West Nile Virus in California:
Guidelines for Human Testing and Surveillance
Within the Regional Public Health Laboratory Network

California Department of Public Health
Richmond, California

May 2011
**West Nile Virus in California: Guidelines for Human Testing and Surveillance**

*Within the Regional Public Health Laboratory Network*

**TABLE OF CONTENTS**

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diagnostic Testing Guidelines</td>
<td>1</td>
</tr>
<tr>
<td>Submitting Specimens to Regional Public Health Laboratory Network for Testing</td>
<td>1</td>
</tr>
<tr>
<td>Viral and Rickettsial Disease Laboratory (VRDL) Testing Algorithm</td>
<td>2</td>
</tr>
<tr>
<td>Laboratory Diagnosis and Test Interpretation</td>
<td>2</td>
</tr>
<tr>
<td>Case Classification: Regional Public Health Laboratory Network</td>
<td>3</td>
</tr>
<tr>
<td>Results from Commercial or Reference Laboratories</td>
<td>3</td>
</tr>
<tr>
<td>West Nile Virus-Associated Fatalities</td>
<td>4</td>
</tr>
<tr>
<td>Reporting</td>
<td>4</td>
</tr>
<tr>
<td>Contacts, Links, and Appendices Index</td>
<td>7</td>
</tr>
<tr>
<td>Appendix A: Instructions for Submitting Specimens</td>
<td>8</td>
</tr>
<tr>
<td>Appendix B: West Nile Virus Specimen Submittal Form</td>
<td>9</td>
</tr>
<tr>
<td>Appendix C: VRDL Testing Algorithm – Serum</td>
<td>10</td>
</tr>
<tr>
<td>Appendix D: WNV Laboratory Testing at VRDL</td>
<td>11</td>
</tr>
<tr>
<td>Appendix E: Revised National Surveillance Case Definition</td>
<td>12</td>
</tr>
<tr>
<td>Appendix F: West Nile Virus Infection Case Report</td>
<td>14</td>
</tr>
<tr>
<td>Appendix G: West Nile Virus Infection Case Report-Supplemental Form</td>
<td>15</td>
</tr>
<tr>
<td>Appendix H: Report of West Nile-Virus Positive Blood Donor</td>
<td>16</td>
</tr>
</tbody>
</table>
West Nile Virus in California: Guidelines for Human Testing and Surveillance Within the Regional Public Health Laboratory Network

Diagnostic Testing Guidelines

West Nile virus (WNV) testing within the regional public health laboratory network (i.e., the California Department of Public Health Viral and Rickettsial Disease Laboratory and participating local public health laboratories) is recommended for individuals with the following symptoms, particularly during West Nile virus “season,” which typically occurs from July through October in California:

A. Encephalitis
B. Aseptic meningitis (Note: Consider enterovirus for individuals ≤ 18 years of age)
C. Acute flaccid paralysis; atypical Guillain-Barré Syndrome; transverse myelitis; or
D. Febrile illness*
   a. Illness compatible with West Nile fever and lasting ≥ 7 days
   b. Must be seen by a health care provider

* The West Nile fever syndrome can be variable and often includes headache and fever (T ≥ 38°C). Other symptoms include rash, swollen lymph nodes, eye pain, nausea or vomiting. After initial symptoms, the patient may experience several days of fatigue and lethargy.

Identification of human cases is important early in the West Nile virus season to assess the burden of human illness and target mosquito control and public education activities to reduce exposure risk. However, depending on the volume of tests requested and laboratory capacity, local public health laboratories may need to consider limiting testing to individuals with neuroinvasive disease once West Nile virus is established in a given area.

Submitting Specimens to Regional Public Health Laboratory Network for Testing

Required specimens:

- Acute serum: ≥ 2cc serum
If a lumbar puncture is performed and residual CSF is available:
  - Cerebral spinal fluid (CSF): 1-2cc CSF for further testing at CDC (please note: these results may not be available for several weeks)
If West Nile virus is highly suspected and acute serum is negative or inconclusive, request:
  - 2nd serum: ≥ 2cc serum collected 3-5 days after acute serum

Paired acute and convalescent serum specimens are useful for demonstration of seroconversion to WNV. Paired samples should be collected whenever WNV is suspected. Although a single acute serum may provide evidence of recent WNV infection, a negative acute serum does not necessarily rule out infection. Occasionally, a specimen may be collected too soon to show antibody related to a current illness (e.g. with immunocompromised individuals).

Specimens must be submitted with a completed specimen submittal form (See Appendix A: Instructions for Submitting Specimens; and Appendix B: West Nile Virus Specimen Submittal Form).
Viral and Rickettsial Disease Laboratory Testing Algorithm

The Viral and Rickettsial Disease Laboratory [VRDL] will test serum samples for West Nile Virus [WNV].

