



Non-Human Mosquito-Borne Disease Monitoring Activities

The ideal mosquito-borne disease surveillance program measures the amount of viral amplification and transmission in nature and reliably provides information on the risk of human disease. A complete surveillance program consists of monitoring arboviral seroconversion rates in sentinel chickens, weather patterns, the abundance of vector and amplification host species, and the incidence of human and animal disease. The ultimate goal of surveillance is to increase our ability to predict when and where arboviral transmission to humans is likely to occur so that vector and disease control activities can be implemented prior to the beginning of an epidemic. Continuous local surveillance is also invaluable in monitoring both the progress and the cessation of periods of epidemic risk to humans.

Sentinel Chickens

Arboviruses are found in mosquitoes throughout Florida during most of the year. Sentinel chickens can be infected with mosquito-borne viruses via the bite of an infected mosquito during any month, but transmission is most often reported between August and November.

Historically, sentinel chickens have been more frequently infected with St. Louis encephalitis virus (SLEV) and West Nile virus (WNV) than Eastern equine encephalitis virus (EEEV). This is likely due to the focal distribution of EEEV in Florida and the low probability that sentinel flocks are located in EEEV transmission zones. Therefore, sentinel chicken surveillance may be less useful for predicting EEEV transmission to humans. However, during years of heavy EEEV transmission in Florida, EEEV was reported in sentinel chickens over a wide area, indicating a generalized risk of transmission to humans throughout the traditional Florida EEEV transmission zone.

Local health and mosquito control agencies should use sentinel chicken flocks to assess local mosquito transmission of WNV and other arboviruses. Local governments without mosquito control or sentinel chicken surveillance capabilities should consider establishing programs in uncovered areas. Testing of sentinel chicken sera for virus or antibody will be conducted by the Bureau of Public Health Laboratories (BPHL) in Tampa, and results will be reported to submitters and participating programs as quickly as possible.

Sentinel chicken programs are maintained by mosquito control districts or county health departments (CHDs), depending on local resources and priorities. Such programs entail determining flock placement, flock care, weekly collection, processing, and shipping of blood specimens, use of electronic ordering for testing, and notification of appropriate agencies and persons regarding seroconversion data. Under certain conditions, “backyard” (i.e., birds maintained for other purposes) juvenile chickens (birds hatched during the sample year) can be monitored.

Under ideal circumstances, sentinel chicken flocks should be located in every Florida county, because mosquito-borne arbovirus transmission can be quite focal and spread rapidly. When flocks are not maintained in a county, that CHD often relies on the results of sentinel chicken

surveillance in contiguous counties to aid in decision-making. Because of the introduction of WNV into Florida in 2001, chicken surveillance should be conducted throughout the state.

Chickens are not known to transmit mosquito-borne viruses directly to people. They are also not effective virus amplifying hosts. Mosquitoes that bite an infected chicken are unlikely to become infected.

Sentinel Chicken Flock Information

- The surveillance site should be permanently located in an area free from public access and vandalism. Mosquito control personnel should be consulted for advice on flock placement in counties where flocks are maintained.
- The location of each flock (i.e., maps and GPS coordinates) should be reported to the Bureau of Epidemiology (BOE) each January and on the Florida's Environmental Health Surveillance System (FLEHS) sentinel site/flock management website: <https://flehs.myfloridaeh.com/>.
- The number of flocks maintained in each county depends on the size of the county and the resources available for maintaining a sentinel chicken surveillance program. However, a minimum of six chickens per flock is suggested to maintain uninterrupted arboviral surveillance around the vicinity of the flock.
- Sentinel flocks should be located in a variety of habitats throughout the county. These should include, but are not limited to, hardwood hammocks, pine flatwoods, coastal habitats, freshwater marshes, saltwater marshes, residential areas, city and county parks, and urban centers.
- Backyard chicken flocks selected for retrospective surveys should be located within two to three miles of mosquito habitat. During a medical alert, chicken flocks within a two-mile radius of a human case may be sampled.
- Female Leghorn, Barred Rock, Rhode Island Red or Minorcan chickens that reach the age of 10–12 weeks before being placed in the field are ideal for surveillance (game chickens are not recommended). All-hen flocks may be preferred in some urban areas when cocks crowing might annoy residents.
- The local county agricultural extension agent can be contacted to obtain information for contacting local chicken breeders. If a local source of chickens is not available, assistance may be obtained from neighboring counties or mosquito control personnel at the Florida Department of Agriculture and Consumer Services (FDACS). The [List of Appendices](#) has information on contacts for establishing sentinel chicken flocks.
- Each chicken must be properly identified by a uniquely numbered wing or leg band (e.g., available from National Band and Tag Company at 859-261-2035, or www.nationalband.com/). Bands should not be reused after a chicken is removed from a flock.
- Animal care workers should take precautionary measures when handling chickens and when conducting routine maintenance of cages. Workers should wear impermeable gloves (e.g. disposable latex-free gloves) to protect against contact with chicken feces. Arboviruses are not transmitted by contact with chicken feces, but other illness-causing organisms can be found in the excreta. Chicken feces should be treated carefully and properly disinfected and disposed. Dust masks may be used to protect against respiratory irritants when performing work with significant dust levels, such as cleaning cages. See the Infection Control and Personal Protective Equipment Guidelines for persons involved in surveillance,

