**Zoonotic Mosquito-Borne Viruses**  
(***Neuroinvasive and Non-Neuroinvasive*)

California serogroup virus  
Eastern equine encephalitis virus  
St. Louis encephalitis virus  
Venezuelan equine encephalitis virus  
West Nile virus  
Western equine encephalitis virus  
Arboviral disease, other

**PROTOCOL CHECKLIST**

- Enter available information into Merlin upon receipt of initial report
- Review background information on the diseases (see Section 2), case definition (see Section 3), and laboratory testing (see Section 4)
- Forward specimens to the Department of Health (DOH) Bureau of Public Health Laboratories (BPHL) for confirmatory laboratory testing (as needed)
- Inform local mosquito control personnel of suspected arbovirus case as soon as possible (if applicable)
- Inform state Arbovirus Surveillance Coordinator on suspicion of locally acquired arbovirus infection or for any other questions
- Contact provider or blood bank (see Section 5A)
- Interview case-patient
  - Review disease facts (see Section 2)
  - Mode of transmission
  - Ask about relevant risk factors (see Section 5. Case Investigation)
    - Travel and outdoor activity history two weeks prior to onset
    - History of mosquito bites
    - History of febrile illness or travel for household members or other close contacts in the month prior to onset
    - History of previous arbovirus infection or vaccination (yellow fever, Japanese encephalitis)
- Provide education on transmission and prevention (see Section 6)
  - Awareness of mosquito-borne diseases
  - Drain standing water at least weekly to stop mosquitoes from multiplying
  - Discard items that collect water and are not being used
  - Cover skin with clothing or Environmental Protection Agency (EPA)-registered repellent such as DEET (N,N-diethyl-meta-toluamide)
  - Use permethrin on clothing according to manufacturer’s directions
  - Cover doors and windows with intact screens to keep mosquitoes out of the house
- Enter additional data obtained from interview into Merlin (see Section 5E)
- Arrange for a convalescent specimen to be taken (if necessary), including one for asymptomatic blood donors
1. DISEASE REPORTING

A. Purpose of reporting and surveillance
   1. Detect and monitor arboviral disease activity rapidly
   2. Keep the public and other stakeholders informed of activity and increased risk
   3. Work with partners to respond rapidly to arbovirus outbreaks
   4. Use surveillance data to monitor success of response
   5. Characterize risk factors for infection to develop targeted preventive messaging

B. Legal reporting requirements
   Laboratories, physicians, and blood banks are required to report suspected cases to the local county health department (CHD) within one working day of identification and diagnosis (Chapter 64D-3, Florida Administrative Code). Any suspected Venezuelan equine encephalitis cases are required to be reported immediately 24/7 by phone. Reporting should not be delayed for convalescent specimen results.

C. CHD investigation responsibilities
   1. Begin investigation on the same day as notification.
   2. Contact commercial laboratories as soon as possible after a case is reported and request that specimens (serum, and if available, cerebral spinal fluid [CSF]) be forwarded to BPHL-Tampa or Jacksonville for confirmatory testing. Both acute and convalescent specimens may be required to confirm the case.
   3. Inform mosquito control personnel of a suspected arbovirus case as soon as possible (if applicable).
   4. Establish patient travel history in the two weeks prior to symptom onset or date of blood donation (asymptomatic blood donors).
   5. Inform state Arbovirus Surveillance Coordinator of suspicion of locally acquired arbovirus infection.

Note: Information on several arboviral diseases is included in this guidance. Determination of neuroinvasive disease is based on clinical symptoms as described in the case definition. All cases of California serogroup virus illness (i.e. La Crosse, California encephalitis, Jamestown Canyon, Keystone, Snowshoe hare, and Trivittatus viruses) should be reported in Merlin as neuroinvasive (Merlin disease code=06250) or non-neuroinvasive (Merlin disease code=06251). All cases of Eastern equine encephalitis should be reported as neuroinvasive (Merlin disease code=06220) or non-neuroinvasive (Merlin disease code=06221). All cases of St. Louis encephalitis should be reported as neuroinvasive (Merlin disease code=06230) or non-neuroinvasive (Merlin disease code=06231). All cases of Venezuelan equine encephalitis should be reported as neuroinvasive (Merlin disease code=06620) or non-neuroinvasive (Merlin disease code=06621).
All cases of West Nile should be reported as neuroinvasive (Merlin disease code=06630) or non-neuroinvasive (Merlin disease code=06631). All cases of Western equine encephalitis should be reported as neuroinvasive (Merlin disease code=06210) or non-neuroinvasive (Merlin disease code=06211). Other arbovirus cases should be reported as arboviral disease, other (Merlin disease code=06000). Dengue, chikungunya, and yellow fever each have their own Florida reporting codes and are not included in this guidance. Guidance for dengue, chikungunya, yellow fever, and Zika are located in their respective Guide to Surveillance and Investigation chapters as well as on the surveillance and investigation guidance website: www.floridahealth.gov/gsi.