• Immunofluorescence assay (IFA) may be done as an adjunct test on serum (IFA is not done on CSF)

• In addition to serum, VRDL encourages submission of CSF if a lumbar puncture is performed and residual CSF is available. VRDL requests that WNV testing on CSF only be requested for individuals who have a screening test positive (serum IgM+). CSF will be sent to CDC for testing; CSF results may not be available for several weeks, but results for serum tested at VRDL can be available within 14 calendar days from receipt of sample.

• When previously untested serum and CSF are received, enzyme immunoassay (EIA) is performed on serum (CSF is stored in case additional confirmatory testing at the CDC is needed)
  – If the IgM is negative in the serum sample but you strongly suspect WNV, another serum sample should be collected 2-3 days after the first serum. WNV IgM is usually present in immunocompetent individuals by day 5 of illness onset.
  – In immunocompromised individuals the WNV antibody response may be delayed. For these patients, additional testing is warranted, please consult with VRDL for guidance.
  – Please consult with VRDL for guidance any time WNV is strongly suspected, regardless of previous test results.

• Plaque reduction neutralization testing (PRNT) is done to resolve indeterminate results, or by request (Note: At VRDL, PRNT is not currently validated for diagnostic purposes; these results are to be used for surveillance purposes only)

• Enterovirus PCR may also be done on CSF specimens on a seasonal basis, depending on the availability of resources at VRDL
  - Call 510-307-8606 to find out whether the most current algorithm includes enterovirus PCR

• See Appendix C: VRDL WNV Testing Algorithm – Serum and Appendix D: WNV Laboratory Testing at VRDL

Laboratory Diagnosis and Test Interpretation

• IFA is a more subjective assay than EIA and should be interpreted with caution

• IgG(+) result only (i.e., negative for IgM) typically indicates previous infection of a flavivirus
  - Check case history for travel to flavivirus-endemic areas, length of time between onset of symptoms and collection of specimen, vaccination history, etc.
  - If current infection is still suspected, obtain convalescent serum to test for seroconversion

• VRDL is always available for consultation on test results with local public health laboratories
<table>
<thead>
<tr>
<th>Tests</th>
<th>Results</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgM</td>
<td>negative</td>
<td>Antibody not detected</td>
</tr>
<tr>
<td>IgG</td>
<td>negative</td>
<td></td>
</tr>
<tr>
<td>IgM</td>
<td>negative</td>
<td>Infection at undetermined time</td>
</tr>
<tr>
<td>IgG</td>
<td>positive</td>
<td></td>
</tr>
<tr>
<td>IgM</td>
<td>positive</td>
<td>Possible evidence of recent or current infection; further testing necessary**</td>
</tr>
<tr>
<td>IgG</td>
<td>negative</td>
<td></td>
</tr>
<tr>
<td>IgM</td>
<td>positive</td>
<td>Evidence of recent or current infection***</td>
</tr>
<tr>
<td>IgG</td>
<td>positive</td>
<td></td>
</tr>
<tr>
<td>IgM</td>
<td>indeterminate</td>
<td>Inconclusive ‡request convalescent serum</td>
</tr>
<tr>
<td>IgG</td>
<td>negative</td>
<td></td>
</tr>
</tbody>
</table>

* Due to heterotypic antibody responses and/or cross-reactions, serologic results should be interpreted on the basis of clinical and epidemiological information
** Note the possibility of a false positive IgM result (EIA)
*** Note that some individuals may have persisting antibodies from the previous WNV season
‡ Paired acute and convalescent serum samples may be useful for demonstration of seroconversion

Case Classification: Regional Public Health Laboratory Network

A case is considered to be WNV positive if the patient has a clinically compatible illness (see Appendix E for case definition) and has the following laboratory results:
- IgM(+) by two different assays (e.g. EIA and IFA); or
- IgM(+) and IgG(+) by EIA; or
- IgM(+) and IgG(+) by IFA; or
- Rising IgG antibodies

Results from Commercial or Reference Laboratories

- California Code of Regulations, Title 17, Section 2505 requires laboratories to report positive West Nile virus test results to the local health department

- Local health departments should follow up on IgM(+) results from commercial labs
  - If a patient has clinically compatible illness and is IgM-positive and IgG-positive, the commercial lab results are sufficient to conclude that patient is infected with WNV – however, for the first few cases of the WNV season, it is recommended that positive results from commercial labs be verified by repeat/confirmatory testing at the local public health lab and/or VRDL
  - If patient is IgM-positive and IgG-negative, be aware that IgM can be falsely positive; follow-up testing is suggested

- IgG-positive result only (i.e., IgM-negative) typically indicates previous infection

