# West Nile and St. Louis Encephalitis Viruses in California: Guidelines for Human Testing, Surveillance, and Reporting


California Department of Public Health
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## Why is Surveillance of WNV/SLEV Important?

West Nile virus (WNV) is the leading cause of mosquito-borne (arboviral) disease in the United States and the most common vector-borne disease in California. It is usually transmitted to people by the bite of an infected *Culex spp.* mosquito. Most WNV infections are asymptomatic; however, clinical disease can range from mild febrile illness to severe neuroinvasive disease. St. Louis encephalitis virus (SLEV) has an indistinguishable clinical presentation and co-circulates with WNV in many California counties and should be considered as a causative agent of both neuroinvasive illness and non-neuroinvasive illness in areas where both viruses are present. Most individuals who develop symptoms present with non-neuroinvasive disease typified by an acute febrile illness that can include such symptoms as headache, myalgia, arthralgia, rash, and/or gastrointestinal symptoms.[1] Neuroinvasive illness due to these viruses is rare and is usually characterized by the acute onset of fever with headache, myalgia, stiff neck, altered mental status, seizures, or limb weakness, leading to either encephalitis, meningitis, acute flaccid paralysis (AFP), or other neurological dysfunction. AFP in these cases can result from anterior ("polio") myelitis, peripheral neuritis, or post-infectious peripheral demyelinating neuropathy (i.e., Guillain-Barré syndrome). Less common neurological manifestations, such as cranial nerve palsies or retinopathy, may also be a manifestation of neuroinvasive illness.[1] Both viruses are in the genus *Flavivirus*, along with dengue, Zika, Japanese encephalitis, yellow fever, and Powassan viruses. These closely related viruses stimulate production of antibodies that cross react with each other. If a patient is a resident of a county with both WNV and SLEV circulating or has a recent travel history to an area where other flaviviruses are endemic, confirmatory testing should be conducted.

### California Department of Public Health

The California Department of Public Health (CDPH) provides guidance to medical providers and local health departments regarding interpretation of laboratory results, disease classification, and case reporting.[2] The Viral and Rickettsial Disease Laboratory (VRDL) at CDPH works in conjunction with local public health laboratory partners to provide laboratory testing for specimen submitters. The Vector-Borne Disease Section (VBDS) at CDPH works with local physicians, communicable disease controllers, epidemiologists, and vector control agencies to ensure prevention, surveillance, and control of vector-borne diseases including, but not limited to, WNV and SLEV.

**Contact Information for CDPH**

| CDPH Program Name | Contact Name | Phone/Fax/Email |
|---|---|---|
| **Viral and Rickettsial Disease Laboratory (VRDL)** | VRDL Main Line | (510) 307-8585 |
| | VRDL Email | VRDL.Submittal@cdph.ca.gov |
| **Vector-Borne Disease Section (VBDS)** | Mary Beth Danforth, PhD<br>WNV/SLEV Epidemiologist | (916) 449-5179<br>Mary.Danforth@cdph.ca.gov |
| | VBDS Fax (for case report forms) | (510) 412-6263 |

[1] CDC WNV Website: https://www.cdc.gov/west-nile-virus/
[2] CDPH WNV Website: https://westnile.ca.gov

## Clinical and Laboratory Criteria for Diagnosis of WNV/SLEV

Identification of human cases early in the WNV/SLEV season is important for guiding mosquito surveillance, control, and public education activities that reduce the risk of additional infections. Thus, WNV and SLEV testing is recommended for individuals with the following clinical syndromes, particularly during WNV/SLEV season, which usually ranges from June through November. The clinical aspects of the CSTE case definition for arboviral disease (neuroinvasive vs. non-neuroinvasive) can be found in Table 1, with laboratory criteria for diagnosis (probable / confirmed) described in Table 2.