eradication, and control of avian influenza outbreaks in birds in Florida located in the [List of Appendices](#).

Husbandry

- Housing should be constructed in such a manner that the chickens can be protected from the elements (shade and protection from rain is required) and from predators. It is recommended that cages be maintained above the ground.
- A raccoon/fox-proof wired (or double wiring) coop with a strong door and a secure lock to the entrance used for feeding and bleeding purposes should be sufficient to protect the chickens. Mosquitoes must have free access to the coop interior.
- Housing should be adapted to the condition of the terrain and should have adequate slope to keep the ground dry.
- Chickens should be fed in accordance with feed manufacturer's recommendations, including the addition of chicken scratch. Sufficient amounts of fresh water should be supplied to the flocks and cages should be cleaned on a regular basis.
- A separate flock of chickens should be kept in a **mosquito-proof building** to replace chickens lost due to seroconversion or mortality.
- Sentinel chicken cages can be fitted with exit traps, which collect female mosquitoes (empty and blood fed) as they exit the sentinel cage, usually early in the morning. Molecular testing of these mosquitoes for arboviruses is encouraged (if testing is available).
- Clusters of morbidity or among flocks should be reported to FDACS, Division of Animal Industry, at 850-410-0900.

Bleeding Schedules/Record Keeping

- Accurate records should be maintained for future reference with detailed information on the location of the site (exact address and GPS coordinates), surrounding vegetation, and weather conditions during the surveillance season.
- All chickens in the flock should be bled every week, with the exception of **new** birds.
- New chickens should be bled when they are placed in the holding site to establish a negative baseline. A second baseline bleed is required once the chickens leave the holding site and arrive at their final destination. After two weeks, the chickens can be bled every week with the rest of the flock.
- All sera samples should be delivered to BPHL Tampa by noon on Wednesday of every week.
- Blood samples are screened initially using the Hemagglutination Inhibition (HAI) test. This test is broadly reactive and will indicate the presence of flavivirus or alphavirus antibodies. The samples with a positive HAI to either alphavirus or flavivirus are then tested using the IgM enzyme linked immunosorbent assay (ELISA). This test will indicate for which virus the sample has antibodies against. Sometimes, the HAI test will be positive and the IgM ELISA test will be negative. When this happens, the samples are tested using serum neutralization.
- The length of time to obtain confirmed negative or positive test results may vary. Please see the testing flow chart in the [List of Appendices](#).

- Antibody-positive chickens may revert to false HAI-negative status on later serum samples; thus, chickens that are reported as confirmed positive should be removed from the flock and replaced with a baseline-negative bird from the holding flock.
- The weekly seroconversion rate is the number of confirmed arbovirus antibody-positive chickens divided by the number of birds tested. Seroconversion rates can be calculated for the state, county, or individual flocks.
- Serologically negative chickens may be bled throughout the season, but all chickens should be replaced annually with new birds early in the year (April–May).
- Chickens that seroconvert or die should be replaced with a non-immune chicken having a **new** band number. The new band number must not duplicate the band number of other chickens at that site. Notify the BPHL and update the FLEHS flock management database website as to dead/missing chickens and their replacements.