- When in doubt, try to obtain either the original specimen or a convalescent sample to forward to the local public health lab or to VRDL for repeat/confirmatory testing

- Public health reporting by commercial laboratories is being facilitated by VRDL (see below)
West Nile Virus-Associated Fatalities

Determining whether or not West Nile virus infection has played a causal role in a fatality can be difficult. West Nile virus may not always be listed as a contributory or underlying cause of death on death certificates. Patients often have many underlying conditions and preexisting medical problems that also may be related to the immediate causes of death. In general, if a patient was diagnosed with West Nile virus and never recovered from the sequelae (e.g. was discharged to convalescent hospital until date of death), a health department may consider designating the patient as a WNV-associated fatality.

Reporting

Since West Nile virus infection is a laboratory diagnosis, and since West Nile surveillance is a multi-component system maintained nationwide through ArboNet (CDC’s source for WNV data), reporting human cases of West Nile virus to the California Department of Public Health is done through slightly different routes than regular disease reporting. The algorithm below outlines the various paths through which West Nile virus infections may be reported.
Important Issues about Reporting

- West Nile virus infection is reportable by both laboratories and providers
- Fax or mail case report forms (See Appendix F: West Nile Virus (WNV) Infection Case Report; Appendix G: West Nile Virus (WNV) Infection Supplemental Form; and Appendix H: Report of West Nile Virus-Positive Blood Donor) to the California Department of Public Health Communicable Disease Emergency Response Branch (CDER) – please indicate, either on form or by phone/email, that individual has tested positive for WNV:
  Fax (510) 620-5896; CDER-West Nile, 850 Marina Bay Parkway, Richmond, CA 94804
- **Only cases reported to CDPH-CDER are entered into ArboNET and posted on the California WNV website** – If a local agency uses AVSS or another local system for their disease surveillance, they will enter West Nile infections separately into those systems, as well as send a case history form to CDPH-CDER
  - The following AVSS classifications can be used to enter cases:
    - ENCP-WNV: For West Nile encephalitis cases
    - MENG-WNV: For West Nile meningitis cases
- WNV-FVR: For West Nile fever cases
- WNV-AFP: For West Nile acute flaccid paralysis cases
- WNV-ASYM: For WNV infections detected via blood bank with no accompanying illness
- WNV-UNK: For cases with unknown or undeterminable clinical status

  - CDPH-CDER will check CalREDIE for new case reports
  - CDPH-CDER will check AVSS for reported WNV infections that may not have been previously reported

- Health departments should notify their local vector control agency of any confirmed human West Nile virus activity as soon as possible, so that enhanced mosquito surveillance and control measures can be implemented
- A line list of locally acquired WNV cases will be maintained and updated biweekly on the California WNV website (http://westnile.ca.gov)
- Report clinical syndrome as West Nile fever, neuroinvasive disease (specify encephalitis, meningitis, acute flaccid paralysis, or other), unknown, or asymptomatic (not a case)
- Contact CDER (510) 620-3987 if local lab or health department knows of a case that is not on website or ArboNET

**Important Issues about VRDL Results**
- All VRDL results are faxed and mailed to submitting local public health lab, and faxed to local health department of patient’s residence
- Non-diagnostic results, or results that are to be used for surveillance purposes only (e.g. PRNT), will be faxed and mailed separately
- Local health departments need to report West Nile virus results to providers
- VRDL results are routinely reported to local health departments/labs
  - Positive results relayed immediately by phone or email, then followed up with fax/mail
  - Negative results faxed/mailed to labs 1-2 times/week
- Fax requests for results (include patient name and identifier e.g. date of birth) to: (510) 307-8599, Attn: West Nile Virus Project
Contacts
Communicable Disease Emergency Response Branch

Cynthia Jean Yen, MPH ........................................... (510) 620-3987
Carol Glaser, DVM, MD (for clinical consultation)....... (510) 307-8613
Payer (510) 720-0078
West Nile Virus Surveillance Project Fax ................. (510) 620-5896

Viral and Rickettsial Disease Laboratory

Maria Salas, MPH.................................................. (510) 307-8606
Katharine King.................................................... (510) 307-8562
Heather Sheriff................................................... (510) 307-8608

Vector Borne Disease Section

West Nile Virus Hotline ....................................... (877) 968-2473

Links

California West Nile Virus Website ...................... http://westnile.ca.gov
CDC West Nile Virus Website ............................. http://www.cdc.gov/ncidod/dvbid/westnile/

Appendices

A. Instructions for Submitting Specimens
B. West Nile Virus Specimen Submittal Form
C. Viral and Rickettsial Disease Laboratory West Nile Virus Testing Algorithm – Serum
D. West Nile Virus Laboratory Testing – Viral and Rickettsial Disease Laboratory
F. West Nile Virus Infection Case Report
G. West Nile Virus Infection Case Report- Supplemental Investigation Form
H. West Nile Virus-Positive Blood Donor Report Form
Instructions for Submitting Specimens

☐ Refrigerated specimens should be sent on cold pack using an overnight courier
  - If CSF needs to be stored ≤72 hours before submittal, store at 2 to 8°C and ship on cold pack.
  - If CSF needs to be stored >72 hours before submittal, freeze at -70°C or colder and ship on dry ice.

