Laboratory Issues and West Nile Virus

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Lansing Michigan
Laboratory Issues and West Nile Virus

- Arboviruses
- Laboratory Diagnosis
- Investigation of 2002 WNV outbreak in Michigan
- Plans for 2003-Lab perspective
The arboviruses

- **Arthropod-borne viruses** (mosquitoes, sand-flies, fleas, ticks, lice, etc)
- Enveloped RNA viruses - 4 families
- **Flaviviridae** - WNV isolated in 1937 in west Nile district of Uganda in Eastern Africa.
Arboviral encephalitides

Mosquito
- eastern equine encephalitis (EEE)
- western equine encephalitis (WEE)
- St. Louis encephalitis (SLE)
- La Crosse (LAC)

Ticks
- Powassan
WNV Transmission

Dead end host

New modes of transmission

Dead end host
Laboratory Issues and West Nile Virus

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Immune Response in WNV Infection

-5 -4 -3 -2 -1 0 1 2 3 4 5 6 7 8 9 10

DAYS POST ONSET

#pfu/ml

IgM

IgG

WN viremia

ELISA P/N

Laboratory Diagnosis of Human cases

- Specimens
  - Cerebrospinal fluid (CSF)
  - CSF and Serum combination
  - Sera- Acute and Convalescent (obtained at least 8 d and 22 d post onset respectively)
  - Least preferred single serum sample

Ref: Antibody Capture ELISA (IgM & IgG) Protocol. CDC Fort Collins, Colorado
CDC Neutralization Test Protocol. CDC Fort Collins, Colorado
Laboratory Diagnosis of Human cases contd

- Laboratory Tests
  - Capture enzyme-linked immunosorbent assay (MAC-ELISA-IgM).
  - Capture enzyme-linked immunosorbent assay (MAC-ELISA-IgG) and
  - Plaque Reduction Neutralization Test (PRNT)

Ref: Antibody Capture ELISA (IgM & IgG) Protocol. CDC Fort Collins, colorado
CDC Neutralization Test Protocol. CDC Fort Collins, colorado
IgM Capture ELISA

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

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

3. Add West Nile Recombinant Antigen
   ➢ 4° Overnight

4. Add HRP anti-Flavivirus McAb
   ➢ 37° 1 Hour

### WN Serological Data

**Typical Human WN Case**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Days post-onset</th>
<th>IgM P/N</th>
<th>IgG P/N</th>
<th>PRNT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>WN</td>
<td>SLE</td>
<td>WN</td>
</tr>
<tr>
<td><strong>Typical WN Case</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>acute serum</td>
<td>8</td>
<td>12.75</td>
<td>4.00</td>
<td>1.37</td>
</tr>
<tr>
<td>conv. serum</td>
<td>31</td>
<td>11.35</td>
<td>4.21</td>
<td>6.38</td>
</tr>
</tbody>
</table>

In primary flavivirus infections:

- *Martin et al 2002*: IgM P/N to WN is 3-5X greater than SLE.
- **2002 data**: Use 2X criteria WN to SLE ratio: only 1 exception in 417 WN confirmed cases.

# Serological Testing Criteria at Michigan

<table>
<thead>
<tr>
<th>CSF</th>
<th>Paired sera</th>
<th>Single serum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(Ac. at least 8 days and conv at least 22 days post onset)</td>
<td></td>
</tr>
<tr>
<td>• P/N ratio &gt;/= 10</td>
<td>• P/N ratio &gt;/=5  presumptive.</td>
<td>• P/N ratio- same as in paired sera.</td>
</tr>
<tr>
<td></td>
<td>• P/N 2-5  equivocal.</td>
<td></td>
</tr>
<tr>
<td>• P/N 2-10  equivocal and requested for a serum sample.</td>
<td>• IgG Tests -A four-fold rise in titre to distinguish a recently acquired infection from a past infection.</td>
<td>• A convalescent serum requested on equivocals .</td>
</tr>
<tr>
<td></td>
<td>• PRNT was performed on all specimens showing a four fold increase in IgG titer.</td>
<td>• PRNT To rule out the cross-reactions between WNV and other arbovirus infections (SLE, EEE and CGV).</td>
</tr>
</tbody>
</table>

• MAC-ELISA IgM performed in Singlet. Positive MAC-ELISA repeated in duplicate
West Nile Virus Nucleic Acid Amplification Tests (NAAT) Diagnostic Testing

- Identify viremic antibody negative "window phase" patients
- Low levels WNV RNA found in clinically ill, immuno-competent patients (i.e., WN fever)
- Higher persistent levels of WNV RNA found in immuno-compromised patients
- Improved sensitivity with better quality of diagnostic samples or virus concentration methods?
- WNV RNA(-) results are meaningless
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Weekly WNV Testing (Aug-Nov 02)

Total Specimens (Jan, 02-Dec, 02)-2910
How we handled the 2002 outbreak in the laboratory?

