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

California Department of Public Health
Richmond, California

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Table of Contents
Why is Surveillance of WNV/SLEV Important?.................................................................2
   California Department of Public Health .................................................................2
   Contact Information for CDPH..............................................................................2
Clinical and Laboratory Criteria for Diagnosis of WNV/SLEV.................................................................3
   Table 1: Clinical Case Definition........................................................................3
   Table 2: Laboratory Criteria for Diagnosis................................................................4
Viral and Rickettsial Disease Laboratory (VRDL) Testing for WNV and SLEV ..........5
   Recommended Specimens for Collection ..............................................................5
   Molecular Testing...................................................................................................5
   Serologic (IgM/IgG) Screening Tests ......................................................................5
   Plaque Reduction Neutralization Test (PRNT) .........................................................6
   Table 3. VRDL Diagnostic versus Surveillance Assays ...........................................6
Factors to Consider when Interpreting Arboviral Laboratory Serology Results ..........6
Transmission of VRDL Laboratory Test Results to Submitters ..............................................7
Imported Arboviral Diseases .....................................................................................7
Reporting Probable or Confirmed Cases of WNV/SLEV to CDPH.............................8
   Asymptomatic WNV Infections including Blood Donors .....................................8
   West Nile Virus (WNV) Case Reporting ...............................................................8
   Saint Louis Encephalitis Virus (SLEV) Case Reporting .......................................9
   CDPH Case Counts ..............................................................................................9
   Vector Control Notification ....................................................................................9
   WNV/SLEV Associated Fatalities .........................................................................10
Appendix A: WNV/SLEV Presumptive Viremic Donors Report Form .......................11
Appendix B: WNV CalREDIE Reporting Flowchart ..................................................12
Appendix C: SLEV CalREDIE Reporting Flowchart ..................................................13
Why is Surveillance of WNV/SLEV Important?
West Nile virus (WNV) is the leading cause of mosquito-borne (arboviral) disease in the United States. It is most commonly spread to people by the bite of an infected Culex spp. mosquito. Most arboviral infections are asymptomatic; however, clinical disease can range from mild febrile illness to severe neuroinvasive disease. Arboviruses from the same genus stimulate production of anti-arboviral antibodies that cross react with each other. In geographic areas where two or more closely related arboviruses occur, serologic testing for more than one virus may be needed to determine the specific causative agent. For example, such testing might be needed to distinguish antibodies resulting from infections within the genus Flavivirus (including but not limited to: West Nile, St. Louis encephalitis, Powassan, Zika, Dengue, Japanese encephalitis, and yellow fever viruses). St. Louis encephalitis virus (SLEV), a virus closely related to WNV, is co-circulating with WNV in many California counties. SLEV should be considered as a causative agent of both neuroinvasive illness and non-neuroinvasive illness. Neuroinvasive illness due to flaviviruses is usually characterized by the acute onset of fever with headache, myalgia, stiff neck, altered mental status, seizures, limb weakness, or cerebrospinal fluid (CSF) pleocytosis, leading to either encephalitis, meningitis, or acute flaccid paralysis (AFP). AFP 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, may also occur. Both WNV and SLEV can also cause non-neuroinvasive disease. This can take the form of an acute febrile illness that can include (among others) headache, myalgias, arthralgia, rash, and/or gastrointestinal symptoms.

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. The Viral and Rickettsial Disease Laboratory (VRDL) at CDPH works in conjunction with local public health laboratory partners to provide laboratory testing for 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 WNV and SLEV.