☐ Each specimen should be clearly labeled with patient name, specimen type, and date of specimen collection

☐ Specimens must be submitted with a specimen submittal form. The following information is asked for on the specimen submittal form because it is important for accurate interpretation of results:
  - Onset date
  - Unusual immunological status of patient, if any
  - County of residence
  - History of travel to flavivirus-endemic areas
  - History of prior vaccination against flavivirus disease
  - Brief clinical summary including clinical diagnosis

☐ Please include any West Nile virus test results obtained by the local public health laboratory or a commercial reference laboratory
  - Other laboratory results affect the VRDL testing algorithm; Specimens that have screened positive or indeterminate for WNV IgM antibodies at another laboratory will be immediately tested with the heterophile subtract procedure

☐ Do not send specimens on Fridays for weekend delivery (VRDL Specimen Receiving Hours M-F 8-5)

☐ Address specimens for VRDL to:
  Specimen Receiving/ West Nile
  850 Marina Bay Parkway
  Richmond, CA  94804
West Nile virus testing is recommended on individuals with the following:

A. Encephalitis
B. Aseptic meningitis (Note: Consider enterovirus for individuals ≤ 18 years of age)
C. Acute flaccid paralysis; atypical Guillain-Barré Syndrome; transverse myelitis; or
D. Febrile illness compatible with West Nile fever* and lasting ≥ 7 days (must be seen by health care provider):

* The West Nile fever syndrome can be variable and often includes headache and fever (T≥38°C). Other symptoms include rash, swollen lymph nodes, eye pain, nausea or vomiting. After initial symptoms, the patient may experience several days of fatigue and lethargy.

1. **Required specimens:**
   - **Acute Serum:** ≥ 2 cc serum
   - **Cerebrospinal Fluid (CSF):** 1-2 cc CSF may be submitted with acute serum for further testing at CDC if lumbar puncture is performed and residual CSF is available (Please note: these results may not be available for several weeks)

2. If West Nile virus is highly suspected and acute serum is negative or inconclusive:
   - **2nd Serum:** ≥ 2 cc serum collected 3-5 days after acute serum

   - Each specimen should be labeled with **date of collection, specimen type, and patient name**
   - Refrigerated specimens should be sent on **cold pack** using an overnight courier
   - Frozen specimens should be sent on **dry ice** using an overnight courier
   - CSF that cannot be shipped within 72 hours of collection should be stored frozen at -70°C or colder.
   - Serum that cannot be shipped within 48 hours of collection may be stored at 4°C or frozen at -20°C or colder.
   - Please do not send specimens on Fridays (Specimen Receiving Hours: M-F 8-5)
   - Send specimens to CDPH VRDL: **Specimen Receiving – West Nile Virus**
     850 Marina Bay Parkway
     Richmond, CA  94804

   - Local Public Health Laboratory West Nile **IFA/EIA IgM results** (or attach copy of results):

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Date Collected</th>
<th>IgM Assay Method</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Negative</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IFA</td>
<td>o IFA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EIA</td>
<td>o IFA</td>
</tr>
</tbody>
</table>

**IMPORTANT: THE INFORMATION BELOW MUST BE COMPLETED AND SUBMITTED WITH SPECIMENS**

<table>
<thead>
<tr>
<th>Patient’s last name, first name:</th>
<th>Patient Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age or DOB:</td>
<td>Address __________________________</td>
</tr>
<tr>
<td>Sex (circle): M F Onset Date:</td>
<td>City________________ Zip_______ County________</td>
</tr>
<tr>
<td>Clinical findings:</td>
<td>Phone Number (_______) ______________</td>
</tr>
<tr>
<td>O Encephalitis O Meningitis O Acute flaccid paralysis</td>
<td>Other information (immunocompromised, travel hx, hx of flavivirus infection, etc.):</td>
</tr>
<tr>
<td>O Febrile illness O Other:</td>
<td>This section for Laboratory use only. Date received by VRDL and State Accession Number</td>
</tr>
<tr>
<td>Other tests requested:</td>
<td>1st Specimen type and/or specimen source Date Collected</td>
</tr>
<tr>
<td>1st</td>
<td>1st Specimen type and/or specimen source Date Collected</td>
</tr>
<tr>
<td>2nd</td>
<td>2nd Specimen type and/or specimen source Date Collected</td>
</tr>
<tr>
<td>3rd</td>
<td>3rd Specimen type and/or specimen source Date Collected</td>
</tr>
</tbody>
</table>