Instructions for Bleeding Chickens

A blood collection kit should be assembled for use in the field. A plastic craft tray or small, light toolbox should contain: needles, syringes, serum separator tubes, gloves, two pencils or sharpie markers, a small tightly closed plastic container of alcohol-soaked cotton balls, a checklist of chicken wingband numbers by site, insect repellent, and waterless hand disinfectant/cleaner for the worker. Hand sanitizers containing 70% alcohol are most effective. In addition, bring a sharps safety disposal container, appropriate disposal bags for waste, and a small cooler of ice (ice is useful for hemostasis if gentle pressure fails to assist with clotting).

Bleeding should be undertaken only by appropriately trained professionals. A person working alone may bleed chickens (a chicken restrainer to facilitate this is described in *Mosquito News* 3(2):357-359, 1986). Two field personnel can make the process easier. Once securely restrained, the bird should be placed on its side and the opposite wing extended for easiest access to the vein that is to be bled:

1. Stretch out a wing to expose its underside. Alternate wings each time the chicken is bled in order to allow healing (some may choose to take samples from jugular veins).
2. When needed, pluck feathers where the wing joins the body to expose the vein. Wet the area with alcohol to make the vein more readily visible and to clean the venipuncture site. Apply pressure to the medial aspect of the vein to increase blood pressure to the vein.
3. Carefully insert into the vein, bevel side up, a 23- or 25-gauge 0.5-inch needle (depending on the size of the vein) fitted to a 3 cc syringe. Use a new needle and syringe for each chicken.
4. Withdraw 1.5 to 2.0 cc of whole blood by drawing on the plunger *slowly* in order to keep the vein from collapsing.
5. Remove the needle and apply gentle pressure with an alcohol-soaked cotton ball at the site of venipuncture for hemostasis. Make sure to release pressure prior to removing the needle from the vein to reduce possibility of hematoma or blood loss into free space next to vein.

Note: Gloves and a face shield or protective eyewear should be worn during the entire bleeding procedure. Hands should be cleaned with an alcohol-based disinfectant after removing gloves and the gloves disposed. Hand sanitizers containing 70% alcohol are most effective. Should a novel strain of avian influenza be detected in the U.S., additional

occupational safety measures will be necessary. Guidelines are available in the [List of Appendices](#).

6. Dispense the blood slowly into a 4-inch commercial serum separator tube (tubes can be purchased from Fisher Scientific, 1-800-766-7000, www.fishersci.com or other scientific/medical supply companies). The use of these tubes precludes the need to transfer serum and label to a second sterile tube, thus reducing the chance of mislabeling a specimen and saving technician time. The use of such tubes reduces the rate of bacterial contamination and produces more useable serum.

Note: To reduce hemolysis, uncork the tube, carefully recap and remove the needle from the syringe, and slowly express the blood into the tube. Needles should only be recapped using a one-handed technique (using the syringe to scoop the cap onto the needle), or by using forceps or a clamp. Uncapped needles can be removed from the syringe by a mechanical unwinding device that deposits the needle directly into the sharps container.

In addition, all needles must be deposited into a sharps container at the point of origin, which is defined as the area where the waste is generated. The sharps containers must be transported by a Department of Health (DOH)-registered transporter (see www.floridahealth.gov/healthy-environments/biomedical-waste/bmw-transporter-list.html) to a permitted storage or treatment facility that has an active permit from DOH. Treatment must be achieved by incineration, steam sterilization, or an alternative treatment process approved by DOH.

If the phlebotomist or assistant is stuck by a needle during the bleeding procedure, the chicken blood must be tested for virus. Contact the BPHL in Tampa at 813-974-8000 for directions. If an arbovirus is detected in the chicken blood, the phlebotomist should contact their local CHD to facilitate testing.

1. Label each vial using a waterproof marking pen or pencil with the following information:
 - a. **Important** – Correct bird number from the permanent wing tag or leg band
 - b. Flock site location/name
 - c. Collection date
2. Lay tubes on their sides (this increases serum yield). Keep tubes on coldpacks to help reduce hemolysis (rupturing of RBCs).
3. If possible, centrifuge for 15 minutes at 1200 rpm, trapping the clot in the bottom of the tube.
4. The tube may be shipped directly to BPHL in Tampa without decanting the serum.

