr>
<tr>
<td>16</td>
<td>12.7</td>
<td>1.5</td>
<td>640</td>
<td>ND</td>
<td>–</td>
<td>NA</td>
<td>20</td>
<td>IL</td>
<td>8/23</td>
</tr>
<tr>
<td>17</td>
<td>16.9</td>
<td>4.8</td>
<td>640</td>
<td>ND</td>
<td>ND</td>
<td>NA</td>
<td>30</td>
<td>IL</td>
<td>6/25</td>
</tr>
</tbody>
</table>

Initial Laboratory Testing at DVBD 2006*

- 35/106 confirmed CHIKV infections from travelers returning from India (N=33) and Reunion Island (N=2)

27 IgM ELISA+ and PRNT + SD and CTK Rapid tests sensitivity low
CDC PCs negative in lateral flow assays

- Days from illness onset to specimen collection: range 4-101

- 8 RT-PCR +
  - Days from illness onset to specimen collection: range 1-6
  - None IgM ELISA +

- 5 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

CDC Diagnostic Testing Algorithm for detection of CHIKV infection

Serum* collected

<Day 6 POI
  ↓
qRT-PCR
  ↓
POS
  ↓
REPORT

≥Day 6 POI
  ↓
IgM ELISA
  ↓
POS
  ↓
PRNT
  ↓
REPORT

NEG
  ↓
REPORT

*Meets Clinical Case Definition: Fever and arthralgia in a person returning from a CHIK-endemic or epidemic region POI, post-onset of illness.
qRT-PCR Interpretation

2 sets Chikungunya primers and probes
CHIKV 3855 (primer 1) GAGCATA CGGTACGCAGATAG
CHIKV 3957c (primer 2) TACTGGGTACACATGGTGGTTTC + TGCTGGTGACACATGGTGGTTTC
CHIKV 3886 FAM (probe) ACGAGTAATCTCGTGACTGGGACGTA + ACGAGTCATCTCGTGATTGGACGCA


CHIK 856 (primer 1) ACCATCGGTGTCCATCTAAAG
CHIK 962c (primer 2) GCCTGGGCTCATCGTTATT
CHIK 908FAM (probe) ACAGTGGTTTCGTGGAGGGCTAC
Designed to Caribbean isolates (Lanciotti unpublished)

Positive: Ct value <38 in duplicate wells (threshold of detection ≈1 PFU)
Equivocal: Ct value <38 in one of two wells.
Negative: Ct values >38 in duplicate wells.

All positive and equivocal samples are repeated with a second set of primer/probes for confirmation. A positive result in any of the negative controls invalidates the entire run. Failure of the positive control to generate a positive result also invalidates the entire run.

Serology

• Samples tested simultaneously by CHKV and DENV MAC-ELISA (not cross-reactive)
• Samples with IgM positive results confirmed by PRNT
Samples tested at CDC in 2014

- 1,452 Samples Received (46% <Day 6)
- 660 Tested by qRT-PCR; 324 POS
- 1120 Tested by IgM ELISA; 433 POS
Results from subset of samples by qRT-PCR and MAC-ELISA tested at CDC from U.S. Virgin Islands (n=174)

<table>
<thead>
<tr>
<th>Category</th>
<th>Total #</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR NEG + SER NEG</td>
<td>46</td>
<td>26.4%</td>
</tr>
<tr>
<td>PCR POS + SER NEG</td>
<td>78</td>
<td>44.8%</td>
</tr>
<tr>
<td>PCR NEG + SER POS</td>
<td>31</td>
<td>17.8%</td>
</tr>
<tr>
<td>PCR POS + SER POS</td>
<td>5</td>
<td>2.9%</td>
</tr>
<tr>
<td>Equivocals</td>
<td>14</td>
<td>8%</td>
</tr>
</tbody>
</table>
Number of qRT-PCR Positive Samples by Days Post-Onset of Illness
CDC Arbovirus Diagnostic Laboratory: Conclusions

- qRT-PCR is the primary diagnostic test

- Few samples (2.9%) are RT-PCR and IgM ELISA positive

- Labs with only qRT-PCR OR MAC-ELISA capacity should test samples at all days POI; negative results reported as inconclusive
CDC reagent production laboratory

- One laboratory scientist makes all antigens and antibodies

- Reagents produced for CDC developed diagnostic testing for approximately 50 arboviruses

- Previous to 2000 made and distributed reference quantities only

- 2002 distributed reagents for large-scale WNV testing

- Currently provide reagents for specific arboviruses with no validated commercial assays

- 2014 distributed >1600 reagent vials in >250 shipments to US state and international labs

- Approximately 1000 vials were for CHIKV diagnosis
CDC does not endorse any particular commercial assay, so why evaluate the assays?