Questions? Call Maria Salas at (510) 307-8606

Submitting Physician_________________ Phone Number (_______) ______________
Submitting Facility_________________________ Phone Number (_______) ______________
LOCAL RESULT: NOT TESTED

EIA
- Focus IgM
- In-house IgG

Focus M(-) heterophile(-) In-house G(-)

Report (Neg)  REIVEW*

Focus M(-) heterophile(-) In-house G(+)

Focus M(+) heterophile(-) In-house G(+)

Focus M(+) heterophile(-) In-house G(+/-)

Repeat EIA
- Focus IgM w/heterophile

Focus M(+) heterophile(-) In-house G(+)

Focus M(+) heterophile(-) In-house G(+/-)

Report (Pos)  REVIEW*

Focus M(-) heterophile(-) In-house G(+)

LOCAL RESULT: POSITIVE/ INDETERMINATE

EIA
- Focus IgM w/heterophile†
- In-house IgG

Focus M(-) heterophile(-) In-house G(-)

• Call LHD

Focus M(+) heterophile(-) In-house G(+)

Focus M(+) heterophile(+) In-house G(+/-)

Focus M(-) heterophile(-) In-house G(+)

Report (Pos)  REVIEW*

• IFA and/or PRNT
• Request conv sample
• TS or designee signs off before reporting

† Heterophile antibodies are "interfering" antibodies that can cause false positive IgM EIA results

* Review the following information:
  • onset date?
  • travel history?
  • old flavivirus?
  • vaccine?
Laboratory diagnosis of human West Nile virus (WNV) infection is a multi-step process. In some cases, physicians send specimens to private commercial laboratories for WNV diagnostic testing. More commonly, specimens are sent to the local or state health department for diagnostic laboratory testing.

Testing available at the California Department of Public Health Viral and Rickettsial Disease Laboratory includes:

**Serologic tests**

**Enzyme Immunoassay (EIA) testing:** The immunoglobulin M (IgM) antibody-capture enzyme immunoassay (EIA) is the frontline test for WNV diagnosis. The EIA is the ideal test because it is both simple and sensitive (i.e., highly likely to find true positives). EIA testing can be completed in 14 calendar days from the time samples arrive at the laboratory. Generally several specimens are tested in each EIA run.

The immunoglobulin G (IgG) EIA test is used as an adjunct test—a single IgG result cannot differentiate between old and new infection; however, paired sera showing significant change in IgG antibody levels can be helpful.

**Immunofluorescence Assay (IFA) testing:** IFA tests for WNV can also test for IgM and IgG antibodies. The advantages of these tests are that they are rapid and amenable to just a few samples. However, the IFA is a more subjective assay than the EIA.

**Molecular tests**

Molecular methods for WNV testing can be used as an adjunct to the serologic tests. For diagnosis of clinical disease, serological tests are more accurate than molecular tests. Reverse Transcriptase - Polymerase Chain Reaction (RT-PCR) is a process that uses nucleic acid amplification techniques. While these tests can be useful in diagnosis, they have low sensitivity for a variety of reasons for WNV, making them inappropriate as the sole test for laboratory diagnostic testing of possible human WNV infections. An advantage of this method is the relatively rapid turn around time. RT-PCRs may be useful for immunocompromised individuals that have a delay in antibody response and prolonged viremia. Additionally, VRDL uses molecular methods to rule out enterovirus.

**Confirmation of results**

**Plaque reduction neutralization test (PRNT)**

Once VRDL has an initial positive result, further testing may be done to confirm that the infection detected is West Nile virus. WNV is a flavivirus, which can be problematic as far as cross-reactivity with other flaviviruses. The flaviviruses include St. Louis encephalitis (SLE) and Japanese encephalitis (JE) viruses, both of which are closely related to WNV, as well as yellow fever (YF) and dengue (DEN) viruses. People who have been recently vaccinated for JE or YF, or who have a recent exposure to JE, YF, SLE, or DEN viruses, may have a positive IgM for WNV, even though they have not actually been exposed to WNV.