Note: **A completed submittal form must be included with serum samples shipped to BPHL.** Serology submittal forms with barcodes for each collection are generated by the specimen submitter using the FLEHS database. The barcodes contain information such as county name, site number, bird number, date collected and whether the bird is new or not. BPHL will assign laboratory numbers and scan the barcodes when the serum samples are received and the information stored in the barcodes will be transferred electronically to the BPHL Laboratory Information System (LIMS) from FLEHS. When BPHL completes a test, results are entered into LIMS. Please contact the Arbovirus Surveillance Coordinator for information on FLEHS and the use of this database. Samples received before noon on Wednesday will have HAI test results reported on the following Friday.

Please see the [List of Appendices](#) for additional recommendations on specimen collection and packaging.

Serum Testing/Data Dissemination

Sentinel chicken sera are tested at BPHL Tampa (contact the laboratory at 813-974-8000). The Tampa laboratory communicates the results weekly to the county coordinator submitting specimens as well as the CHD, BOE, and the FDACS Bureau of Scientific Evaluation and Technical Assistance.

Dead Bird Reporting and Testing

WNV infection causes morbidity and mortality in many bird species in the U.S. 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 of bird mortality is considered a tool for WNV surveillance.¹ The Florida Fish and Wildlife Conservation Commission (FWC) coordinates the monitoring efforts of bird mortality in the state. Dead bird sightings may be reported on their website: <http://legacy.myfwc.com/bird/>. The data are used to detect focal areas with intense WNV activity.

Because of the understanding we have gained about the mortality rates of different bird species infected with WNV, under most circumstances dead bird testing is not warranted. Instead, ask the public to report bird mortality sightings on the <http://legacy.myfwc.com/bird/> website. CHD staff and other agency personnel should assist with the reporting process as needed.

BPHL Tampa accepts dead bird specimens. When there is a need to verify the cause of an increased corvid or overall bird mortality, a representative sample may be submitted to the BPHL Tampa for WNV testing. When bird carcasses are in the appropriate condition for WNV diagnostic testing, the carcass and an Arbovirus Surveillance: Necropsy and Virus Isolation form may be submitted by CHDs, FDACS, FWC, mosquito control staff, veterinarians, or wildlife rehabilitators to the BPHL Tampa to be sampled and tested using a polymerase chain reaction (PCR) assay or virus isolation. The laboratory submission form is located in the [List of Appendices](#). Initial testing should take about one week. Clusters of mortality of single non-corvid species or families of birds such as doves, ducks or brown pelicans are usually not caused by WNV and should not be submitted for WNV testing. However, the findings of these dead birds need to be reported. FWC tracks all clusters of wild bird mortality in the state and investigates selected mortality clusters reported at the website above. General precautionary measures should be observed when handling a dead bird.

When collecting a dead bird to submit for testing:

Avoid touching the bird with your bare hands. Wear disposable gloves or place a plastic bag over your hand to pick up the bird. After the bird is placed in a plastic bag, seal it tightly. Remove the gloves or plastic bag from your hands by turning them inside out. Dispose of the

¹ West Nile Virus in the United States: Guidelines for Surveillance, Prevention, and Control. CDC. www.cdc.gov/westnile/resources/pdfs/wnvGuidelines.pdf.

gloves or plastic bag in a trash bag. Place the bag containing the bird in a second plastic bag and tie securely. Place the double-bagged bird in a cooler with blue ice. Wash your hands thoroughly with soap and water. Ship the bird in either a hard-sided cooler or a Styrofoam cooler placed in a cardboard box. It is important to specify that the package be shipped via ground transportation. The shipping company should let you know if the package is unable to be shipped by ground to a certain location. If this is the case, different packaging may be needed to ship the package via air.

When disposing of a dead bird:

Avoid touching the bird with your bare hands. Wear disposable gloves or place a plastic bag over your hand to pick up the bird. Bury the bird two feet deep **or** place the bird in a plastic bag and tie securely. Remove the gloves or plastic bag from your hands by turning them inside out. Dispose of the gloves or plastic bag in a trash bag. Place the bag containing the bird in a second bag and tie securely. Place the double-bagged bird in the garbage. Wash your hands thoroughly with soap and water. Wash any clothing that has come into contact with the bird with normal household detergent at normal temperatures. Do not eat, drink, or smoke while working with or handling dead birds.