- Reagents are evaluated and validated for CDC protocols only
- CDC protocols optimized for specific reagents and supplies
- Commercially available reagents and supplies (buffers, ELISA plates, etc) must be purchased by each lab
- CDC MAC-ELISA protocol requires standardization in each lab, which not all labs have capacity to do
- CDC produces small lots; each new lot of reagents needs to be standardized
## Evaluations of Commercial CHKV IgM Detection Assays

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Location</th>
<th>Assay name and format</th>
<th>Reference no.</th>
<th>No. samples per kit</th>
<th>Price</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Plate ELISA</strong></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>IBL International*</td>
<td>Germany</td>
<td>CHIK IgM micro-capture ELISA</td>
<td>RE58841</td>
<td>91</td>
<td>$525</td>
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<tr>
<td>CTK Biotech</td>
<td>USA/China</td>
<td>RecombiLISA CHIK IgM Test</td>
<td>E0315</td>
<td>91</td>
<td>unknown</td>
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<td>Genway (NovaTec)</td>
<td>Germany</td>
<td>CHIKV IgM μ-capture ELISA</td>
<td>40-521-475066</td>
<td>91</td>
<td>$585</td>
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<tr>
<td>Abcam (NovaTec)</td>
<td>Germany</td>
<td>Anti-CHIKV IgM human ELISA kit</td>
<td>ab177848</td>
<td>91</td>
<td>$475</td>
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<td>SD Diagnostics</td>
<td>Korea</td>
<td>CHIKa IgM ELISA</td>
<td>16EK10</td>
<td>91</td>
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<td>Euroimmun</td>
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<td>E1293a-9601M</td>
<td>93</td>
<td>$341</td>
</tr>
<tr>
<td>Inbios</td>
<td>USA</td>
<td>CHIKjj Detect MAC-ELISA</td>
<td>Research use only</td>
<td>92</td>
<td>NA</td>
</tr>
<tr>
<td><strong>Rapid test</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTK Biotech</td>
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<td>On-site CHIK IgM Combo Rapid test</td>
<td>R0066C</td>
<td>30</td>
<td>unknown</td>
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<td>SD BIOLINE Chikungunya IgM</td>
<td>46FK10</td>
<td>25</td>
<td>unknown</td>
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<tr>
<td><strong>IFA</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Euroimmun</td>
<td>Germany</td>
<td>Anti-CHIKV IIFT (IgM)</td>
<td>FI293a-1010 G/M</td>
<td>50</td>
<td>$250</td>
</tr>
</tbody>
</table>

*Not evaluated at CDC.*
Sample panel for evaluation (n≈90)

- **CHKV Asian strain:**
  - 48 CDC CHKV IgM+ and PRNT+ sera from Yap (2013), Philippines, and Caribbean (2014) outbreaks

- **CHKV East/Central/South African (ECSA) strain:**
  - 4 CDC CHKV IgM+ and PRNT+ sera from Comoros (2005) and travelers to India (2006)

- **CDC CHKV IgM- sera**
  - 3 Normal control serum
  - 30 CDC CHKV IgM- sera from fever patients from Thailand, Yap, and Caribbean
  - 2 DENV IgM+ sera
  - 3 CDC IgM Positive control serum for other alphaviruses (O'nyong-nyong, Venezuelan equine encephalitis, North American eastern equine encephalitis).
1. Assay first tested with 10-20 sample subset consisting of PCs from India, Comoros, and Caribbean; NC; and CHKV IgM+ and IgM- samples from Caribbean and Yap

2. PCs used as IHC for each test run.

3. If all PCs positive in test, proceed to test complete panel

4. If PCs negative in test, retest. If PCs negative in 2\textsuperscript{nd} test, test with alternate conditions suggested by manufacturer (eg., change incubation time and temp, handwash vs autowash) or contact manufacturer

