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

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

The California Department of Public Health (CDPH) Viral and Rickettsial Disease Laboratory (VRDL) provides laboratory support, technical assistance, and consultations on West Nile virus (WNV) test results to local public health laboratories. VRDL also serves as a reference laboratory for counties without public health laboratory services.

Diagnostic Testing Guidelines

West Nile virus testing is recommended for individuals with the following clinical syndromes, particularly during WNV “season,” which typically occurs from July through October in California:

- Encephalitis
- Aseptic meningitis (Note: Consider enterovirus for individuals ≤ 18 years of age)
- Acute flaccid paralysis; atypical Guillain-Barré Syndrome; transverse myelitis; or
- Febrile illness*
  - Illness compatible with West Nile fever and lasting ≥ 7 days
  - 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 WNV season to target mosquito control and public education activities to reduce exposure risk. However, once WNV is established in a given area for the season, local public health laboratories may consider limiting testing to individuals with neuroinvasive disease if testing volume and laboratory capacity are limited. Please consult with VRDL for guidance any time WNV is strongly suspected, regardless of previous test results.

Viral and Rickettsial Disease Laboratory Testing for West Nile Virus

Laboratory diagnosis of human West Nile virus (WNV) infection is a multi-step process due to the high degree of serological cross-reactivity among flaviviruses and the relative lack of sensitivity of molecular diagnosis for WNV. Testing available at the CDPH VRDL includes serologic and molecular tests.

See Appendices A and B, A: Instructions for Submitting Specimens to VRDL and B: VRDL West Nile Virus Testing Algorithm.
Appropriate Clinical Specimens for WNV Laboratory Testing at VRDL

<table>
<thead>
<tr>
<th>Specimen</th>
<th>IgM* †</th>
<th>IgG</th>
<th>PRNT§</th>
<th>PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum (≥2 ml)</td>
<td>Yes</td>
<td>Paired Acute/Convalescent sera only</td>
<td>IgM (+) sera only</td>
<td></td>
</tr>
<tr>
<td>CSF** (1-2 ml)</td>
<td>Yes</td>
<td></td>
<td>As needed</td>
<td>Yes</td>
</tr>
</tbody>
</table>

* Serum collected within 3 days of symptom onset may not have detectable levels of IgM related to the current illness. If the IgM is negative in the serum sample but WNV is strongly suspected, another serum sample should be collected 3-5 days after the first serum. WNV IgM is usually present in immunocompetent individuals by day 5 after illness onset.
† Most sera and all CSF are tested for IgM by the Focus WNV IgM enzyme immunoassay (EIA); an in-house immunofluorescence assay (IFA) is also available for testing sera.
§ The WNV Plaque Reduction Neutralization Test (PRNT) is not validated for clinical use and is for surveillance purposes only. CSF will be tested on a per case basis.
** Since enteroviruses and WNV can cause similar clinical manifestations, enterovirus PCR may also be done on CSF specimens.

Serologic tests

**Enzyme Immunoassay (EIA) testing:** The immunoglobulin M (IgM) antibody-capture enzyme immunoassay (EIA) is the frontline test for WNV at VRDL. EIA testing can be completed within 14 calendar days from when samples arrive at the laboratory.

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

**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.

**Plaque reduction neutralization test (PRNT):** Due to the high degree of serological cross-reactivity among flaviviruses, additional laboratory testing is required to confirm that a WNV IgM detection is specific for West Nile virus. Other flaviviruses include dengue (DEN), St. Louis encephalitis (SLE) and Zika viruses, as well as yellow fever (YF), and Japanese encephalitis (JE) viruses. People who have been recently vaccinated for JE or YF, or who have a recent exposure to another flavivirus, may have a false positive IgM for WNV, even though they have not actually been exposed to WNV.

The PRNT is the most specific serological test available for distinguishing between the arthropod-borne flaviviruses. Since WNV PRNT testing is not currently validated for diagnostic purposes at the VRDL, final results that include PRNT results are reported out within 21 days from detection of WNV IgM and may not be used for clinical purposes.