### Table 1: Clinical Case Definition[3]

| Clinical Description | Clinical Criteria |
|---|---|
| **Neuroinvasive** Disease | Meningitis **(Note: enterovirus should also be considered for individuals ≤ 18 years of age)**, 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.<br><br>Other clinically compatible symptoms of neuroinvasive arboviral disease include headache, myalgia, rash, arthralgia, vertigo, vomiting, paresis, and/ or nuchal rigidity (neck stiffness). |
| **Non-neuroinvasive** Disease | Fever (chills) as reported by the patient and documented by a health-care provider, **and** |
| | Absence of neuroinvasive disease, **and** |
| | Absence of a more likely clinical explanation.<br><br>Other clinically compatible symptoms of non-neuroinvasive arboviral disease include headache, myalgia, arthralgia, rash, or gastrointestinal symptoms. |

[3] CSTE Case Definition for Arboviral Diseases: https://ndc.services.cdc.gov/case-definitions/arboviral-diseases-neuroinvasive-and-non-neuroinvasive-2015/

## Table 2: Laboratory Criteria for Diagnosis[3]

| Case Classification | Clinical Description | Laboratory Criteria |
|---|---|---|
| **Probable** | **Neuroinvasive Disease:** A case that meets the above clinical criteria for neuroinvasive disease and the following laboratory criteria: | • Virus-specific IgM antibodies in CSF or serum but with no other testing completed, in a county where both WNV and SLEV were detected that calendar year.[4] |
| | **Non-neuroinvasive Disease:** A case that meets the above clinical criteria for non-neuroinvasive disease and the following laboratory criteria: | • Virus-specific IgM antibodies in serum but with no other testing.[5] |
| **Confirmed** | **Neuroinvasive Disease:** A case that meets the above clinical criteria for neuroinvasive disease and ***one or more*** of the following laboratory criteria: | • Detection of specific nucleic acid or isolation of virus from serum, blood, CSF, other body fluids, **or**<br>• Four-fold or greater change in virus-specific antibody titers in paired sera, **or**<br>• Virus-specific IgM antibodies in serum with confirmatory virus-specific neutralizing antibodies in the same or a later specimen, **or**<br>• 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 |
| | **Non-Neuroinvasive Disease:** A case that meets the above clinical criteria for non-neuroinvasive disease and ***one or more*** of the following laboratory criteria: | • Detection of specific nucleic acid or isolation of virus from serum, blood, other body fluids, **or**<br>• Four-fold or greater change in virus-specific antibody titers in paired sera, **or**<br>• Virus-specific IgM antibodies in serum with confirmatory virus-specific neutralizing antibodies in the same or a later specimen. |

[4] Case-patients meeting probable case definition for both WNV and SLEV (i.e., IgM against both WNV and SLEV are detected, but neutralizing antibody against both WNV and SLEV are detected or neutralizing antibody against neither WNV and SLEV are detected), should be reported as probable WNV cases.

## Viral and Rickettsial Disease Laboratory (VRDL) Testing for WNV and SLEV

Please visit our VRDL website for detailed information about testing guidance for WNV and SLEV.[5]

### Recommended Specimens for Collection

**Note:** CSF *must* be submitted with an accompanying serum sample

- ≥3-5 cc **Serum** (Red top or serum separator tubes acceptable)
- 1-2 cc **Cerebral Spinal Fluid (CSF)** (Sterile screwcap tube)

VRDL has developed a real-time RT-PCR test to detect WNV in acute whole blood samples of cases suspected of WNV disease. This assay has improved sensitivity approaching 90% for detection of WNV RNA in blood samples collected within 2-3 weeks of onset. VRDL is currently validating this whole blood protocol for diagnostic use as a laboratory developed test. If possible, please also include the following for all specimen submissions:

- ≥3-5 cc **Whole Blood** (Purple Top - EDTA tubes)

See Table 3 for a listing of diagnostic versus surveillance use only tests provided by VRDL and the projected turnaround time for results. **Note: Tests designated for surveillance use *may not* be used for clinical diagnoses.**
See **Appendix A: WNV and SLEV Testing Algorithm**

### Serologic (IgM) Screening Tests

**Enzyme Immunoassay (EIA) serology testing:** An EIA test may be used for frontline screening of IgM antibodies for WNV at VRDL. A positive flavivirus antibody finding in CSF is valuable in cases with neuroinvasive disease; **however, a negative result does not rule out flavivirus infection**, as antibody levels in CSF tend to be lower than those in serum, and thus may be below assay detection limits. **Because of this, submission of CSF samples without accompanying serum is discouraged.**