5. Repeat testing with different lot # kits
**CDC IHPC** had negative result in test.

** CDC IHPC had positive result in test.

ND, not done as specificity and agreement could not be calculated.
Abcam CHIK MAC-ELISA performance by lot#

- Overnight incubation
- 30 min incubation
- Tested on same plate

Positive >11
Equivocal 9-11
Negative <9
# Summary

## CDC evaluations of CHIKV IgM detection assays

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Assay format</th>
<th>Performance /concordance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Plate ELISA</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTK Biotech</td>
<td>RecombiLISA CHIK IgM Test</td>
<td>Low /ND</td>
</tr>
<tr>
<td>Genway (NovaTec)</td>
<td>CHIKV IgM μ-capture ELISA</td>
<td>Low /0%</td>
</tr>
<tr>
<td>Abcam (NovaTec)</td>
<td>Anti-CHIKV IgM human ELISA kit</td>
<td>Inconsistent quality/0%-95%</td>
</tr>
<tr>
<td>SD Diagnostics</td>
<td>CHIKa IgM ELISA</td>
<td>Low /&lt;50%</td>
</tr>
<tr>
<td>Euroimmun</td>
<td>Anti-CHIKV ELISA (IgM)</td>
<td>High/&gt;90%</td>
</tr>
<tr>
<td>Inbios</td>
<td>CHIKjj <em>Detect</em> MAC-ELISA</td>
<td>High/&gt;90% (research format)</td>
</tr>
<tr>
<td><strong>Rapid test</strong></td>
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<tr>
<td>CTK Biotech</td>
<td><em>On-site</em> CHIK IgM Combo Rapid test</td>
<td>Low/ND</td>
</tr>
<tr>
<td>SD Diagnostics</td>
<td>SD BIOLINE Chikungunya IgM</td>
<td>Low/ND</td>
</tr>
<tr>
<td><strong>IFA</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Euroimmun</td>
<td>Anti-CHIKV IIFT (IgM)</td>
<td>High/98%</td>
</tr>
</tbody>
</table>
Summary (continued):

- **Euroimmun Anti-CHIKV ELISA (IgM) Anti-CHIKV IIFT (IgM) kits show highest performance**
  - Only one lot of kits has been evaluated at CDC
  - Euroimmun working towards FDA approval
  - IgG depleted in sample dilution buffer; antigen coated on plate

- **Abcam Anti-CHIKV IgM human ELISA kit lot-to-lot variability**
  - Does not use CHKV IgM+ as PC
  - Assay PC is biotin labeled IgM, so test valid as long as the SP conjugate, TMB and stop solution are added
  - Only way to determine if assay working correctly is to use in-house control
  - Have worked with manufacturer (Novatec) through Abcam to correct, but test has not been re-formatted

- **SD and CTK CHK MAC-ELISAs low sensitivity**
  - CDC PCs negative in ELISA
  - Antigen coated on plate
  - Serum IgG likely competitively bound IgG

- **SD and CTK Rapid tests sensitivity low**
  - CDC PCs negative in lateral flow assays

- **Euroimmun IIFA high performance**
  - Requires experienced technician to “read” slides
  - Immunosorb sample dilution buffer with IgG depletion sold separately
  - Incomplete washing results in false positives
Future directions

- CDC and NML Canada will publish results of CHK IgM kit evaluations to provide guidance to laboratories

- CDC will provide reagents through PAHO for CDC CHK MAC-ELISA (antigen, conjugate, PC) until commercially available kit is validated

- CDC provides protocol, primers/probe sequences, and CHKV PC RNA lysate for qRT-PCR through PAHO

- In 2015 CDC initiated a CHKV proficiency testing program; PT panels available through PAHO
  - **Serology Panel**
    - Blind-coded mix of IgM/IgG Pos & Neg specimens
    - Verified non-infectious
    - Positive control serum included
  - **Nucleic Acid Panel**
    - Blind-coded mix of RNA Pos & Neg specimens in Qiagen lysis buffer
    - Verified non-infectious
    - Varying levels of RNA in Pos specimens
    - Positive control RNA lysate included
Thank you!