2. THE DISEASES AND THEIR EPIDEMIOLOGY

A. Etiologic agent
More than 130 arboviruses are known to cause human disease. Most arboviruses of public health importance belong to one of three virus genera: Flavivirus, Alphavirus, and Orthobunyavirus. Mosquito-borne arboviruses that are endemic to Florida include St. Louis encephalitis virus (SLEV), West Nile virus (WNV), and Eastern equine encephalitis virus (EEEV). Both SLEV and WNV are flaviviruses while EEEV is an alphavirus. See section 2I for more information on arboviruses in Florida. Additional arboviruses that are endemic in the United States include other alphaviruses: Western equine encephalitis virus (WEEV) and Everglades virus (EVEV); and orthobunyaviruses: California serogroup viruses (La Crosse [LACV], California encephalitis, Jamestown Canyon [Florida], Keystone [Florida], Snowshoe hare, and Trivittatus viruses [Florida] and Tensaw [Florida]). Exotic arboviral viruses to be aware of include: Venezuelan equine encephalitis (VEE), Zika (ZIKV), Ross River, Japanese encephalitis (JEV), Cache Valley, Murray Valley encephalitis, and Rift Valley fever (RVF) viruses. This guide will focus on the viruses that are endemic to Florida. However, both imported and possible locally acquired cases of any of the arboviruses are reportable diseases in Florida.

B. Description of illness
The clinical spectrum of these infections includes asymptomatic infection, mild febrile illness, aseptic meningitis, and encephalitis that can progress to coma and death. Other clinically compatible symptoms of arbovirus disease may include headache, myalgia, rash, arthralgia, vertigo, nausea, vomiting, paresis, altered mental status, seizures, limb weakness, nuchal rigidity, hemorrhagic disease, and ocular involvement. For the purposes of surveillance and reporting, based on their clinical presentation, arboviral disease cases are often categorized into two primary groups: neuroinvasive disease and non-neuroinvasive disease. Past outbreaks in Florida of WNV and SLEV infection have indicated that individuals over age 60 are at increased risk for severe disease. While EEEV transmission is sporadic, it can result in high rates of mortality, and those individuals under 16 and over 50 years of age have been shown to be at increased risk for severe disease. LACV, an arbovirus not present in Florida but identified regularly as imported cases, can also result in neuroinvasive disease, primarily in children under 16.

C. Reservoirs
Zoonotic arboviruses are maintained in complex life cycles involving a non-human primary vertebrate host and a primary mosquito vector. These viruses usually cycle undetected until humans encroach on a natural focus or the virus escapes this focus via a secondary vector or vertebrate host as the result of some ecologic change. For some arboviruses (WNV,
EEEV, SLEV), humans and domestic mammals can develop clinical illness, but usually are dead-end hosts because they do not produce significant viremia, and are not a source of virus for biting mosquitoes. For example, horses have a high mortality rate for EEEV but do not contribute to the disease life cycle. In addition, other animal species such as emus/ostriches (EEEV) and alligators (WNV) are at risk of exposure and can develop a high enough viremia to infect mosquitoes as well as support non-mosquito-borne transmission. Unlike dengue and chikungunya, infected people pose minimal risk for introduction of these viruses into Florida. Many arboviruses that cause encephalitis have a variety of different vertebrate hosts; however, avian hosts are most commonly associated with the arboviruses that circulate in Florida. For LACV, small mammals such as chipmunks or squirrels serve as vertebrate hosts.

D. **Modes of transmission**
Transmission is through the bite of an infected mosquito. While there are many different mosquito species present in Florida, not all of them can transmit these arboviruses and the mosquito species involved in transmission vary between viruses. The main mosquito species that transmit WNV and SLEV belong to the *Culex* genus. Mosquito species from several genera may be involved with EEEV transmission, with transmission between birds and mosquitoes facilitated by *Culiseta melanura* and species from additional genera involved in the transmission to dead-end hosts. Many of these species prefer to feed at dawn and dusk. Other modes of transmission for some arboviruses include blood transfusion, organ transplantation, perinatal transmission, breastfeeding, and laboratory exposures. Since 2003, all blood donations are screened for the presence of WNV prior to transfusion. According to the Centers for Disease Control and Prevention (CDC), scientists have found no evidence that a mother’s West Nile Virus infection harms her breastfeeding infant. Therefore, CDC recommends that women with West Nile Virus illness continue breastfeeding because the benefits of breast milk are thought to outweigh the theoretical risk of harm to the infant. In the few documented cases of West Nile Virus transmission through breastfeeding (one case in the year 2002; three cases in the year 2003), none resulted in recognizable illness in the infant.

Emus and ostriches are highly vulnerable to EEEV infection and exposure to body fluids (particularly feces, saliva, and blood) from infected birds can expose additional birds, other animals, and people. Alligators may have the potential to transmit WNV through feces and infected tissues. Proper personal protective equipment (PPE) such as disposable or washable outerwear, shoe covers, gloves (cut resistant if working with sharp tools), full face shield, and an N95 fit-tested respirator or surgical mask should be worn when working with emus or ostriches that might be infected with EEEV or alligators with potential WNV infection. Many zoonotic viruses may also be present in the central nervous system of infected dead-end hosts such as WNV or EEEV infected horses. Cut-resistant gloves and PPE to prevent splashes to eyes, mucous membranes, and open wounds are recommended if collecting or handling central nervous system tissues or fluids.