Additional laboratory testing may be required to rule out the false-positive reactions that result from an exposure to a related flavivirus. The PRNT is the most specific test available for distinguishing between and among the arthropod-borne flaviviruses. Because exposure to other flaviviruses is possible in many areas of WNV activity, initial IgM positive results may need to be confirmed by PRNT. The PRNT usually takes up to 8 days if testing for both WNV and SLE viruses is required. The process may take even longer if testing with YF or Dengue viruses is necessary. This additional testing (e.g., the PRNT) may require growth of the virus and may take a week or more (plus shipping time) to conduct. Since PRNT testing is not currently validated for diagnostic purposes at the VRDL, PRNT results are reported out separately and should be used for surveillance purposes only.

**Tests in development**

The VRDL is in the process of developing tests for more rapid confirmation of WNV, e.g. the Western Blot.
West Nile virus infection is reportable to local health departments under Title 17 of the California Code of Regulations. Below is the case definition for West Nile virus disease as summarized by the Centers for Disease Control and Prevention (CDC) [available at http://www.cdc.gov/ncidod/dvbid/westnile/clinicians/surveillance.htm#casedef]. Blood donors that test positive for West Nile virus through blood bank screening should also be reported to CDPH, regardless of clinical presentation.

**CASE DEFINITION: West Nile Virus**

*NOTE: This definition is for public health surveillance purposes only. It is not intended for use in clinical diagnosis.*

**Clinical description**

Arboviral infections may be asymptomatic or may result in illnesses of variable severity sometimes associated with central nervous system (CNS) involvement. When the CNS is affected, clinical syndromes ranging from febrile headache to aseptic meningitis to encephalitis may occur, and these are usually indistinguishable from similar syndromes caused by other viruses. Arboviral meningitis is characterized by fever, headache, stiff neck, and pleocytosis. Arboviral encephalitis is characterized by fever, headache, and altered mental status ranging from confusion to coma with or without additional signs of brain dysfunction (e.g., paresis or paralysis, cranial nerve palsies, sensory deficits, abnormal reflexes, generalized convulsions, and abnormal movements). West Nile fever syndrome can be variable and often includes headache and fever (T≥38°C or 100.4°F). Other symptoms include rash, swollen lymph nodes, eye pain, nausea or vomiting. After initial symptoms, the patient may experience several days of fatigue and lethargy.

**Laboratory Criteria for Diagnosis**

- Fourfold or greater change in virus-specific serum antibody titer, or
- Isolation of virus from or demonstration of specific viral antigen or genomic sequences in tissue, blood, cerebrospinal fluid (CSF), or other body fluid, or
- Virus-specific immunoglobulin M (IgM) antibodies demonstrated in CSF by antibody-capture enzyme immunoassay (EIA), or
- Virus-specific IgM antibodies demonstrated in serum by antibody-capture EIA and confirmed by demonstration of virus-specific serum immunoglobulin G (IgG) antibodies in the same or a later specimen by another serologic assay (e.g., neutralization or hemagglutination inhibition).

**Case Classification**

- **Probable:** A case occurring during a period when arboviral transmission is likely and with the following supportive serology: 1) a single or stable (less than or equal to twofold change) but elevated titer of virus-specific serum antibodies; or 2) serum IgM antibodies detected by antibody-capture EIA but with no available results of a confirmatory test for virus-specific serum IgG antibodies in the same or a later specimen.
- **Confirmed:** An encephalitis or meningitis case that is laboratory confirmed.

**Comment**

Because closely related arboviruses exhibit serologic cross-reactivity, positive results of serologic tests using antigens from a single arbovirus can be misleading. In some circumstances (e.g., in areas where two or more closely related arboviruses occur, or in imported arboviral disease cases), it may be epidemiologically important to attempt to pinpoint the infecting virus by conducting cross-neutralization
tests using an appropriate battery of closely related viruses. This is essential, for example, in determining that antibodies detected against St. Louis encephalitis virus are not the result of an infection with West Nile (or dengue) virus, or vice versa, in areas where both of these viruses occur. Because dengue fever and West Nile fever can be clinically indistinguishable, the importance of a recent travel history and appropriate serologic testing cannot be overemphasized. In some persons, West Nile virus-specific serum IgM antibody can wane slowly and be detectable for more than one year following infection. Therefore, in areas where West Nile virus has circulated in the recent past, the co-existence of West Nile virus-specific IgM antibody and illness in a given case may be coincidental and unrelated. In those areas, the testing of serially collected serum specimens assumes added importance.