Laboratory Testing Protocol

At BPHL Tampa, sera collected from sentinel chicken flocks and wild birds and animals are tested for antibodies to EEEV, SLEV, and WNV with three different serological assays according to the following algorithm: All specimens are screened using an HAI assay to detect alphavirus (EEEV or highlands J virus), or flavivirus (SLEV or WNV) antibodies. Sentinel chicken sera that are flavivirus-positive are tested in an SLEV and WNV IgM ELISA assay. Sentinel sera that are alphavirus-positive in the HAI assay are tested for IgM antibody to EEEV. IgM antibody-negative sera and IgM antibody-equivocal sera may be assayed by SN for confirmation of etiology. HAI flavivirus antibody-positive wild bird or mammalian sera are assayed by SN to confirm the etiological agent. Dead bird specimens are processed and assayed by PCR for detection of EEEV, WNV and SLEV nucleic acids. A portion of tissue is also placed in cell culture for virus isolation attempts. Reports are sent to the county where the bird was collected and to the submitter. Forms for submission of samples are located in the [List of Appendices](#).

Veterinary Surveillance

Cases of equine and other animal arboviral diseases are also used to assess the impact of WNV and EEEV in the state. Veterinarians should send equine and other veterinary animal sera or brain tissue to the FDACS Bronson Animal Disease Diagnostic Laboratory in Kissimmee for evaluation (321- 697-1400). Results should be available within one week. Positive animals are reported to DOH by FDACS.

Veterinary Case Definition

A **confirmed case** of an arboviral infection is illness in an equine, emu, or other veterinary case with clinical signs, plus one or more of the following in an ante-mortem test:

- Isolation of an arbovirus from tissue, blood, or cerebrospinal fluid (CSF)

- An associated four-fold or greater change in neutralizing or HAI antibody titer to an arbovirus in appropriately timed, paired sera (nonvaccinated or known vaccine history)
- Detection of IgM antibody to an arbovirus by IgM Antibody Capture ELISA (MAC-ELISA)

In a post-mortem sample, a confirmed arbovirus case is positive by either:

- PCR for arbovirus genomic sequences in tissue, blood, or CSF
- Positive immunohistochemistry for arbovirus antigen in tissue
- Isolation of an arbovirus from those samples

Clinical signs should include one or more of the following: depression, ataxia (including stumbling, staggering, wobbly gait, or incoordination), weakness, inability to stand, death, elevated rectal temperature, change in mentation, and cranial nerve abnormalities (primarily weakness of the tongue). Horses are also commonly hyperaesthetic for one to several days. In certain arbovirus infections, horses can present with rapid onset of head pressing, coma, aimless wandering, and blindness. Emus and ostriches may present with a usually fatal hemorrhagic gastroenteritis. High levels of virus are present in these birds and their stool.

Suspected arbovirus cases in non-equines can only be confirmed by a positive PCR for arbovirus genomic sequences or virus isolation in tissue, blood, or CSF.

All samples must be submitted with an Arbovirus Encephalitis Case Information Form FDACS 09125 for appropriate classification of test results. This form is located in the [List of Appendices](#).

Mosquito Monitoring

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. Therefore, it is the quality of collections, not the quantity, which is important. Ideally, the method of surveillance and sampling sites should remain constant from year-to-year to allow comparison between years.

Mosquito trapping data for *Aedes aegypti* and *Aedes albopictus* surveillance should be entered by mosquito control districts into the Centers for Disease Control and Prevention (CDC) database, MosquitoNet, to help further define the distribution of these species: www.cdc.gov/Arbonet/MosquitoNET/Default.aspx. CDC further expanded the database so that surveillance for all mosquito species could be included. Please reach out to FDACS Bureau of Scientific Evaluation and Technical Assistance for additional information or support on submitting to MosquitoNet.

Laboratory testing of pooled mosquitoes is available from BPHL. However, it is important to note that such testing need only be conducted when specific aims of the surveillance program have been defined, and it has been determined by DOH that the testing is necessary to enhance the ultimate goal of risk reduction.