Contact Information for CDPH

<table>
<thead>
<tr>
<th>CDPH Program Name</th>
<th>Contact Name</th>
<th>Phone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viral and Rickettsial Disease Laboratory (VRDL)</td>
<td>VRDL Main Line</td>
<td>(510) 307-8585</td>
</tr>
<tr>
<td></td>
<td>VRDL Fax</td>
<td>(510) 307-8599</td>
</tr>
<tr>
<td>Vector-Borne Disease Section</td>
<td>Robert Snyder WNV Epidemiologist/ Subject Matter Expert</td>
<td>(916) 445-2491</td>
</tr>
<tr>
<td></td>
<td>VBDS Fax (for case report forms)</td>
<td>(510) 412-6263</td>
</tr>
</tbody>
</table>

1 [CDC WNV Website]: https://www.cdc.gov/westnile
2 [CDPH WNV Website]: http://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 CSTE case definition of a clinically compatible case of arboviral disease can be found in Table 1, with laboratory criteria for diagnosis being defined in Table 2.

Table 1: Clinical Case Definition

<table>
<thead>
<tr>
<th>Clinical Description</th>
<th>Clinical Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>NEUROINVASIVE Disease</td>
<td>Meningitis (NOTE: enterovirus should also be considered for individuals ≤ 18 years of age), encephalitis, acute flaccid paralysis; atypical Guillain-Barré Syndrome; transverse myelitis, or other acute signs of central or peripheral neurologic dysfunction, as documented by a physician, AND Absence of a more likely clinical explanation. Other clinically compatible symptoms of neuroinvasive arboviral disease include headache, myalgia, rash, arthralgia, vertigo, vomiting, and/ or nuchal rigidity (neck stiffness).</td>
</tr>
<tr>
<td>NON-NEUROINVASIVE Disease</td>
<td>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. Other clinically compatible symptoms of non-neuroinvasive arboviral disease include: headache, myalgia, rash, arthralgia, vertigo, vomiting, paresis and/ or nuchal rigidity (stiff neck).</td>
</tr>
</tbody>
</table>

Table 2: Laboratory Criteria for Diagnosis

<table>
<thead>
<tr>
<th>Case Classification</th>
<th>Clinical Description</th>
<th>Laboratory Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PROBABLE</strong></td>
<td><strong>Neuroinvasive Disease:</strong> A case that meets the above clinical criteria for neuroinvasive disease and the following laboratory criteria:</td>
<td>• 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.</td>
</tr>
<tr>
<td></td>
<td><strong>Non-neuroinvasive Disease:</strong> A case that meets the above clinical criteria for non-neuroinvasive disease and the following laboratory criteria:</td>
<td>• Virus-specific IgM antibodies in serum but with no other testing.</td>
</tr>
<tr>
<td><strong>CONFIRMED</strong></td>
<td><strong>Neuroinvasive Disease:</strong> A case that meets the above clinical criteria for neuroinvasive disease and <strong>ONE OR MORE</strong> of the following laboratory criteria:</td>
<td>• Detection of specific nucleic acid or isolation of virus from serum, blood, CSF, other body fluids, OR • Four-fold or greater change in virus-specific antibody titers in paired sera, OR • Virus-specific 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.</td>
</tr>
<tr>
<td></td>
<td><strong>Non-Neuroinvasive Disease:</strong> A case that meets the above clinical criteria for non-neuroinvasive disease and <strong>ONE OR MORE</strong> of the following laboratory criteria:</td>
<td>• Detection of specific nucleic acid or isolation of virus from serum, blood, other body fluids, OR • Four-fold or greater change in virus-specific antibody titers in paired sera, OR • Virus-specific IgM antibodies in serum with confirmatory virus-specific neutralizing antibodies in the same or a later specimen.</td>
</tr>
</tbody>
</table>

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4 [CSTE Case Definition for Arboviral Diseases](https://wwwn.cdc.gov/nndss/conditions/arboviral-diseases-neuroinvasive-and-non-neuroinvasive/case-definition/2015/)

5 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.6

**Recommended Specimens for Collection**

**NOTE:** CSF or whole blood specimens should be submitted with an accompanying serum sample

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

Laboratory diagnosis of human WNV and SLEV infections is a multi-step process. A laboratory cannot use serology or molecular tests alone to distinguish WNV from SLEV because of the serological cross-reactivity between these flaviviruses and the low sensitivity for detecting viral RNA in serum and CSF samples. Currently, VRDL is evaluating the sensitivity of a WNV and SLEV real-time PCR assay using whole blood samples based upon published data indicating that it may be more sensitive than serum or CSF.7 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 diagnosis. Diagnostic Test have a turnaround time for results of 14 calendar days. Surveillance Only test have a turnaround time of up to 21 calendar days.