**Molecular tests**

RT-PCR has a relatively rapid turn-around time but a low sensitivity for WNV, making it inappropriate as the sole test for laboratory diagnosis of symptomatic WNV infections. At VRDL, RT-PCR is available for diagnosis only on CSF specimens. For diagnosis of clinical disease, serological tests are more accurate than molecular tests.
Laboratory Diagnosis, Test Interpretation, and Case Classification

- A positive RT-PCR result confirms WNV infection; however, due to low sensitivity, a negative RT-PCR result does not exclude WNV infection.

- In the absence of a positive RT-PCR result, a positive WNV IgM result in a patient with a clinically compatible illness is sufficient to meet a probable case definition (Appendix C).

- To be considered a confirmed case, all WNV IgM positive results must be confirmed by PRNT (Table 1).
  - A repeat positive IgM result at another laboratory is not sufficient to confirm WNV. For the first few cases of the WNV season, it is recommended that positive results from commercial or public health laboratories be verified by confirmatory testing, i.e., RT-PCR or PRNT at the local public health laboratory and/or VRDL.
  - When in doubt, obtain either the original specimen or a convalescent sample to forward to the local public health laboratory or VRDL for repeat or confirmatory testing.

- In the absence of a positive IgM result, a positive IgG result only indicates previous infection with a flavivirus.

- In immunocompromised individuals, the WNV antibody response may be delayed. For these patients, additional testing is warranted. Please consult with VRDL for guidance.

Table 1. Interpretation of West Nile virus antibody test results*

<table>
<thead>
<tr>
<th>Tests</th>
<th>Test Interpretation</th>
<th>Overall Interpretation</th>
<th>Reflex Testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgM</td>
<td>negative</td>
<td>Antibody not detected</td>
<td>None</td>
</tr>
<tr>
<td>IgG</td>
<td>negative</td>
<td></td>
<td>Request follow up serum if specimen collected &lt;3 days post onset</td>
</tr>
<tr>
<td>IgM</td>
<td>negative</td>
<td>Flavivirus infection at undetermined time</td>
<td>None</td>
</tr>
<tr>
<td>IgG</td>
<td>positive</td>
<td></td>
<td>Request follow up serum if specimen collected &lt;3 days post onset</td>
</tr>
<tr>
<td>IgM</td>
<td>indeterminate</td>
<td>Inconclusive;</td>
<td>None</td>
</tr>
<tr>
<td>IgG</td>
<td>negative</td>
<td>Request convalescent serum</td>
<td>Request follow up serum if specimen collected &lt;3 days post onset</td>
</tr>
<tr>
<td>IgM</td>
<td>positive</td>
<td>Possible evidence of recent or current infection; further testing necessary*</td>
<td>PRNT</td>
</tr>
<tr>
<td>IgG</td>
<td>negative</td>
<td></td>
<td>PRNT</td>
</tr>
<tr>
<td>IgM</td>
<td>positive</td>
<td>Possible evidence of recent or current infection; further testing necessary‡</td>
<td>PRNT</td>
</tr>
<tr>
<td>IgG</td>
<td>positive</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Serologic results should be interpreted on the basis of clinical and epidemiological information
‡ Due to heterotypic antibody responses and/or cross-reactions, IgM detections may be falsely positive for WNV. Specimen will be reflexed to PRNT.
‡ Some individuals may have persisting antibodies from the previous WNV season. A recent or current infection may be confirmed with a four-fold or greater rise in IgG titer between acute and convalescent paired sera by EIA and/or PRNT.
Case Reporting: See Appendices D-F

Acute WNV infection is a reportable disease. Under Title 17 of the California Code of Regulations, Section 2505, laboratories are required to report positive WNV test results to the local health department where the patient resides. To determine whether an individual should be reported to CDPH as a WNV case, local health departments should refer to the case definition for WNV (Appendix C). Please note this case definition is for public health surveillance purposes only and is not intended for use in clinical diagnosis.