The seasonality of arboviral transmission is variable and depends on the geographic location of exposure, the specific cycles of viral transmission, and local climatic conditions. Reporting should be etiology-specific (see below; the six diseases printed in bold are nationally reportable to CDC):

- St. Louis encephalitis virus disease
- West Nile virus disease
- Powassan virus disease
- Eastern equine encephalitis virus disease
- Western equine virus disease
- California serogroup virus disease (includes infections with the following viruses: La Crosse, Jamestown Canyon, snowshoe hare, trivittatus, Keystone, and California encephalitis viruses)

Asymptomatic West Nile Virus Infection: Asymptomatic infection with WNV, which is generally identified in blood donors, is also reportable. WNV-positive blood donors detected by blood banks are reported directly to local health departments. Blood donors who test positive for WNV may not necessarily be ill, nor will they initially have positive IgM or IgG antibody test results. Local health departments should report blood donors who meet the following criteria for being a presumptively viremic donor to CDPH-CDER:

A presumptively viremic donor (PVD) is a person with a blood donation that meets at least one of the following criteria:

a) One reactive nucleic acid-amplification (NAT) test with signal-to-cutoff (S/CO) ≥ 17  
b) Two reactive NATs

Additional serological testing is not required. Local health departments should follow up with the donor after two weeks of the date of donation to assess if the patient subsequently became ill. If the donor did become ill as a result of WNV infection, an updated case report form should be sent to CDER so that the blood donor may be reclassified as a clinical case.

----------------------------------------------------------------------------------------------------------

Note: Due to the continued risk of unintentional or intentional introduction of exotic arboviruses into the United States (e.g., Venezuelan equine encephalitis virus), or the reemergence of indigenous epidemic arboviruses (e.g., St. Louis encephalitis and western equine encephalitis viruses), physicians and local public health officials should maintain a high index of clinical suspicion for cases of potential exotic or unusual arboviral etiology, and consider early consultation with arboviral disease experts at state health departments and CDC.
Hypertension:
Past medical history:

Glucose: ____
Protein: ____ Plt: ____
%Diff: ______________ HCT: ____
WBC: ____ %Diff: _____________
RBC: ____ WBC: ____

Date: ____/____/____ Date: ____/____/____

In ICU ……………………

Do the following apply anytime during current illness:

Encephalitis ………………
Clinical syndrome:
Aseptic meningitis …………
Acute flaccid paralysis …
Febrile illness ………………
Asymptomatic ……………..
Other _________________________________________

Do the following apply anytime during current illness:

In ICU ……………………
Fever ≥38°C………………
Headache …………………
Rash ……………………..
Stiff neck …………………
Muscle pain/weakness …
Altered consciousness …
Seizures …………………

Sex:  □ Male  Ethnici ty:  □ Hispanic  Race:  □ White  □ Asian/ Pacific Islander
□ Female  □ Non-Hispanic  □ Black  □ American Indian/Alaskan Native
□ Unknown  □ Unknown  □ Unknown  □ Other:

Physician Information (Mandatory):
Name: ___________________________ Facility: ___________________________
Pager/Phone: (_____ ) ______ Fax: (_____ ) ______ Email: ______________________

Date of first symptom(s): ____/____/____ □ Hospitalized or □ ER / Outpatient
If hospitalized, admit date: ___/____/_____ Discharge date: ___/____/_____ If patient died, date of death: ___/____/____

Travel/Exposures within 4 wks of onset (specify details):

Mosquito bites/exposure ………… [□ Yes  □ No  □ Unk]
Dates/Locations: __________________________

Travel outside of California ………… [□ Yes  □ No  □ Unk]
Dates/Locations: __________________________

Travel outside the U.S. ……………… [□ Yes  □ No  □ Unk]
Dates/Locations: __________________________

Donated blood …………………… [□ Yes  □ No  □ Unk]
Date: ____/____/____

Donated organ …………………… [□ Yes  □ No  □ Unk]
Date: ____/____/____

Received blood transfusion ………… [□ Yes  □ No  □ Unk]
Date: ____/____/____

Received organ transplant: ………… [□ Yes  □ No  □ Unk]
Date: ____/____/____

Currently pregnant …………………… [□ Yes  □ No  □ Unk]
Week of gestation: ____

Ever traveled outside the U.S. …..…….. [□ Yes  □ No  □ Unk]
Dates/Locations: __________________________

Ever rec’d yellow fever vaccine….. [□ Yes  □ No  □ Unk]
Date: ____/____/____

Knowledge of WNV prior to illness:
Did patient do anything to avoid mosquito bites?
If yes, [□ Yes  □ No  □ Unk]
- used insect repellent? [□ Yes  □ No  □ Unk]
- drained standing water near home? [□ Yes  □ No  □ Unk]

Other significant history/exposures: __________________________

Other lab results (MRI/CT, etc.): __________________________

West Nile Virus Test Results:

<table>
<thead>
<tr>
<th>Testing Laboratory</th>
<th>Specimen Type</th>
<th>Coll Date</th>
<th>Test Type</th>
<th>Result</th>
</tr>
</thead>
</table>

FAX this form: (510) 620-5896 or MAIL to: CDPH–West Nile Virus, 850 Marina Bay Parkway, Richmond CA 94804

Page 14 of 16  May 2011
Beginning in 2008, the Centers for Disease Control and Prevention (CDC) will collect surveillance data on selected underlying medical conditions and therapies that have previously been identified as risk factors for severe illness, hospitalization, and/or death among persons with WNV disease. Initial reports of WNV infections should be sent to the California Department of Public Health immediately after they have been confirmed. However, this supplemental investigation form is not time-sensitive and can be submitted at any time after a case has been reported.