E. **Incubation period**
The incubation period for these arboviruses varies depending on which virus is involved, but the onset of symptoms tends to be within two weeks from the time of the mosquito bite and ranges between 2–21 days. The specific incubation periods for endemic arboviruses in Florida is 2–14 days for WNV illness, 5–15 days for SLEV, and 3–10 days for EEEV. The incubation period for LACV is 5–15 days.
F. Period of communicability
Humans are dead-end hosts because they do not produce significant viremia to infect mosquitoes and do not contribute to the transmission cycle for most zoonotic arboviruses, including those endemic to Florida.

G. Treatment
Because the zoonotic arboviruses are viral diseases, antibiotics are not helpful for treatment, and the effectiveness of antiviral agents for arboviruses in the U.S. has not been shown. Treatment is supportive, attempting to deal with problems such as swelling of the brain, respiratory paralysis, and other treatable complications.

H. Prophylaxis
There are currently no commercially available human vaccines for these diseases, though several types of WNV vaccine are in development. However, actions to reduce risk for mosquito bites described in Section 6 are recommended. A vaccine against EEEV is licensed for horses and has been used off-label for ratites (ostriches and emus) and camelids (alpacas and llamas). Equine vaccines protecting against WNV have been on the market since 2001. Vaccination against WNV for camelids is an off-label use as well.

There is a vaccine available for Japanese encephalitis virus for individuals traveling to endemic areas.

I. Arboviral diseases in Florida
Most cases of zoonotic arboviral disease occur June through September, when mosquitoes are most active. Prior to 2001, SLEV was the predominant arbovirus circulating in Florida, and many outbreaks with high numbers of cases have been documented in the past. Since the introduction of WNV to Florida in 2001, the number of human cases of SLE have decreased dramatically. Two human cases of SLE were reported in 2014, representing the first human cases identified since 2003. WNV is now the predominant arbovirus in Florida, with over 300 cases reported between 2001–2017. EEEV infections are sporadic, and there have not been more than five cases within any given year. These three viruses are considered endemic in Florida. In Florida, environmental surveillance for these viruses includes serologic testing of sentinel chickens and other animals, identification of veterinary cases, monitoring of dead bird reports, and testing of mosquito vectors. Additional information on environmental surveillance is included in Section 6B.

Other zoonotic arboviral diseases of minor public health significance in Florida are caused by Everglades, La Crosse, Keystone, and Jamestown Canyon viruses. Both Keystone and Jamestown Canyon viruses have had one case identified as Florida-acquired. While serologic evidence of Everglades virus infection has been documented in south Florida, only three clinical cases have ever been identified. Cases of La Crosse encephalitis are often imported from the Appalachian and Midwestern regions of the United States, and the virus is not believed to be present in Florida. To date, no human cases of WEE have been reported as acquired in Florida. Additional information on these viruses can be found in Chapter 2 of the guide. Exotic arboviruses may be imported into Florida via infected mosquito or animal reservoirs; however, the potential for ongoing transmission of these viruses exists only when competent vectors and reservoirs are present.
3. CASE DEFINITION

Zoonotic Arboviral Diseases (Neuroinvasive and Non-Neuroinvasive)

A. Clinical description

Most arboviral infections are asymptomatic. Clinical disease ranges from mild febrile illness to severe encephalitis. Other clinically compatible symptoms of arbovirus disease may include headache, myalgia, rash, arthralgia, vertigo, vomiting, paresis, altered mental status, seizures, limb weakness, or nuchal rigidity. For the purposes of surveillance and reporting, based on their clinical presentation, arboviral disease cases are often categorized into two primary groups: neuroinvasive disease and non-neuroinvasive disease.

Neuroinvasive disease: Many arboviruses cause neuroinvasive disease such as aseptic meningitis, encephalitis, or acute flaccid paralysis (AFP). These illnesses are usually characterized by the acute onset of fever with headache, myalgia, stiff neck, or CSF pleocytosis (increase in white blood cell count). AFP may 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, also occur.

Non-neuroinvasive disease: Most arboviruses can cause an acute systemic febrile illness (e.g., West Nile fever) that may include headache, myalgia, arthralgia, rash, or gastrointestinal symptoms. Some viruses also can cause more characteristic clinical manifestations, such as severe polyarthralgia or arthritis due to Ross River virus.