- All VRDL results are faxed to the submitting local public health lab and to the local health department where the patient resides.
- Local health departments are expected to forward test results to the appropriate health care provider.
- Local health departments should follow up on all IgM-positive results from commercial labs.

Reporting West Nile Virus Cases and Presumptive Viremic Donors

- **How to Report:**
  - Local health departments must report cases of WNV illness and WNV-positive blood donors via CalREDIE or by FAX to 510-412-6263.
  
  Note: case report forms may also be mailed to Vector-Borne Disease Section, ATTN: WNV Human Forms, California Department of Public Health, 850 Marina Bay Parkway, Bldg G1-004, Richmond, CA 94804, but this may result in reporting delays.
  - See Appendix D: West Nile Virus (WNV) Infection Case Report and Appendix E: Report Form for Presumptive Viremic Donors. Jurisdictions reporting through CalREDIE, see Appendix F: CalREDIE Reporting Flowchart.

- **Reporting guidelines:**
  - Report the case as
    - West Nile virus - Non-neuroinvasive (specify syndrome as febrile illness or other clinical presentation [if non-febrile]);
    - West Nile virus - Neuroinvasive (specify syndrome[s] as encephalitis, meningitis, acute flaccid paralysis, and/or other neuroinvasive presentation); or
    - West Nile virus - Asymptomatic (specify syndrome as asymptomatic).
  - Neuroinvasive symptoms and presence of fever/chills must be specified.
  - WNV laboratory results must be included in the case report.

- **Official case counts:**
  - Case counts are updated every Friday during the WNV season on the California WNV website ([http://westnile.ca.gov](http://westnile.ca.gov)). Cases reported to CDPH by close of business on Tuesday will be included in the Friday update.
Cases reported via CalREDIE that meet the following criteria will be included in CDPH case counts and reports, as well as reported to the CDC ArboNET reporting system (See Appendix F: CalREDIE Reporting Flowchart):

- Process Status: Closed by LHD
- Disease: West Nile virus – Neuroinvasive, non-neuroinvasive, or asymptomatic
- Resolution Status: Confirmed or Probable

Cases that do not meet the above criteria will NOT be counted and reported (e.g., cases listed as Under Investigation, West Nile virus – Unspecified, or Suspect)

If a local health department is aware of a case missing from the case count on the CDPH website or in ArboNET, please contact Jacklyn Wong at (510) 412-4650.

- **Vector control notification:** Health departments should notify their local vector control agency of any confirmed human WNV activity as soon as possible, so that enhanced mosquito surveillance and control measures can be implemented.

**West Nile Virus-Associated Fatalities**

Determining whether or not WNV has caused a fatality can be difficult. WNV 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 WNV and never recovered from the sequelae (e.g., was discharged to a convalescent hospital until date of death), a health department may consider designating the patient as a WNV-associated fatality.

**Contacts**

**Viral and Rickettsial Disease Laboratory**
Diana Singh............................................................. (510) 307-8608
Maria Salas, MPH.................................................... (510) 307-8606
VRDL Fax................................................................. (510) 307-8599

**Vector-Borne Disease Section**
Jacklyn Wong, PhD.................................................. (510) 412-4650
VBDS Fax............................................................... (510) 412-6263
West Nile Virus Hotline ............................................. (877) 968-2473

**Useful Links**

California West Nile Virus Website........... [http://westnile.ca.gov](http://westnile.ca.gov)
CDC West Nile Virus Website............... [https://www.cdc.gov/westnile](https://www.cdc.gov/westnile)
Appendix A: Instructions for Submitting Specimens to VRDL

Recommended Specimens:

- ≥2 cc acute serum
- If a lumbar puncture is performed, 1-2 cc cerebral spinal fluid (CSF)

If West Nile virus is highly suspected and acute serum is negative or inconclusive, request:

- ≥2 cc convalescent serum collected at least 3-5 days after acute serum.