### Plaque Reduction Neutralization Test (PRNT)

The Plaque Reduction Neutralization Test (PRNT) can detect virus-specific neutralizing antibodies in a sample. Due to the high degree of serological cross-reactivity among flaviviruses, the PRNT is the most specific serological test available for distinguishing between WNV and SLEV, as well as other flaviviruses. Nevertheless, PRNT results may be confounded by vaccination/previous exposure to any flaviviruses due to the production of generic cross-reactive, flavivirus-neutralizing antibodies.
**Note:** IgG antibodies and neutralizing antibodies are not always the same. Neutralizing antibodies represent a subset of total antibodies (IgA, IgD, IgE, IgG, and IgM) that, via binding to a virus, interfere with its ability to infect a cell (i.e., neutralization), whereas not all IgG antibodies will neutralize virus. IFA and EIA tests aim to measure the quantity of antibodies which bind to a specific viral antigen, whereas the PRNT measures the antibodies' ability to neutralize virus in culture.
See Appendix B: **WNV and SLEV PRNT Results Interpretation**

[5] VRDL WNV-SLEV Testing Guidance:
https://www.cdph.ca.gov/Programs/CID/DCDC/CDPH%20Document%20Library/WNV-SLEV-TestingGuidance.pdf
**Note:** An electronically-completed VRDL General Purpose Specimen Submittal Form must be submitted for each sample submitted for testing.

## Molecular Testing

Molecular tests, such as real-time PCR, are generally not recommended as the primary test for laboratory diagnosis of symptomatic arboviral infections due to the low sensitivity for detection of WNV and SLEV nucleic acid in serum and CSF. This is likely because most individuals present to healthcare providers after symptoms develop, which is when fewer viral particles are expected to be circulating in their systems. However, studies have documented that real-time PCR of whole blood may be more sensitive and may detect viral nucleic acids for longer after symptom onset than other specimen types. **If WNV or SLEV nucleic acids are detected, the result is highly specific to the virus and confirmatory serology testing is not required**. For this reason, VRDL has developed a real-time RT-PCR test to detect WNV in acute whole blood samples of cases suspected of WN disease and demonstrated that this assay has significantly improved sensitivity (~90%) in blood samples collected within 2-3 weeks of onset. VRDL is validating this method as a clinical diagnostic test for WNV. Additionally, VRDL is assessing a RT-PCR assay for SLEV to determine if SLEV RNA can be detected in whole blood with similar sensitivity. VRDL encourages submission of whole blood samples in suspect WNV or SLEV cases.

### Table 3. VRDL Assays for Diagnostic and Surveillance Use (TAT in business days)

| Virus | Specimen | Real-time PCR | IgM Serology | PRNT |
|---|---|---|---|---|
| **WNV** | ***Serum*** | Not an acceptable sample type | Diagnostic (10) | Surveillance (16) |
| | ***CSF*** | Diagnostic (10) | Diagnostic (10) | Surveillance (16) |
| | ***Whole blood*** | Surveillance (10) | Not an acceptable sample type | Not an acceptable sample type |
| **SLEV** | ***Serum*** | Not an acceptable sample type | Not available | Surveillance (16) |
| | ***CSF*** | Surveillance (10) | Not available | Surveillance (16) |
| | ***Whole blood*** | Surveillance (10) | Not an acceptable sample type | Not an acceptable sample type |

## Factors to Consider when Interpreting Arboviral Laboratory Serology Results

**Arboviral serologic assays:** Assays for the detection of IgM antibodies commonly include enzyme-linked immunosorbent assay (ELISA), microsphere immunoassay (MIA), or immunofluorescence assay (IFA). These assays provide a presumptive diagnosis and should have confirmatory testing performed. Confirmatory testing involves the detection of arboviral-specific neutralizing antibodies utilizing assays such as plaque reduction neutralization test (PRNT).

**Other information to consider.** Vaccination history, detailed travel history, date of onset of symptoms, and knowledge of potentially cross-reactive arboviruses known to circulate in the geographic area should be considered when interpreting results.