B. Laboratory criteria for diagnosis

Confirmatory:

Neuroinvasive disease

- Isolation of virus from, or demonstration of specific viral antigen or nucleic acid in tissue, blood, CSF, or other bodily fluid (e.g., culture, immunohistochemistry [IHC], polymerase chain reaction [PCR])*

OR

- Four-fold or greater change in virus-specific quantitative antibody titers in paired sera (e.g., enzyme-linked immunosorbent assay [EIA/ELISA], microsphere immunoassay [MIA], or immunofluorescence assay [IFA])

OR

- Both of the following:
  - Virus-specific IgM antibodies in serum or CSF (e.g., EIA/ELISA, MIA, or IFA)
  - And confirmatory virus-specific neutralizing antibodies in the same or a later specimen (e.g., EIA/ELISA with serum neutralization [SN] or plaque reduction neutralization [PRNT])

OR

- Both of the following:
  - Virus-specific IgM antibodies in CSF (e.g., EIA/ELISA, MIA, or IFA)
  - And negative, equivocal, or indeterminate result for other IgM antibodies in CSF for arboviruses endemic to the region where exposure occurred
Non-neuroinvasive disease

- Isolation of virus from, or demonstration of specific viral antigen or nucleic acid in tissue, blood, or other bodily fluid, excluding CSF

OR

- Four-fold or greater change in virus-specific quantitative antibody titers in paired sera

OR

- Both of the following:
  - Virus-specific IgM antibodies in serum
  - And confirmatory virus-specific neutralizing antibodies in the same or a later specimen

*Excluding West Nile virus (WNV) nucleic acid test (NAT) from blood bank screening.

Presumptive:

Neuroinvasive and non-neuroinvasive disease

- Virus-specific IgM antibodies (e.g., EIA/ELISA, MIA, or IFA) in serum with no other testing for arboviruses endemic to the region where exposure occurred

Neuroinvasive disease only

- Virus-specific IgM antibodies (e.g., EIA/ELISA, MIA, or IFA) in CSF with no other testing for arboviruses endemic to the region where exposure occurred

Supportive:

Neuroinvasive and non-neuroinvasive disease

One or more of the following:

- Both of the following:
  - Positive WNV nucleic acid test (NAT) from blood bank screening
  - And isolation of virus from, or demonstration of specific viral antigen or nucleic acid in, tissue, blood, or other body fluid (e.g., culture, IHC, PCR) by a state public health laboratory (PHL) or the Centers for Disease Control and Prevention (CDC);

OR

- Both of the following:
  - Positive WNV NAT from blood bank screening
  - And Virus-specific IgM antibodies in serum or CSF (e.g., EIA, MIA, IFA);

OR

- All of the following:
  - Positive WNV NAT from blood bank screening,
  - And absence of a negative PCR from a PHL or the CDC,
  - And absence of a negative result for WNV IgM antibodies (e.g., EIA, MIA, IFA).

C. Case classification

Confirmed:

Neuroinvasive disease

Illness clinically compatible with neuroinvasive disease in a person with confirmatory laboratory evidence
**Non-neuroinvasive disease**
Illness clinically compatible with non-neuroinvasive disease in a person with confirmatory laboratory evidence

**Probable:**

**Neuroinvasive disease**
Illness clinically compatible with neuroinvasive disease in a person with presumptive laboratory evidence

**Non-neuroinvasive disease**
An illness clinically compatible with non-neuroinvasive disease in a person with presumptive laboratory evidence.

**Suspect:** A person with confirmatory, presumptive, or supportive laboratory evidence.

**Comments**

Note that in Florida, West Nile virus (WNV) and St. Louis encephalitis virus (SLEV) are endemic, and testing should be performed for both viruses. Dengue EIA/ELISA or PCR are recommended in non-neuroinvasive disease cases to rule out local dengue introductions. Chikungunya testing may also be recommended for some non-neuroinvasive disease cases.

**Interpreting arboviral laboratory results**

- **Serologic cross-reactivity:** In some instances, arboviruses from the same genus produce cross-reactive antibodies. In geographic areas where two or more closely related arboviruses occur, serologic testing for more than one virus may be needed and results compared to determine the specific causative virus. For example, such testing might be needed to distinguish antibodies resulting from infections (or vaccinations) within genera (e.g., flaviviruses) such as West Nile, St. Louis encephalitis, Powassan, dengue, yellow fever, or Japanese encephalitis viruses.

- **Rise and fall of IgM antibodies:** For most arboviral infections, IgM antibodies are generally first detectable at three to eight days after onset of illness and persist for 30 to 90 days, but longer persistence has been documented (e.g., up to 500 days for West Nile virus). Serum collected within 8 days of illness onset may not have detectable IgM, and testing should be repeated on a convalescent-phase sample to rule out arboviral infection in those with a compatible clinical syndrome.

- **Persistence of IgM antibodies.** Arboviral IgM antibodies may be detected in some patients months or years after their acute infection, particularly WNV. Therefore, the presence of these virus-specific IgM antibodies may signify a past infection and may be unrelated to the current acute illness. Finding virus-specific IgM antibodies in CSF or a four-fold or greater change in virus-specific antibody neutralizing 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 also should be carefully considered.

- **Persistence of IgG and neutralizing antibodies.** Arboviral IgG and 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 illnesses with the presence of IgG, but not IgM,
Zoonotic Mosquito-Borne Viruses

should be evaluated for other etiologic agents except for some dengue infections. In addition, a virus neutralization test (SN or PRNT) is required to differentiate virus-specific IgG within the flavivirus genus, although commercial laboratories often incorrectly report IgG results for a specific flavivirus. For instance, EIA results reported as positive for WNV IgG antibody should be reported as positive for flavivirus antibody IgG.

- **Other information to consider.** When interpreting results, 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.