Questions to Assess Underlying Medical Conditions and Medication Use

Patient Name (Last, First): ___________________________________________ DOB: ___/___/____

Clinical syndrome: □ Neuroinvasive disease □ West Nile fever □ Other clinical □ Asymptomatic infection

1. Before your West Nile virus infection, did a health care provider ever tell you that you had any of the following medical conditions?
   - Diabetes ........................................................... □ Yes □ No □ Unknown
   - High blood pressure (hypertension) ........................ □ Yes □ No □ Unknown
   - Heart attack (myocardial infarction) ......................... □ Yes □ No □ Unknown
   - Angina or coronary artery disease ........................... □ Yes □ No □ Unknown
   - Congestive heart failure (CHF) ............................. □ Yes □ No □ Unknown
   - Stroke ............................................................. □ Yes □ No □ Unknown
   - Chronic obstructive pulmonary disease (COPD) ....... □ Yes □ No □ Unknown
   - Chronic liver disease ........................................ □ Yes □ No □ Unknown
   - Kidney failure or chronic kidney disease ............ □ Yes □ No □ Unknown
   - Alcoholism ..................................................... □ Yes □ No □ Unknown
   - Bone marrow transplant ..................................... □ Yes □ No □ Unknown
   - Solid organ transplant ....................................... □ Yes □ No □ Unknown

   If yes: What organ was transplanted?: _________________________________________

   What year was the transplant?: ____________________________________________

   Cancer ............................................................. □ Yes □ No □ Unknown

   If yes: What type(s)?: ___________________________________________________

   What year were you diagnosed?: __________________________________________

   Are you currently being treated for cancer?: □ Yes □ No □ Unknown

2. Before your West Nile infection, did a health care provider ever tell you that you had a medical condition that limited your ability to fight an infection?
   □ Yes □ No □ Unknown

   If yes: What condition(s)?: ______________________________________________

   What year were you being treated for: □ Yes □ No □ Unknown

3. At the time you were diagnosed with West Nile virus infection, were you taking any of the following types of prescription medications or treatments?
   - Chemotherapy ..................................................... □ Yes □ No □ Unknown
   - Other treatments for cancer ................................... □ Yes □ No □ Unknown
   - Hemodialysis ...................................................... □ Yes □ No □ Unknown
   - Other treatments for kidney disease ........................ □ Yes □ No □ Unknown
   - Oral or injected steroids (not inhaled or topical) .... □ Yes □ No □ Unknown
   - Insulin or other medications to treat diabetes ...... □ Yes □ No □ Unknown
   - Medications to treat high blood pressure .......... □ Yes □ No □ Unknown
   - Medications to treat coronary artery disease ...... □ Yes □ No □ Unknown
   - Medications to treat congestive heart failure ..... □ Yes □ No □ Unknown
   - Medications that suppress the immune system .... □ Yes □ No □ Unknown

4. Which of the following sources provided the information above? (check all that apply)
   - Patient □ Yes □ No
   - Family member/friend □ Yes □ No
   - Provider □ Yes □ No
   - Medical record □ Yes □ No
**Report of West Nile Virus-Positive Blood Donor to the California Department of Public Health**

1. Blood Collection Facility:
   a. Name:_______________________________________
   b. Address: __________________________________ Zip Code_________
   c. Telephone number: (_____) _________ - _______________
   d. Contact person: ______________________________

2. Blood Unit Identification Number: ___________________________

3. Date of Collection: _______/ ______/ ______________

4. Donor’s name:________________________________________

5. Case identification number assigned by the blood center_________________
   (This tracking code should be different from the index blood unit identification number or other operational identification numbers. It is to be used to track the case investigation)

6. Donor’s date of birth: __/__/____

7. Donor’s gender: M/F

8. Donor’s Address ______________________________________
   ZIP code: _ _ _ _ _ Tel: (______)_____________________

9. This test was confirmed: Y/N  If Y, confirmatory test and result:_______________________

10. NAT #1 S/CO:_____

11. NAT #2 S/CO:_____ (if done)

12. Blood testing laboratory (optional): Name:_______________________________
    Address:________________________________________________________________
    Phone: (_____)(____)_____

13. Comments_______________________________________________________________
    ______________________________________________________________________
    ______________________________________________________________________