**Molecular Testing**

Molecular tests, such as real-time PCR, are not recommended as the primary test for laboratory diagnosis of symptomatic arboviral infections due to the low sensitivity for WNV and SLEV in serum and CSF. This is likely because most individuals present to healthcare providers when 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 also detect viral nucleic acids for longer post 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 these reasons, VRDL is currently assessing a real-time PCR test for the detection of WNV/SLEV RNA in whole blood, and recommends submission of whole blood.

**Serologic (IgM/IgG) Screening Tests**

**Immunofluorescence Assay (IFA) or Enzyme Immunoassay (EIA) serology testing:** Either an IFA or EIA test may be used for frontline screening of IgM antibodies for WNV and SLEV 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

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

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 are discouraged.**

IgG serology can be used as an adjunct test for WNV/SLEV when paired acute and convalescent sera are submitted for testing. **Paired sera** showing a significant rise (≥4x) in IgG antibody titer may indicate a recent infection, whereas a **single IgG** titer result cannot differentiate between old and new infections.

**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. PRNT results may be confounded by vaccination/previous exposure to 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 antibody's ability to neutralize virus in culture.

<table>
<thead>
<tr>
<th>Virus</th>
<th>Specimen</th>
<th>Real-time PCR</th>
<th>Serology</th>
<th>PRNT</th>
</tr>
</thead>
<tbody>
<tr>
<td>WNV</td>
<td>Serum</td>
<td>Not an acceptable sample type</td>
<td>Diagnostic</td>
<td>Surveillance</td>
</tr>
<tr>
<td></td>
<td>CSF</td>
<td>Diagnostic</td>
<td>Diagnostic</td>
<td>Surveillance</td>
</tr>
<tr>
<td></td>
<td>Whole blood</td>
<td>Surveillance</td>
<td>Not an acceptable sample type</td>
<td>Not an acceptable sample type</td>
</tr>
<tr>
<td>SLEV</td>
<td>Serum</td>
<td>Not an acceptable sample type</td>
<td>Surveillance</td>
<td>Surveillance</td>
</tr>
<tr>
<td></td>
<td>CSF</td>
<td>Surveillance</td>
<td>Surveillance</td>
<td>Surveillance</td>
</tr>
<tr>
<td></td>
<td>Whole blood</td>
<td>Surveillance</td>
<td>Not an acceptable sample type</td>
<td>Not an acceptable sample type</td>
</tr>
</tbody>
</table>

**Factors to Consider when Interpreting Arboviral Laboratory Serology Results**

- **Arboviral serologic assays:** Assays for the detection of IgM and IgG 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 to 90 days, but
longer duration has also been documented (e.g., ≥3 years for WNV\textsuperscript{8}). 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\textsuperscript{9}.

- **Persistence of IgM antibodies:** Arboviral IgM antibodies may be detected in some patients 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 IgG and other neutralizing antibodies:** Arboviral IgG and other 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 IgG, but not IgM, should be evaluated for other etiologic agents.

**Reporting 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 fax. Local public health laboratories or health departments receiving results from VRDL are expected to forward test results to the submitter and share 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 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 exotic 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.

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\textsuperscript{9} CDC WNV Diagnostic Testing Information for Healthcare Providers:
https://www.cdc.gov/westnile/healthcareproviders/healthCareProviders-Diagnostic.html
Reporting Probable or Confirmed Cases of WNV/SLEV to CDPH

Acute 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 test results to the local health department where the patient resides. Please also report probable and confirmed cases of SLEV in the same manner. To determine whether an individual should be reported to CDPH as a case, local health departments should refer to the case definition for WNV/SLEV\(^{10}\), and reach out to Robert Snyder (916-445-2491; Robert.snyder@cdph.ca.gov), the WNV subject matter expert at CDPH, 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.