**Rise and fall of IgM antibodies:** For most infections, IgM antibodies are detectable between 3- and 8-days post symptom onset and generally persist for 30-90 days, but longer duration has also been documented (e.g., ≥5 years for WNV). Serum collected within 3 days of symptom onset may not have detectable IgM antibodies from the current illness. If the serum sample is IgM negative but WNV is

strongly suspected, another serum sample should be collected 3-5 days after the first serum for repeat testing.[6]

**Persistence of IgM antibodies:** Arboviral IgM antibodies may be detected in some patients for months or years after their acute illness. Therefore, the presence of these virus-specific IgM antibodies may indicate a past infection and be unrelated to the current illness. Finding virus-specific IgM antibodies in CSF or a fourfold or greater change in virus-specific antibody titers between acute- and convalescent-phase serum specimens provides additional laboratory evidence that the arbovirus was the likely cause of the patient's recent illness. Clinical and epidemiologic history should also be carefully considered.

**Persistence of neutralizing antibodies:** Arboviral neutralizing antibodies can persist for many years following a symptomatic or asymptomatic infection. Therefore, the presence of these antibodies **alone** is only evidence of previous infection and clinically compatible cases with the presence of neutralizing antibodies, but not IgM, should be evaluated for other etiologic agents.

### Transmission of VRDL Laboratory Test Results to Submitters

Following completion of testing, laboratory results are sent to the local public health laboratory or local health department through secure email or the VRDL Laboratory Web Portal. Local public health laboratories or health departments receiving results from VRDL are expected to forward test results and share all significant findings with the epidemiologists and health department where the patient resides. **Note: Local health departments should follow up on all IgM-positive results.**

### Imported Arboviral Diseases

Human disease due to dengue, Zika, chikungunya, or yellow fever viruses are nationally notifiable to CDC using specific case definitions. However, many other exotic arboviruses (e.g., Japanese encephalitis, tick-borne encephalitis, Venezuelan equine encephalitis, and Rift Valley fever viruses) are important public health risks in the United States because competent vectors exist here that could permit transmission of imported arboviral pathogens. Health-care providers and public health officials should maintain a high index of clinical suspicion for cases of potentially imported or unusual arboviral etiology, particularly in international travelers. If a suspected case occurs, it should be immediately reported to appropriate local and state health authorities.

## Reporting Probable or Confirmed Cases of WNV/SLEV to CDPH

Acute symptomatic WNV/SLEV infection are nationally notifiable conditions. Under Title 17 of the California Code of Regulations, Section 2505, laboratories are required to report positive WNV and SLEV test results to the local health department where the patient resides. Both probable and confirmed cases are reportable to CDPH and are included in case tallies. To determine whether an individual should be reported to CDPH as a case, local health departments should refer to the case definitions for WNV/SLEV,[3] and/or reach out to Mary Beth Danforth, PhD, (916-449-5179; Mary.Danforth@cdph.ca.gov), the CDPH WNV and SLEV subject-matter expert, with any questions or concerns. Please note that this case definition is intended for public health surveillance purposes only and should not be used for clinical diagnoses.

[6] CDC WNV Clinical Testing and Diagnosis for WNV Disease: https://www.cdc.gov/west-nile-virus/hcp/diagnosis-testing

Local health departments must report cases of WNV/SLEV illness and **WNV-positive blood donors [Presumptive Viremic Donors (PVD)]** via CalREDIE, by FAX to 510-412-6263, or by secure email to VBDS@cdph.ca.gov. WNV case report forms are available in CalREDIE. If your jurisdiction does not use CalREDIE, please contact the Vector-Borne Disease Section for a PDF form.

## Asymptomatic WNV Infections including Blood Donors

Asymptomatic infection with WNV, which is generally identified in blood donors but also in organ donors, is also reportable. Blood or organ donors who test positive for WNV via molecular assays may not necessarily be ill or have positive IgM or IgG antibody test results. Local health departments should report blood donors that meet at least one of the following criteria as a presumptively viremic donor to CDPH-VBDS:

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

Additional serological testing is not required if either of these criteria are met. Local health departments should follow up with the donor two weeks after the date of donation to assess if the patient subsequently developed symptoms and reclassify the infection per their clinical presentation.