- **Differentiating between dengue and West Nile infections in patients with positive flavivirus labs.**
  - WNV IgM titers are negative or low positive in dengue fever patients (or vice versa); however, the WNV IgG can be quite elevated in dengue patients since IgG strongly cross-reacts between flaviviruses.
  - Neuroinvasive disease is relatively uncommon with dengue infections and more likely to be WNV infection. Confusion differentiating WNV and dengue infections is most likely in patients without symptoms of neuroinvasive disease (fever patients).
  - Travel to a dengue-endemic country in the two weeks prior to febrile illness onset or travel of a household member to a dengue-endemic country in the four weeks prior to patient illness should increase suspicion of dengue.
  - Joint pain is often much more severe in cases of dengue fever compared to WNV fever.
  - Thrombocytopenia and leukopenia are more common in cases of dengue fever compared to WNV fever.

**Imported arboviral diseases**

Human disease cases due to dengue, chikungunya, or yellow fever viruses are nationally notifiable to the CDC using specific case definitions. However, many other exotic arboviruses (e.g., Zika, Japanese encephalitis, tick-borne encephalitis, Venezuelan equine encephalitis, and Rift Valley fever viruses) are important public health risks for the U.S. as competent vectors exist that could allow for sustained transmission upon establishment 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 reported to the appropriate local and state health agencies and CDC. Arboviral encephalitis cannot be distinguished clinically from other central nervous system (CNS) infections.

**Reporting:** Next business day; laboratories and physicians are required to report cases to the local county health department (CHD) within one working day of identification and diagnosis. Any suspected cases of Venezuelan equine encephalitis are required to be reported immediately 24/7 by phone. Reports should not be delayed for convalescent specimens as final laboratory confirmation.

**Acute and convalescent sera from reported cases must be forwarded to the Bureau of Public Health Laboratories for confirmatory testing.**
4. LABORATORY TESTING

A. Criteria for diagnosis
Confirming the diagnosis of arboviral disease can be made using a variety of testing methods. BPHL provides confirmatory laboratory testing services for patients with clinical signs of arboviral disease. BPHL supports testing for all specimens forwarded for confirmation that meet epidemiological criteria, for suspected local cases, and for individuals without health insurance.

1. Confirm all positive private laboratory test results for these diseases at BPHL.
2. Many of these arboviruses cross-react, particularly the flaviviruses.
3. Testing for arboviruses endemic to the region where exposure occurred is required.
4. Health care providers should submit acute serum specimens from imported and locally acquired cases to either the BPHL-Tampa or -Jacksonville for endemic arbovirus cases.
5. Submit acute serum without waiting for the convalescent specimen.
6. If the individual has neuroinvasive symptoms, send the CSF as well.
7. Routinely send the convalescent specimen (drawn two weeks later) to confirm negative and positive results.
8. Send initial serum specimens from asymptomatic blood donors to BPHL for confirmation and collect a second specimen drawn two weeks after donation date.

B. Services available at BPHL
BPHL can test clinical specimens for any of the endemic arboviruses (WNV/SLEV/EEEV) by viral isolation and detection, antibody detection by serologic assays such as the plaque reduction neutralization test (PRNT), or enzyme-linked immunosorbent assay (ELISA). Only the Tampa laboratory has the capacity to perform the PRNT test. Please contact the Arbovirus Surveillance Coordinator if requesting testing for other non-endemic arboviruses (excluding dengue, chikungunya, and Zika viruses) and provide relevant travel and symptom information. While testing at BPHL for zoonotic arboviruses not commonly found in Florida is limited, additional testing at CDC may be requested as appropriate.

C. Testing requests
1. Submitting specimens and isolates to BPHL
   a. BPHL staff should be notified of specimen submission, and all submissions should be accompanied by a Clinical Laboratory Submission Form DH1847 found at: www.floridahealth.gov/diseases-and-conditions/disease-reporting-and-management/disease-reporting-and-surveillance/surveillance-and-investigation-guidance/_documents/dh1847clinicalabsubmissionform.pdf.
   b. Decide which tests you are going to request and fill in the Clinical Lab Submission Form DH1847. Select option:
      • 1510 for arbovirus antibody
      • 1670 for arbovirus culture
      • 1500 for arbovirus IgM
      • 1680 for arbovirus PCR
      Fill out additional mandatory information in the box below the virology section as indicated on the form.
   c. Include clinical history, onset and specimen collection dates, and travel history.
2. Packaging and shipping
   a. Specimens can be sent to the assigned BPHL (Jacksonville or Tampa) for testing.
   b. Specimen type and labeling
i. If the specimen is acute (collected five or fewer days post onset), the serum, CSF, or tissue should be shipped frozen on dry ice in an insulated cooler. Hold specimen in an insulated container with dry ice or an ultra-low freezer until shipped. This is best for virus isolation, but viral RNA may still be detectable in freshly collected acute serum that is immediately sent overnight to the laboratory with frozen gel ice in an insulated cooler.

ii. If the specimen is convalescent (collected six or more days post onset), the serum or CSF may be shipped frozen on dry ice or cold with frozen gel ice in an insulated cooler because the serum or CSF will be tested for antibody only. Hold serum or CSF in a refrigerator until shipped.