Trapping Mosquitoes

Current methodologies for trapping mosquitoes are available from the Florida Coordinating Council on Mosquito Control or local mosquito control agencies. Printed copies of *Florida*

Mosquito Control: The State of the Mission as Defined by Mosquito Controllers, Regulators, and Environmental Managers are available from the Florida Medical Entomology Laboratory, University of Florida/IFAS, 200 9th Street SE, Vero Beach, Florida 32962, 772-778-7200; or downloaded from the FMEL web page: <https://fmel.ifas.ufl.edu/fmel---gen---info---v3/fmc-white-paper/>.

Collections of flying mosquitoes (mostly host-seeking females) can be made by utilizing many different trap designs (CDC, New Jersey, and updraft to name a few). Traps can be run with or without added carbon dioxide (CO₂) and other secondary attractants such as octenol. Ovipositing female mosquitoes can be collected in gravid traps. Host-baited traps, including lard can traps and Trinidad traps, can be used to collect host-seeking female mosquitoes. Sentinel chicken cages can be fitted with exit traps, which collect female mosquitoes (empty and blood fed) as they exit the sentinel cage, usually early in the morning. Resting mosquitoes can be collected with backpack aspirators and large, medium, or small hand-held aspirators.

Once collections are identified and counted, the number of mosquitoes in each group for each species should be entered into a database for graphical presentation or plotted manually so that day-to-day changes in mosquito abundance can be readily seen. Age determinations allow for identification of periods in which the risk of viral transmission is highest.

Collection Techniques

1. Traps
 - a. CDC (with or without CO₂)
 - b. Gravid, ABC traps (with CO₂), MM-X traps (a.k.a. pickle jar) (with CO₂)
 - c. Lard can
 - d. Mosquito Magnet traps
 - e. BG-Sentinel Mosquito trap (Biogents)
2. Traps may be set any place arboviral transmission is suspected to be ongoing. Arboviral transmission can be extremely focal in widely dispersed habitats, other trap sites and collection techniques should be considered, including ground aspirator collections at mosquito daytime resting sites, avian roosts, and areas of past virus activity.
3. Maintain accurate and detailed nightly records for each collecting bag and each resulting mosquito pool.
4. Priority: ornithophilic (mainly feeds on birds) and opportunistic mosquitoes that are known vectors of human disease.
 - a. *Culex*
 - b. *Culiseta*
 - c. *Coquillettidia*
 - d. *Aedes*

Sample Processing

1. Prior to sending the samples, BPHL Tampa must be contacted.
2. Hold samples on wet ice in field or transport traps in coolers.
 - a. Do not use dry ice to kill or anesthetize collections, because the CO₂ acidifies the sample and may kill the virus, thus interfering with tests designed to isolate live virus. However, it is desirable to ship mosquitoes that are sealed within proper tubes to BPHL Tampa on dry ice (see instructions below).