## West Nile Virus (WNV) Case Reporting

Report the case as:

- West Nile virus – non-neuroinvasive (specify clinical syndrome as 'febrile illness' or 'other clinical presentation' [if non-febrile])
  - Non-neuroinvasive cases should **not** have any neuroinvasive symptoms indicated (e.g. seizures, paresis/paralysis, coma, ataxia, etc.)
- West Nile virus – neuroinvasive (specify clinical syndrome[s] as 'encephalitis', 'meningitis', 'acute flaccid paralysis', and/or 'other neuroinvasive presentation')
  - Primary clinical syndrome must be neuroinvasive (can also include others as secondary and tertiary syndromes)
  - Please consult the CDPH epidemiologists when considering 'other neuroinvasive presentation' as this should only be indicated in the strict absence of other neuroinvasive symptoms
- West Nile virus – asymptomatic (specify as asymptomatic)
  - WNV laboratory results must be included in the case report
  - Specimen collection date must be included in the case report form in CalREDIE as well as Blood Donor Identification Number (e.g., W1234567890)

See **Appendix C: WNV CalREDIE Reporting Flowchart**

## St. Louis Encephalitis Virus (SLEV) Case Reporting

Report the case as "St. Louis encephalitis virus"

- If the case was initially entered as a WNV incident, change "Disease Being Reported" to "St. Louis Encephalitis Virus Infection"
- The SLEV incident must indicate symptoms and whether or not disease was neuroinvasive (clinical syndrome[s] 'encephalitis', 'meningitis', 'acute flaccid paralysis', and/or 'other neuroinvasive presentation'), or non-neuroinvasive ('febrile illness' or 'other clinical presentation' [if non-febrile])

- Both SLEV and WNV laboratory results must be included in the SLEV case report
- Onset date must be included in the case report.

See **Appendix D: SLEV CalREDIE Reporting Flowchart**

## CDPH Case Counts

- All WNV and SLEV cases reported to CDPH by 5:00 PM Wednesday during the WNV and SLEV transmission season (May through December) will be included in the CDPH's weekly update. Those case counts will be posted every Friday during arbovirus season on the California WNV website (http://westnile.ca.gov). SLEV cases are reported on the SLEV tab of the California WNV website.
- Cases reported via CalREDIE that meet the following criteria will be included in CDPH case counts and reports, and reported to the CDC ArboNET reporting system each week:
    - Process Status: Closed by LHD
    - Disease: West Nile virus – neuroinvasive, non-neuroinvasive, or asymptomatic, **or** St. Louis Encephalitis virus
    - Resolution Status: Confirmed or Probable
    - Onset date (if blood/organ donor, then date of specimen collection/donation)

**Note:** Cases that do not meet the above criteria will ***not*** be counted / reported (e.g. cases listed as Under Investigation or Suspect). If you believe a case is missing from the case count on the CDPH website or elsewhere for that season (or previous seasons), please contact VBDS WNV epidemiologist, Mary Beth Danforth, PhD, at (916) 449-5179 or Mary.Danforth@cdph.ca.gov.