iii. At least 2 ml of serum or CSF are requested for testing.

iv. Fluids are stored in standard sterile airtight tubes or a serum separator tube (separated prior to refrigeration and shipping) and tissue in an airtight sterile container without added media or fixative.

v. Each specimen must be labeled with the patient’s name, date of birth, and date of collection.

vi. Unseparated whole blood is an unsatisfactory specimen and should not be shipped to the laboratory.

c. A DOH Clinical Laboratory Submission Form must be included for each patient, listing all specimens. Follow packaging and shipping guidelines for diagnostic specimens (Biological Substance, Category B, UN3373). All suspect diagnostic specimens must be shipped and packaged according to International Air Transport Association (IATA) and Department of Transportation (DOT) Packaging Instructions 650 for biological substance, category B agents. Per these regulations, anyone who handles, offers for transport, or transports specimens must be trained and certified to do so. Specifications state specimens must be packed in a basic triple packaging system consisting of a primary watertight container wrapped with absorbent material, secondary watertight container, and an outer shipping package. Enclose an itemized list of contents between the secondary packaging and the outer packaging.

d. Contact BPHL for packaging and shipping training dates. BPHL conducts approximately 20 face-to-face trainings per year statewide, free of charge. DOH employees must register for the classes in the DOH online training system TRAIN-FL. For shipping guidance, contact BPHL. Additional shipping trainings are also available commercially through vendors.

e. To expedite receipt of specimens at the laboratory, overnight or two-day express shipment is suggested. If sera are shipped on Friday, the package must be clearly marked for “Saturday Morning Delivery.”


D. Interpretation of results
For any questions about lab results from BPHL or other labs, consult the Arbovirus Surveillance Coordinator or BPHL-Tampa or -Jacksonville. Interpretation of each of the tests is dependent upon the time of specimen collection relative to the date of symptom onset, the patient's previous arbovirus infection history, and serum cross-reactivity within the antigenic complex. In Florida, previous WNV, DENV, or ZIKV infection or previous yellow fever or Japanese encephalitis vaccination are the most common factors that can complicate the interpretation of flavivirus antibody tests. In addition, current infections with herpes simplex
virus (HSV), Epstein-Barr virus (EBV), *Streptococcus*, influenza or other pathogens may also complicate the interpretation of antibody tests.

5. **CASE INVESTIGATION**

A. **Contact the physician or hospital**
   1. **Florida Confidential Vector-Borne Disease Case Report form (required):**
      This form can be used to guide the interview and can be completed during the interviews with the health care provider and the patient.
      
   2. Confirm that an arbovirus infection has been diagnosed in the reported case.
   3. Obtain the following from the health care provider or facility:
      a. Date of onset
      b. Signs and symptoms
      c. Travel history for the entire incubation period (2 weeks for many zoonotic arboviruses)
      d. Predisposing conditions (e.g., immunosuppression)
      e. Tests performed (including EIA, PCR, culture or any other test performed)
      f. Treatment for pre-existing conditions (e.g., rheumatic arthritis)
   4. Ask what information has been given to the patient, including whether the patient knows about the diagnosis and risk factors.
   5. Obtain as much demographic information as possible, including contact information (home, cellular, pager, and work numbers). Ask how and where the patient can be contacted (e.g., at hospital or home).
   6. Notify the physician that you will be contacting the case as DOH follows up on all cases of arboviral disease to assess risks factors, to better characterize the occurrence of these infections in Florida, and to identify potential means for preventing further illness. It may also be appropriate at this point to determine if the physician has any concerns about the health department contacting the case.
   7. The CHD designee will arrange acute and convalescent blood sample collection and submission to BPHL, as appropriate, to confirm infection with a vector-borne disease.
   8. If the potential case meets the case definition for a confirmed, probable, or suspect case, the CHD is responsible for reporting all required information in Merlin under the appropriate disease code.
   9. Have acute specimens forwarded to BPHL for testing.

B. **Interview the case**
   1. Contact the case or the case’s proxy to complete an interview as soon as possible after the case is reported to optimize recall.
      a. Make at least three phone call attempts to reach the case.
      b. Calls should be made at different times of the day, with at least one attempt in the evening.
      c. If unable to reach by phone or certified letter, a field visit to the home should be made for suspected locally acquired cases.
   2. **Florida Confidential Vector-Borne Disease Case Report form (required):**
      This form can be used to guide the interview and can be completed during the interview.
3. Items to cover during interview  
   a. Provide brief background on disease, including mode of transmission, incubation period, symptoms, etc.  
   b. Ask for travel and activity history.  
      i. Travel outside county of residence, state, or country  
      ii. Occupation and homeless status  
      iii. Hobbies (e.g., gardening, fresh water fishing, hunting)  
      iv. Other outdoor activities (smoking outside, etc.)  
      v. Use of preventive measures (intact screens, regular use of repellents, drain standing water, etc.)  
   c. Collect history of blood transfusions or organ transplants in the past 6 months and any blood donations in the 2 weeks prior to symptom onset.  
   d. As part of the interview, provide basic education to the cases about personal protection measures to prevent mosquito bites and the “Drain and Cover” message.  