- b. Make sure mosquitoes are kept alive by keeping them in a humid environment with access to cotton balls soaked with 5% sugar water.
 - c. Once mosquitoes are killed, they must be kept in a freezer maintained at -70°C or colder.
- 3. Use a chill table to sort the specimens. Triethylamine (TEA) can also be used to anesthetize the insects for the sorting process.
- 4. Group female mosquitoes into pools of 50 individual mosquitoes by species, site, and night of collection. Be careful not to contaminate the sample by including loose body parts (e.g. legs) belonging to other mosquito pools.
- 5. Mosquitoes should be live or recently (<2 hr) dead, non-fed or gravid females only. Do not pool blood-fed mosquitoes because if positive it is impossible to tell whether the virus originated in the mosquito or in the blood meal.
- 6. Do not combine mosquitoes trapped on different nights, different sites, or in different types of traps at the same site, even if they are the same species.
- 7. Make sure each mosquito pool is clearly and accurately labeled with a unique identifier number. This information, plus any notes or comments for each pool, should appear on a master data sheet, which is copied and maintained in two separate locations. Information on the pool should include:
 - a. Mosquito species
 - b. Number of specimens
 - c. Mosquito data (sex and empty or gravid for females)
 - d. Collection date
 - e. Collection location
 - f. Collection method (attractant trap type or non-attractant collection; if traps used, note attractant used as this indicates bias for particular age classes)
- 8. Accurate species identification is essential. If you are unsure of the species identification do not guess. Either have the specimen accurately identified or discard it. Unidentified pools will not be tested by BPHL Tampa.
- 9. Label tubes (preferably 2.0 ml plastic, snap-cap microcentrifuge tubes (Fisher Catalog # 02-681-258) with the unique identification number or with the following information: species name and number, site, collection date, and numbers of mosquitoes. Seal the tube with plastic film (or plastic electrical tape) and store it at -70°C. A proper seal is essential to prevent intrusion by carbon dioxide gas when the specimens are shipped on dry ice. Maintain accurate records.
- 10. Complete the Arbovirus Surveillance Mosquito form in the [List of Appendices](#) and send with the submitted pools to BPHL Tampa.
 - a. Contact the laboratory prior to sending samples.
 - b. Drive to BPHL Tampa or send by overnight mail on dry ice.
 - c. Laboratory address:
 - Virology
 - DOH Bureau of Public Health Laboratories
 - 3602 Spectrum Blvd
 - Tampa, FL 33612
 - Tel: 813-974-8000
- 11. To benefit arboviral surveillance programs, mosquitoes should be pooled and shipped to BPHL Tampa within 24 hours of collection. In addition, the shipments need to arrive at the laboratory on a weekday to make sure staff is available to process the specimens. Results will be reported back to the collector within two weeks.

Viral Assay of Mosquitoes

Samples are screened in a molecular assay (RT-PCR) for WNV and, when appropriate, EEEV. Positive pools are reported by email to the submitter. When molecular screening is completed, a report is emailed to the submitter and to BOE. Samples are then inoculated onto cell cultures for arbovirus isolation. When an isolate is detected, it is identified using multiple primer sets and probes. Gene sequencing may be performed. Virus isolates are reported by email to the submitter. When isolation attempts are complete, a report is emailed to the submitter.

Mosquito pools testing at the BPHL Tampa will be given priorities and tested based upon the following guidelines:

- Priority 1 – Validation and confirmation of commercial testing (VecTest®, RAMP®, PCR, etc.).
- Priority 2 – Pilot testing, such as well-designed transmission studies. Such studies must have prior approval through the Arbovirus Surveillance Program.
- Mosquito testing due to clustering of animal or human cases of disease (e.g. to determine local minimum infection rates (MIRs)).
- Routine mosquito surveillance testing and testing for other purposes will be available at the submitter's expense, and only on a space-available basis.

In Florida, no surveillance has been done to prospectively evaluate SLEV, EEEV or WNV infection rates in mosquitoes. It is clear that during epidemic periods, high SLEV or WNV infection rates can be demonstrated in *Cx. nigripalpus* mosquitoes.

If implemented, surveillance based on viral assay of mosquitoes would require several years of operation to evaluate its sensitivity and specificity for detecting periods of elevated risk of arbovirus transmission. Surveillance of mosquito infections should not supplant other sources of information pertinent to arbovirus activity (e.g., transmission to sentinel or wild vertebrates, real-time monitoring of local *Cx. quinquefasciatus* or *Cx. nigripalpus* population dynamics, and rainfall data).

Each organization performing mosquito viral assays should provide test results to the DOH Arbovirus Surveillance Coordinator for inclusion in the statewide database. This should include assay method for positive pools, number of pools and number of individuals per pool, species, date, site collected, and pathogen detected. For negative pools, number of pools of each species should be provided. For further information on using mosquito testing for arbovirus surveillance, see Donald Shroyer's 2001 Wing Beats article.² For further guidance on commercial assays for WNV and EEEV in mosquitoes see the article by Burkhalter, KL, et al. 2006.³ If independent testing is being performed (outside BPHL), any positive results should be reported to the Arbovirus Surveillance Coordinator and positive samples should be forwarded for confirmatory testing at BPHL Tampa.

It is essential that laboratories conducting viral surveillance with mosquitoes (including, for example, RAMP or VecTests) provide appropriate safety procedures for working with Biosafety Level (BSL)-2 and BSL-3 pathogens. For appropriate standards of practice, refer to *Biosafety in*

² Shroyer, D.A. 2001. The collection and processing of mosquitoes for arbovirus assay: some fundamental considerations. *Wing Beats*; 12(2):4.