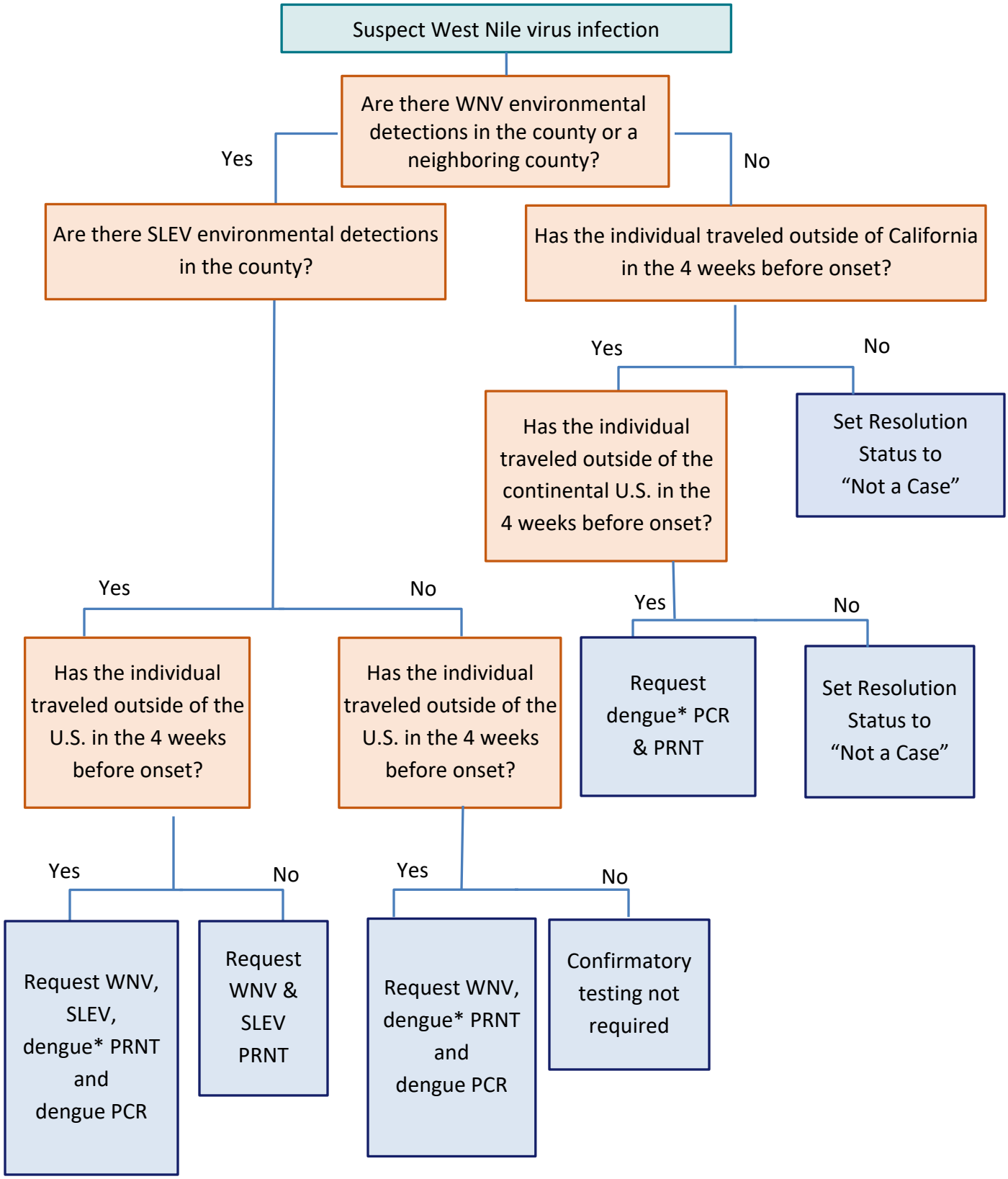
## Vector Control Notification

Health departments should notify their local vector control agency of any human WNV activity as soon as possible, so that enhanced mosquito surveillance and control measures can be implemented to reduce the risk of additional transmission. CDPH encourages reporting, at a minimum, the disease onset date and the cross street where the case patient resides, or if known, where the suspect mosquito exposure occurred. Please contact Mary Beth Danforth, PhD, with any questions or concerns related to communication of cases with local vector control agencies. Ideally this contact will occur prior to laboratory confirmation and closure of the case, as prevention efforts should be undertaken as soon as possible.

## WNV/SLEV Associated Fatalities

Determining whether WNV or SLEV has led to the death of a patient can be difficult. WNV/SLEV may not always be listed as a contributory or underlying cause of death on patients' death certificates, and fatalities can occur well after acute infection. Many case-patients also have underlying conditions that could contribute to the immediate causes of death. In general, if a patient was diagnosed with WNV/SLEV and never recovered from the sequelae (e.g., they were discharged to a convalescent hospital until date of death), a health department should consider designating the patient as a WNV or SLEV associated fatality.

## Appendix A: WNV and SLEV Testing Algorithm




* Other arboviruses may be considered depending on specific travel history and current virus activity.