4. Arrange for a convalescent sample to be drawn, if needed.  

C. Inform local mosquito control personnel of suspected case (if applicable)  
   For counties with a mosquito control district, notification should occur for suspect locally acquired cases (both Florida and non-Florida residents potentially exposed in Florida). Imported cases do not need to be reported to mosquito control. It is not required to provide the exact address of the case to mosquito control for these arbovirus infections.  

D. Inform the Arbovirus Surveillance Coordinator on suspicion of locally acquired arbovirus infection  
   CHDs will immediately inform the Arbovirus Surveillance Coordinator of the suspicion of arbovirus disease in their area and coordinate with Division of Disease Control and Health Protection staff and local mosquito control personnel to ensure timely vector surveillance and control. The Arbovirus Surveillance Coordinator can also provide additional information on equine and other animal cases as well as sentinel chicken surveillance results for endemic arboviruses in nearby areas (if possible).  

E. Merlin data entry  
   1. Create a case in Merlin under the appropriate disease code based on laboratory testing and clinical symptoms. Cases should be created for both imported and locally acquired cases. Cases should also be created for non-Florida residents who were exposed or tested in Florida.  
      a. California serogroup virus illness should be reported in Merlin as neuroinvasive (Merlin disease code=06250) or non-neuroinvasive (Merlin disease code=06251).  
      b. Eastern equine encephalitis should be reported as neuroinvasive (Merlin disease code=06220) or non-neuroinvasive (Merlin disease code=06221).  
      c. St. Louis encephalitis should be reported as neuroinvasive (Merlin disease code=06230) or non-neuroinvasive (Merlin disease code=06231).  
      d. Venezuelan equine encephalitis should be reported as neuroinvasive (Merlin disease code=06620) or non-neuroinvasive (Merlin disease code=06621).  
      e. West Nile should be reported as neuroinvasive (Merlin disease code=06630) or non-neuroinvasive (Merlin disease code=06631).  
      f. Western equine encephalitis should be reported as neuroinvasive (Merlin disease code=06210) or non-neuroinvasive (Merlin disease code=06211).  
      g. Other arbovirus cases should be reported as arboviral disease, other (Merlin disease code=06000).
2. Enter the data collected into Merlin, being sure to include all required fields on the Basic Data screen; complete the Case Symptoms, Travel History, and Extended Data screens; and attach all relevant medical records. Please associate ALL positive results from any laboratory and negative results from BPHL received via electronic laboratory reporting (ELR) to the case. For questions regarding lab results, please contact the Arbovirus Surveillance Coordinator.

3. All WNV-positive asymptomatic blood donors should be entered into Merlin under the outbreak module (Outbreak ID 1780). Any WNV transplant recipients should be entered in a separate outbreak module (Outbreak ID 1807).

6. CONTROLLING FURTHER SPREAD

A. Patient/household education on prevention recommendations
1. Awareness of mosquito-borne diseases
2. Drain standing water to stop mosquitoes from multiplying
   a. Drain water from garbage cans, house gutters, buckets, pool covers, coolers, toys, and flower pots, or any other containers where sprinkler or rain water has collected.
   b. Discard old tires, drums, bottles, cans, pots and pans, broken appliances, and other items that are not being used.
   c. Empty and clean birdbaths and pet water bowls at least once or twice a week.
   d. Protect boats and vehicles from rain with tarps that do not accumulate water.
   e. Maintain swimming pools in good condition and appropriately chlorinated. Empty plastic swimming pools when not in use.
3. Cover skin with clothing or repellent
   a. CLOTHING: Wear shoes, socks, and long pants and long sleeves. This type of protection may be necessary for people who must work in areas where mosquitoes are present.
   b. REPELLENT: Apply mosquito repellent to bare skin and clothing.
   c. Always use repellents according to the label. Repellents with DEET, picaridin, oil of lemon eucalyptus, para-methane-diol, and IR3535 are effective. See the repellent frequently asked questions document in the List of Appendices for more information.
   d. Use mosquito netting to protect children younger than 2 months old.
4. Cover doors and windows with intact screens to keep mosquitoes out of the house.
   a. Repair broken screening on windows, doors, porches, and patios.

B. Environmental evaluation
1. Non-Human Arboviral Disease Surveillance in Florida
   Sentinel chickens can be infected with mosquito-borne viruses via the bite of an infected mosquito. Mosquitoes that bite an infected chicken are unlikely to become infected, and chickens are not known to transmit mosquito-borne viruses directly to people. Florida sentinel chicken programs are maintained by mosquito control districts or CHDs, depending on local resources and priorities. Programs entail determining flock placement, flock care, weekly collection, processing, and shipping of blood specimens; and notification of appropriate agencies and persons regarding seroconversion data. At BPHL-Tampa, sera collected from sentinel chicken flocks, wild birds, and other animals are tested for antibody to EEEV, SLEV, and WNV.
   WNV infection causes morbidity and mortality in many bird species in the United States. In some species, especially crows and blue jays (corvids), there has been substantial mortality due to WNV infection. Detection of local bird mortality may indicate the
presence of the virus in a geographic area. Thus, monitoring bird mortality is considered a tool for WNV surveillance. The Florida Fish and Wildlife Conservation Commission (FWC) coordinates bird mortality monitoring efforts in the state. Dead bird sightings are reported on their website at http://legacy.myfwc.com/bird/default.asp.