Local health departments must report cases of WNV/SLEV illness and **WNV-positive blood donors [Presumptive Viremic Donors (PVD)]** via CalREDIE or by FAX to 510-412-6263. **NOTE:** Case report forms may also be mailed to Vector-Borne Disease Section, however this is discouraged as paper-based reporting can result in significant reporting delays.

**ATTN: WNV Human Forms, California Department of Public Health**

850 Marina Bay Parkway, Bldg G1-004
Richmond, CA 94804


**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, nor will they initially 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’)
  - Clinical syndrome must be neuroinvasive (can also include others as secondary and tertiary syndromes)

\(^{10}\) [CSTE Case Definition for Arboviral Diseases](https://wwwn.cdc.gov/nndss/conditions/arboviral-diseases-neuroinvasive-and-non-neuroinvasive/case-definition/2015/)
• 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 as well as Blood Donor Identification Number (e.g. W#########)

See Appendix B: WNV CalREDIE Reporting Flowchart

Saint Louis Encephalitis Virus (SLEV) Case Reporting
Report the case as “Unusual/Other Disease”
  ▪ If there are any associated WNV incidents, close these incidents as ‘Not A Case’ and indicate that it is an SLEV case, also indicating the Unusual/Other Disease CalREDIE incident number in the ‘Case Investigation’ tab under ‘Notes/Remarks’
  ▪ Either the WNV incident or 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 C: SLEV CalREDIE Reporting Flowchart

CDPH Case Counts
  ▪ Case counts are updated every Friday during the WNV season on the California WNV website (http://westnile.ca.gov). SLEV cases are reported on the SLEV tab of the California WNV website. All WNV and SLEV cases reported to CDPH by 9:00 AM Thursday will be included in the subsequent Friday’s update.
  ▪ 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 an SLEV case indicated as “Unusual/other disease”
    ▪ 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 and 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, Robert Snyder at (916) 445-2491.

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 of (at a minimum) cross street where the suspect mosquito exposure occurred. Please contact Robert Snyder with any questions or concerns related to communication of cases with local vector control agencies. Ideally this contact will occur prior to confirmation 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 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/SLEV Presumptive Viremic Donors Report Form

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

Report of WNV/SLEV-Positive Blood Donor to the CDPH

1. Blood Collection Facility:
   a. Name:__________________________
   b. Address: ___________________________ Zip Code: __________
   c. Telephone number: (_____) ________________________
   d. Contact person: ______________________________

2. Blood Unit Identification Number: _W __________________________

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 name: ________________________________________________
    Address: ________________________________________________________________
    Phone: (_____)_________

13. Comments:______________________________________________________________
    ________________________________________________________________________
Appendix B: WNV 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

Do test results meet laboratory criteria for diagnosis?

Yes

Set Resolution Status to Probable or Confirmed

No

Set Resolution Status to Not a Case

Ready to report?

Yes

Set Process Status to Closed by LHD

No

Set Resolution Status to Confirmed

Set Resolution Status to Not a Case

Yes

Ready to report?

Yes

Set Process Status to Closed by LHD

No

Asymptomatic infections are included in reports on www.westnile.ca.gov and reported to CDC ArboNET. Note that asymptomatic cases are not formally considered ‘cases’ but are reportable.
Appendix C: SLEV CalREDIE Reporting Flowchart

1. **Do laboratory results indicate SLEV?**
   - Yes: Set the disease condition to: Unusual Disease / Other Condition
   - No: Follow WNV reporting algorithm as needed (Appendix B)
2. Close associated WNV incidents and note unusual disease / other condition incident number in additional remarks
3. Set Resolution Status to Probable or Confirmed
   - Yes: Ready to report?
   - No: Set Process Status to Closed by LHD
4. Case is included in case counts and reports on www.westnile.ca.gov and reported to CDC ArboNET