## Appendix B. WNV and SLEV PRNT Results Interpretation

**Scenario 1:**
Neutralizing Antibody to SLEV: Not Detected
Neutralizing Antibody to WNV: Detected

- Close as WNV confirmed

**Scenario 2:**
Neutralizing Antibody to SLEV: Detected
Neutralizing Antibody to WNV: Not Detected

- Close as SLEV confirmed

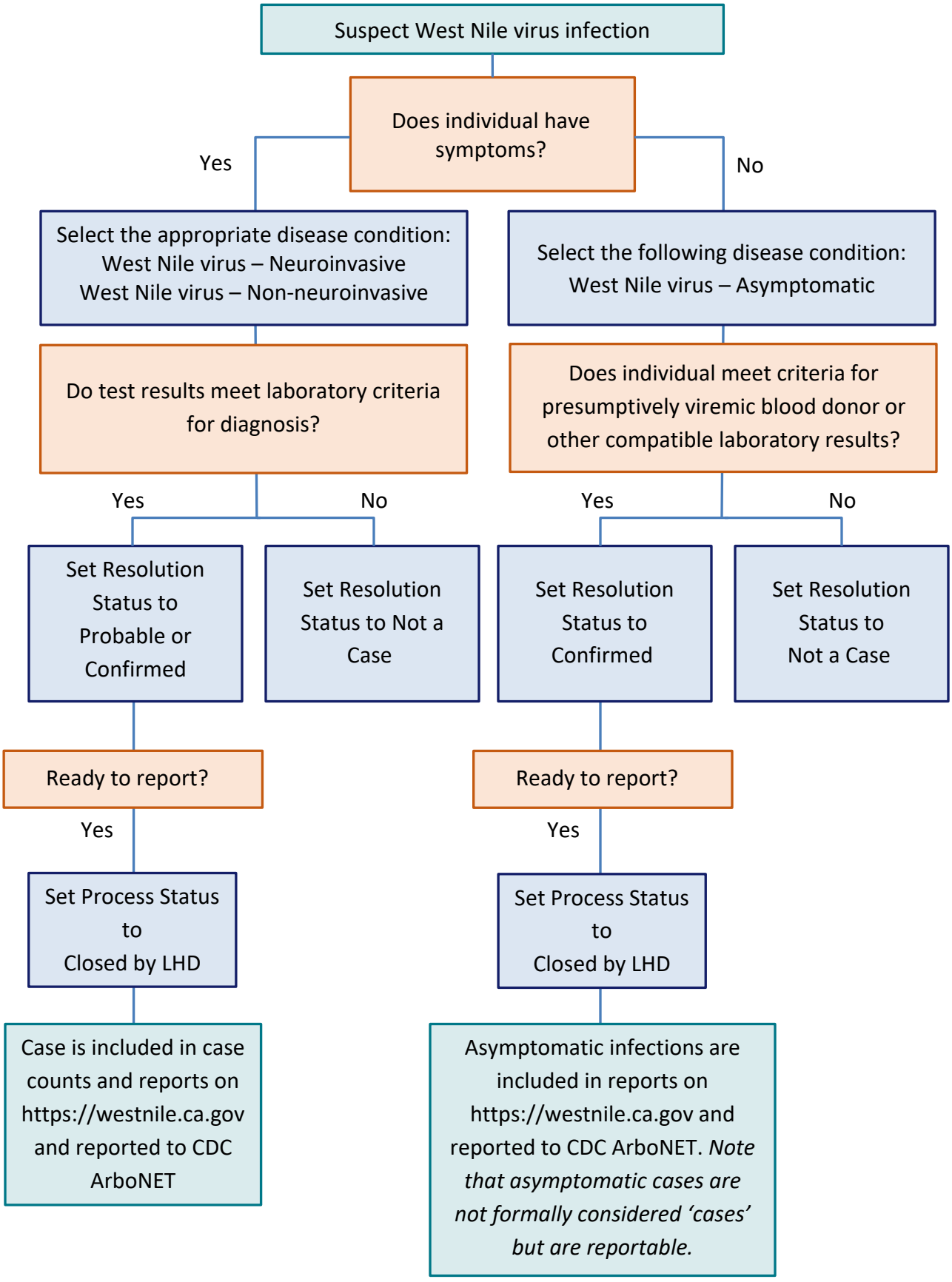
**Scenario 3:**
Neutralizing Antibody to SLEV: Detected
Neutralizing Antibody to WNV: Detected

- Close as probable for whichever virus has been detected in more mosquito pools in the county in the county where the patient resides

**Scenario 4:**
Neutralizing Antibody to SLEV: Not Detected
Neutralizing Antibody to WNV: Not Detected

- Contact VBDS WNV epidemiologist, Mary Beth Danforth, PhD, at (916) 449-5179 or Mary.Danforth@cdph.ca.gov

## Appendix C: WNV CalREDIE Reporting Flowchart



## Appendix D: SLEV CalREDIE Reporting Flowchart