Instructions:

- Refrigerate all specimens at 2 to 8°C and ship on cold pack using an overnight courier.
  - If CSF needs to be stored >72 hours, 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 General Purpose Specimen Submittal Form Lab300
  https://archive.cdph.ca.gov/programs/vrdl/Documents/VRDL_General_Human_Specimen_Submittal_Form_Lab300.pdf. The submittal form must be completed electronically; handwritten submittal forms will not be accepted. The following information is required for accurate interpretation of results:
  - Onset date
  - Unusual immunological status of patient, if any
  - County of residence
  - History of travel to domestic or international flavivirus-endemic areas (include country/locale)
  - History of prior vaccination against flavivirus disease (e.g., YF or JE)
  - Include any known West Nile virus test results
- Do not send specimens on Fridays for weekend delivery (Specimen Receiving hours are M-F, 8-5)
- Address specimens for VRDL to:
  Specimen Receiving/ West Nile
  850 Marina Bay Parkway
  Richmond, CA  94804
Appendix B: Viral and Rickettsial Disease Lab West Nile Virus Testing Algorithm

**SERUM or CSF**

**LOCAL IgM RESULT:**
- **NOT TESTED OR UNKNOWN OR INDETERMINATE**
  - **EIA**
    - Focus IgM
      - **Focus M**(-)
        - Report (Neg)
      - **Focus M**(+)
        - **Focus M** (+) heterophile(-)
          - Report (Pos)
        - **Focus M** (+) heterophile(+)
          - Report (Unsatisfactory)

  - PRNT (not diagnostic)

**LOCAL IgM RESULT:**
- **POSITIVE**
  - PRNT (not diagnostic)

**Additional available tests:**
- **Paired Acute/Convalescent Sera:**
  - EIA IgG (in-house)
- **CSF:**
  - RT-PCR

† Heterophile antibodies are “interfering” antibodies that can cause false positive EIA IgM results.
* Review the following information:
  - onset date
  - travel history
  - prior flavivirus exposure
  - vaccination history
West Nile virus infection is reportable to local health departments under Title 17 of the California Code of Regulations. 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.

Symptomatic Cases (adapted from 2015 CSTE case definition)

Clinical criteria for diagnosis
Neuroinvasive disease
- Meningitis, encephalitis, acute flaccid paralysis, or other acute signs of central or peripheral neurologic dysfunction, as documented by a physician, AND
- Absence of a more likely clinical explanation.

Non-neuroinvasive disease
- Fever (chills) as reported by the patient or a health-care provider, AND
- Absence of neuroinvasive disease, AND
- Absence of a more likely clinical explanation.

Case classification
Confirmed = A case that meets the above clinical criteria and one or more of the following laboratory criteria for a confirmed case:
- Isolation of virus from, or demonstration of specific viral antigen or nucleic acid in, tissue, blood, CSF, or other body fluid, OR
- Four-fold or greater change in virus-specific quantitative antibody titers in paired sera, OR
- Virus-specific immunoglobulin M (IgM) antibodies in serum with confirmatory virus-specific neutralizing antibodies in the same or a later specimen, OR
- Virus-specific IgM antibodies in CSF and a negative result for other IgM antibodies in CSF for arboviruses endemic to the region where exposure occurred.

Probable = A case that meets the above clinical criteria and the following laboratory criteria:
- Virus-specific IgM antibodies in CSF or serum but with no other testing.

Presumptive Viremic Donors (Asymptomatic)
Asymptomatic infection with WNV, which is generally identified in blood donors, is also reportable. 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-VBDS:

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, the disease incident should be reclassified as “West Nile virus – Non-neuroinvasive” or “West Nile virus – Neuroinvasive,” depending on the individual’s clinical symptoms.
Appendix D: West Nile Virus (WNV) Infection Case Report

Patient Information:

Last Name: ________________________________ First Name: ________________________________ DOB: / / Age: ___ Med Rec #: ________
Address: __________________________________________________________ City: ________________ Zip Code: __________
Phone: Home (_____) Work (_____): ____________________________ Occupation: __________________________
Sex:   □ Male  Ethnicity:   □ Hispanic  Race:   □ White  □ Asian/ Pacific Islander
□ Female  □ Non-Hispanic  □ Black  □ American Indian/Alaskan Native
□ Unknown  □ Unknown  □ Other: __________________________________________

Diabetes Type ________  Hypertension: __________________________________________
Glucose: ____  Protein: ____  %Diff: __________________________ WBC: ____
RBC: ____  %Diff: __________________________ HCT: ____  Plt: ____
Date: ____/____/____  Seizures ………………….
Stiff neck ………………….
Rash ………………………
Headache ………………...
Fever …………………..…
In ICU …………

Do the following apply anytime during current illness:
Other ________________________________________.

Asymptomatic ……………  Febrile illness …………..…
Acute flaccid paralysis ……………………….
Aseptic meningitis ………..
Clinical syndrome:
Encephalitis ……………… □ Yes  □ No  □ Unk
Aseptic meningitis ………… □ Yes  □ No  □ Unk
Acute flaccid paralysis … □ Yes  □ No  □ Unk
Febrile illness ……………… □ Yes  □ No  □ Unk
Asymptomatic ……………… □ Yes  □ No  □ Unk
Other ____________________________________________

Do the following apply anytime during current illness:
In ICU ……………… □ Yes  □ No  □ Unk
Fever ≥38°C……………… □ Yes  □ No  □ Unk
Headache ……………… □ Yes  □ No  □ Unk
Rash …………………..… □ Yes  □ No  □ Unk
Stiff neck ……………… □ Yes  □ No  □ Unk
Muscle pain/weakness … □ Yes  □ No  □ Unk
Altered consciousness … □ Yes  □ No  □ Unk
Seizures ………………… □ Yes  □ No  □ Unk

Travel/Exposures within 4 wks of onset (specify details):
Mosquito bites/exposure ……………… □ Yes  □ No  □ Unk
Dates/Locations: __________________________

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

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

Mosquito bites/exposure ……………… □ 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, LFTs, etc.): __________________________________________

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

CSF Results:
Date: / / __________  RBC: __________
WBC: __________  %Diff: __________
Protein: __________  Glucose: __________

CBC Results:
Date: / / __________  WBC: __________
HCT: __________  Plt: __________

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

West Nile Virus Test Results:
Testing Laboratory  Specimen Type  Coll Date  Test Type  Result
Testing Laboratory  Specimen Type  Coll Date  Test Type  Result

FAX this form: (510) 412-6263
or MAIL to: CDPH/Vector Borne Disease Section, 850 Marina Bay Parkway, Richmond CA 94804
Appendix E: Report Form for Presumptive Viremic Donors

California Department of Public Health
Vector-Borne Disease Section
850 Marina Bay Parkway, Richmond, CA 94804
(510) 412-4650 Fax (510) 412-6263

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_______________________________________________________
    ________________________________________________________________
    ________________________________________________________________

Please include this form in the patient’s CalREDIE electronic filing cabinet or fax to (510) 412-6263
Appendix F: CalREDIE Reporting Flowchart

Suspect West Nile virus infection

Does individual have symptoms?

Yes

Select the appropriate disease condition:
West Nile virus – Neuroinvasive
West Nile virus – Non-neuroinvasive

No

Select the following disease condition:
West Nile virus – Asymptomatic

Do test results meet laboratory criteria for diagnosis?

Yes

Set Resolution Status to Probable or Confirmed

No

Set Resolution Status to Not a Case

Do individual meet criteria for presumptively viremic blood donor?

Yes

Set Resolution Status to Confirmed

No

Set Resolution Status to Not a Case

Ready to publish?

Yes

Set Process Status to Closed by LHD

Case is included in case counts and reports on www.westnile.ca.gov and reported to CDC ArboNET

Ready to publish?

Yes

Set Process Status to Closed by LHD

Blood donor is included in reports on www.westnile.ca.gov and reported to CDC ArboNET but is NOT included in case counts