Triaged the samples

Virology section and intersection staff worked collectively 6 days a week
CSF at MDCH 0 day

IgM ELISA 1st Run

Report via EPIC

Neg

Pos

IgM ELISA 2nd Run

Quantity Sufficient

Confirmed positive reports Via EPIC 5 6day

Equivocal

Request a convalescent serum sample

QNS

To report a Probable case request a serum sample

Investigation

Notification Via EPIC to Submitter, LDH & EPI 4 day

Pos
IgM ELISA 1st run → Pos
IgM ELISA 2nd run → Pos
No 4x increase in titer
Arboviral Panel
Confirmed positive reports Via EPIC
Immune Response in WNV Infection

-5 -4 -3 -2 -1 0

IgM

150

#pfu/ml

IgG

ELISA P/N

WN viremia

1

2

3

4

5

6

7

8

9

10

DAYS POST ONSET

illness

Single serum with documented CNS symptoms or paired sera without 4X increase in titer

IgM ELISA (+) PRNT (-)

Test for SLE, EEE and CGV

Neg

Single serum collected too early (0-8 d) post onset

Paired sera

Probable WNV Case

No WNV Case
Laboratory Issues and West Nile Virus

- Arboviruses
- Laboratory Diagnosis
- Investigation of 2002 WNV outbreak in Michigan
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Getting Ready for summer of 2003
Plans for 2003

Human Surveillance

- No triaging of samples
- Test whole panel
- Two LT positions in virology for WNV testing
- Cross training
- IgM capture ELISA testing on CSF and Paired serum
- Attempt culture on for WNV
## Testing reagents for 2003

### CDC Reagent Production

<table>
<thead>
<tr>
<th>Year</th>
<th>Reagent Requests/Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>1995 - 1999</td>
<td>100 – 150</td>
</tr>
<tr>
<td>2002</td>
<td>560 reagent requests</td>
</tr>
</tbody>
</table>

- No change in personnel or policy
- Commercial Partners – patent license agreements for WN antigen production.
Manufacturers with WNV Antibody Assays in development

- Focus Technologies
- Ortho Clinical Diagnostics
- Abbott Laboratories
- Chiron (recombinant antigens)
- Pan -Bio
Future of new assays for WNV

Synergy between

- Industry
- Regulatory agencies
- Public and
- Private laboratories
Getting Ready for summer of 2003 contd

Issues with Persistence of IgM antibodies
## Longevity of Human WN Virus-Reactive IgM in Serum

<table>
<thead>
<tr>
<th>Days P.I.</th>
<th>N</th>
<th>Positive MAC-ELISA</th>
<th>Total (%)</th>
<th>Ave. P/N (Range)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Positive (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Equivocal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>22</td>
<td>13 (60)</td>
<td>17 (77)</td>
<td>6.0 (3.0-10.8)</td>
</tr>
<tr>
<td>300-400</td>
<td>21</td>
<td>9  (43)</td>
<td>11 (52)</td>
<td>4.0 (31.6-6.5)</td>
</tr>
<tr>
<td>500</td>
<td>12</td>
<td>5  (42)</td>
<td>6  (60)</td>
<td>5.0 (3.1-6.9)</td>
</tr>
</tbody>
</table>

Hold and request for the Convalescent serum.

No CNS symptoms +

Approval of Virology Lab Manager

Report

Neg

IgM ELISA 1st Run

Pos

IgM ELISA 2nd Run

Pos

PRNT on acute serum

Pos

2003 Testing

Acute Serum at MDCH

No

Yes

PRNT (IgG) on pair

4x rise in titer

Confirmed positive reports Via EPIC

Arboviral Panel

No 4x increase
Persistence of IgM antibodies

- In CSF-No studies

Published-47d

MDCH observations-Three cases with IgM positive

- 110d
- 141d
- 199d
Bird Testing in 2003

- Strict IATA regulations
- Availability of a field assay - Vec Test
- Validation of IHC and PCR-2002
- IHC vs Vec Test at MSU lab in 2003
- Implementation of vec test ??
Over wintering adult *Culex* mosquitoes

To Sum Up......

Human Cases?