³ Burkhalter, KL, et al., 2006. Evaluation of Commercial Assays for Detecting West Nile Virus. *Journal of the American Mosquito Control Association*; 22(1):64-69.

Microbiological and Biomedical Laboratories (BMBL) 5th edition, at www.cdc.gov/biosafety/publications/bmb15/index.htm. It is important to remember that homogenization can produce dangerous aerosols and appropriate protective measures should be observed. Note that the test kit homogenization reagent may not kill all pathogens present in the specimen.

Laboratories also conducting PCR should be aware that the reagents, even if not contaminated by virus, may be hazardous materials requiring appropriate chemical hazard protocols and disposal. In addition, EEEV is considered a select agent (potential to pose a severe threat to public health and safety) by the Department of Health and Human Services (HHS) and the United States Department of Agriculture (USDA). CDC and USDA are responsible for the regulation of these agents. Restrictions on having EEEV in the laboratory can be found at: www.selectagents.gov/.

The DOH laboratory submission form Arbovirus Surveillance: Mosquito for mosquito testing can be found in the [List of Appendices](#).

Weather Analysis – Rainfall Monitoring

Daily rainfall and groundwater accumulations are important meteorological factors when attempting to predict changes in vector abundance as well as viral amplification and transmission. Monitoring daily rainfall is important for three reasons. First, the length of the Florida dry season is an important factor in determining the potential survival of overwintering and potentially infected mosquito vectors. During years with a long dry season (i.e., January through June), there is a lower potential for virus transmission during the following autumn. If the dry season is short, as in 1990, viral amplification and transmission can begin as early as May or June. Second, once the dry season ends, heavy spring rains allow a quick, early season buildup of vector mosquitoes. Finally, daily rainfall patterns are responsible for driving the overall behavior of *Culex* vectors by determining when and where eggs are laid, when host seeking and biting occurs, and when the virus is transmitted. This theory is applicable to SLEV and WNV. The same may not apply to north Florida, since vectors, habitat, and environmental conditions are very different in this part of the state.

Rainfall data are available from the National Weather Service (National Oceanographic and Atmospheric Administration; NOAA). For more localized information, however, it is often necessary to use independent measurements. To monitor daily rainfall, fence-post-style rain gauges are read, emptied, and the amount of rainfall recorded at roughly the same time each day. Annual rainfall records include the timing, amount, and intensity of rain at the beginning of the wet season. This alerts personnel to a potential buildup of the vector population. Daily rainfall records throughout the wet season may show patterns of heavy rain (>2 inches) followed by 10- to 14-day droughts. These conditions are ideal for completion of extrinsic incubation of the virus in infected vectors and for synchronizing vector egg laying, blood feeding, and potential virus transmission. Finally, it is important to know when the dry season begins, as this may mark the end of virus transmission for that year.

Meteorological conditions predispose regions to epidemic arboviral conditions. Specifically, droughts during the amplification (April–June) and early transmission (July–September) phases of the annual Florida arboviral cycle greatly enhances the probability of epidemic transmission.⁴ Real-time measures of drought are critically important for assessing epidemic risk in Florida.

⁴ Day J, F. 2001. Predicting St. Louis encephalitis virus epidemics: Lessons from recent, and not so recent, outbreaks, *Annu Rev Entomol* 46:111-138.

The Keetch-Byram Drought Index (KBDI) is currently used to assess daily surface wetness conditions throughout the state. It has recently become evident that modeled water table data (WTD) provide a much more sensitive measure of groundwater pooling and *Culex* reproductive behavior. One of the most reliable epidemic signatures is modeled WTD that can be tracked throughout the year in real time and used to predict arboviral transmission.⁵ Unfortunately, modeled WTD are not presently available to workers in the field. This may change in the near future, and once the WTD become available for general use, they will provide a powerful tool for monitoring and predicting arboviral epidemics.

⁵ Shaman, J. et al. 2004. Seasonal forecast of SLEV transmission, Florida, Emerging Infectious Diseases, 10:802-809. https://wwwnc.cdc.gov/eid/article/10/5/03-0246_article.