Cases of equine and other animal arboviral disease are also used to assess the impact of WNV and EEEV in the state. Veterinarians send equine and other veterinary animal sera and brain tissue to the Florida Department of Agriculture and Consumer Services (FDACS) Bronson Animal Disease Diagnostic Laboratory in Kissimmee for evaluation. Please notify the Arbovirus Surveillance Coordinator immediately if notified of an emu or ostrich testing positive for EEEV or suspected to have EEEV infection or any alligators suspected or known to have WNV infection. The Coordinator will provide additional guidance and notify relevant partners (FDACS) as needed.

The accurate measurement of vector abundance and population structure is a critical component of arboviral surveillance. Factors such as vector movement, blood feeding, egg laying, and the age of the population determine whether there is a high or low risk of viral transmission and the potential for human infection. The number of mosquitoes collected is not as important as the day-to-day changes in the number collected. Laboratory testing of pooled mosquitoes is available from BPHL on an as-needed basis.

2. Local mosquito control personnel may conduct an immediate assessment of the area and take measures to reduce exposure of residents to vectors. A Mosquito Control Environmental Assessment Form template can be found at the following link: www.floridahealth.gov/diseases-and-conditions/mosquito-borne-diseases/_documents/mosquito-control-environmental-assessment-form.docx.

3. Information on environmental surveillance is available at https://fmel.ifas.ufl.edu/fmel---gen---info---v3/fmc-white-paper/.

4. To receive automated alerts for animal cases or positive mosquito pools for your county, please contact the Arbovirus Surveillance Coordinator for access to the Florida Environmental Health Surveillance System (FLEHS) at https://flehs.myfloridaeh.com/.

C. Issue a mosquito-borne illness advisory or alert as necessary

The need for mosquito-borne illness advisories and alerts is determined by the CHD Director or Administrator after consultation with local mosquito control experts and DOH Central Office using the below criteria. See Chapter 11 of the guide for more detailed information.

1. Advisory criteria (at least one is required):
   a. One sporadic, locally acquired human case or asymptomatic blood donor
   b. Two or more confirmed horse cases over a two-week period
   c. 10% higher-than-baseline seroconversion rate in sentinel chickens in a single county (11% current year vs. 1% baseline) over a two-week period
   d. 50% seroconversion rate in sentinel chickens in a single flock over a one-week period
   e. 50% of mosquito pools collected over a 1–2 week period test positive*

*The use of mosquito pools as advisory criteria will be reviewed on a case-by-case basis. Mosquitoes should be grouped into pools of 10–50 mosquitoes by species. At this time, priority for testing will be given to mosquito pools containing human disease vector species, such as Culex quinquefasciatus or Culex nigripalpus mosquitoes. At least five pools must be submitted for testing to meet this criterion. Mosquito pools must be tested or confirmed at BPHL.
2. Alert criteria:
   a. Cluster of two or more locally acquired human cases or blood donors, or
   b. 50% higher-than-baseline seroconversion rate in sentinel chickens in a single county over a two-week period

3. Templates for advisory or alert
   Templates for advisories or alerts can be found in the List of Appendices. Templates are available in English and Spanish.

D. Education
   1. Education messages should be targeted to at-risk populations (i.e. immigrant populations, outdoor workers, tribal representatives, and homeless people) in languages appropriate to the local population. Media should be used, including radio, newspaper, and television public service announcements. A one-page document on “WNV Illness - Special Considerations for Homeless Populations” can be found in the List of Appendices.
   2. Educational materials and fact sheets should be provided in English and in appropriate languages if there are immigrant populations in the affected area.
   3. Post an EpiCom message indicating the details of locally acquired mosquito-borne disease cases.
   4. Send information to local health care providers about clinical signs and symptoms of arboviral diseases at the beginning of the transmission season and during periods of increased activity. A one-page document for medical providers “West Nile Fever and Neuroinvasive Disease – Information for Clinicians” can be found in the List of Appendices.
   5. Send information to local health care providers about clinical signs and symptoms of arboviral diseases when there is an unusual number of imported cases or an increased trend of imported cases compared to baseline for the county. Review the Florida Weekly Arbovirus Surveillance report for current arboviral activity in Florida.

7. IMPORTANT LINKS

A. Florida Confidential Vector-Borne Disease Case Report Form:  

B. Florida Department of Health Mosquito-Borne Disease in Florida:  

C. Surveillance and Control of Selected Mosquito-Borne Diseases in Florida Guidebook  

D. Mosquito-Borne Disease Surveillance Reports  

E. Mosquito-Borne Illness Response Plan

F. CDC FAQ: Insect Repellent Use and Safety
   www.cdc.gov/westnile/faq/repellent.html

G. Florida Resident’s Guide to Mosquito Control

8. REFERENCES

