

# Mosquito Control Program Vector Surveillance Submission Guidance

## *Summer 2022*

### Executive Summary

The purpose of this document is to provide guidance for county mosquito control agencies (CMCAs) to help target state supported surveillance efforts on vectors of concern to prevent human illness. Recommendations for additional mosquito surveillance to support identified research priorities will be provided separately as needed. While this document lists key species to target for each pathogen of interest, there are other species that CMCAs may also want to consider as part of their surveillance program. Decisions on which species to target and the number of pools/pathogen to submit should be based on local mosquito populations and local/regional arboviral activity. The State Mosquito Control Commission (SMCC) through the Office of Mosquito Control Coordination (OMCC) and/or the New Jersey Agricultural Experiment Station (NJAES) through the Rutgers Center for Vector Biology (CVB) will consult with CMCAs on emerging surveillance needs and strategies.

### Mosquito-Based Surveillance

Mosquito-based surveillance consists of the systematic collection and identification of mosquito samples and screening them for arboviruses. The principal functions of a mosquito-based surveillance program are to:

- Collect data on mosquito population abundance and virus infection rates in those populations.
- Provide indicators of the threat of human infection and disease and identify geographic areas of high-risk.
- Support decisions regarding the need for and timing of intervention activities (i.e., enhanced vector control efforts and public education programs).
- Monitor the effectiveness of vector control efforts.

### Arboviruses of Concern

In New Jersey, West Nile virus (WNV) is the most common mosquito-borne disease with an annual endemic average of 7 human cases and periodic epidemic years with 16-61 human cases. Eastern equine encephalitis (EEE) is also detected annually in mosquito pools and horses, but human cases to date have been rare. The New Jersey Department of Health (NJDOH) started testing for Jamestown Canyon (JCV) in 2019, which has resulted in a low number of detections to date; only two human cases of this emerging infection have been reported in N.J. Although more common in states to the south of N.J., human cases of La Crosse virus (LAC) have been reported in the past in N.J. and have been detected in mosquitoes in recent years. Saint Louis encephalitis (SLE) is a mosquito-borne virus disease that was widely distributed in the continental U.S. Large outbreaks in N.J. occurred in 1964 and 1975 with activity documented in Burlington, Camden, Middlesex, and Monmouth counties. Fortunately, this virus has not been a major concern in recent years, but sporadic human cases are reported in Pennsylvania. After the global Zika pandemic in 2016, awareness of the need to prevent travel-associated arboviruses [Chikungunya (CHIK), Dengue (DENV), Zika (ZIKV)] from entering local mosquito populations has increased and requires different mosquito surveillance and control strategies.

## Trapping

When selecting a mosquito trap for disease surveillance, it should be noted that host-seeking traps including CO<sub>2</sub>-baited light traps, New Jersey Light Traps (NJLT), CDC miniature light traps (CDC), Biogents – Sentinel traps (BGS), etc. introduce a bias and will collect mostly (65-80%) unfed nulliparous females, resulting in a large proportion of the mosquitoes sampled testing negative. Traps that target gravid females (mosquitoes looking to lay eggs), will collect a much larger proportion of mosquitoes that have taken a blood-meal, increasing the likelihood of viral exposure obtained through these blood feeding behaviors.

## Arboviral Testing

Arboviral testing in mosquito pools is performed at two public health laboratories, the N.J. Public Health and Environmental Laboratories (PHEL) and the Cape May County Department of Mosquito Control Biosafety Level 3 Laboratory. There are three multiplex testing options available for CMCA's when submitting mosquito pools:

1. WNV Panel (WNV, EEE, SLE, JCV)
2. La Crosse Panel (WNV, LAC)
3. Exotic Aedes Panel (CHIK, DENV, ZIKV)

Owing to the use of multiplex testing panels, species may be tested for pathogens other than the ones that are solicited. As a result, species may test positive for pathogens that aren't thought to be directly involved in transmission, e.g., *Cx. pipiens* that test positive for EEE. CMCA's are asked to sample from areas of local concern and submit 15-20 mosquito pools per week during the active mosquito surveillance season. N.J.'s vector control season has expanded, consistent with a longer warm weather climate. Prior to 2021, due primarily to fiscal constraints, arbovirus surveillance efforts started at the beginning of June. This decision was adopted following several years of data that established clear WNV seasonal activity trends. In response to earlier virus detections, however, in 2021 vector surveillance will start in mid-May, to better characterize recent early season activity. The mosquito surveillance season end date is variable and is based on environmental factors, mosquito abundance, and arboviral pathogen activity, but is usually between late-October and mid-November.

## Summary of Surveillance by Human Pathogen

### WNV & SLE Surveillance:

- Targeted species: *Cx. pipiens*, *Cx. restuans*, *Cx. salinarius*, *Ae. taeniorhynchus*, *An. quadrimaculatus*
- Timing: Throughout surveillance season
- Geography: All N.J. counties
- Traps: Gravid, CO<sub>2</sub>-baited light traps, BGS
- Volume: 10-15 pools/week
- Testing: WNV Panel

#### EEE Surveillance:

- Targeted species: *Cs. melanura*, *Cq. perturbans*, *Cx. erraticus*
- Timing: Throughout surveillance season
- Geography: All N.J. counties
- Traps: Resting boxes with un-baited light trap(s) (preferred), gravid traps
- Volume: 2-5 pools/week (apart from state resting box sites)
- Testing: WNV Panel

#### JCV Surveillance:

- Targeted species: Early season *Aedes* species and others as described in JCV section
- Timing: Late April/early May and throughout the season
- Geography: All N.J. counties
- Traps: CO<sub>2</sub>-baited light traps
- Volume: 2-4 pools/week
- Testing: WNV Panel

#### LAC Surveillance:

- Targeted species: *Ae. triseriatus*
- Timing: Late April/early May through end of surveillance season
- Geography: All N.J. Counties
- Traps: CO<sub>2</sub>-baited BGS, CO<sub>2</sub>- baited light traps (set low to the ground)
- Volume: 1-2 pools/week
- Testing: LAC Panel

#### Exotic Aedes Surveillance Summary:

- Targeted species: *Ae. albopictus*, *Ae. aegypti*
- Timing: Throughout surveillance season, and/or in response to human cases
- Geography: Statewide surveillance; focused testing only
- Traps: BGS
- Surveillance: 1-2 traps/week
- Testing Volume: No minimum; in response to human case notifications, or local needs
- Testing: Exotic Aedes Panel

## Vector Surveillance Guidance by Pathogen of Concern

### West Nile & St. Louis Encephalitis Virus Surveillance

Human WNV disease cases have been reported during every month of the year in the United States, but as is characteristic of zoonotic arboviruses in temperate climates, intense transmission is limited to the summer and early fall months. Ninety-four percent of human cases have been reported from July through September, and approximately two-thirds of reported cases occur during a 6-week period from mid-July through the end of August. Weather, especially temperature, is an important modifier of WNV transmission and has been correlated with increased incidence of human disease at regional and national levels. Weather likely drives the annual fluctuations in numbers of cases reported at the national level. In New Jersey, West Nile virus (WNV) is the most common mosquito-borne disease with an annual endemic average of 7 human cases and periodic epidemic years with 16-61 human cases.

West Nile virus cycles between mosquitoes (especially *Culex* species) and birds. Some infected birds can develop high levels of the virus in their bloodstream and mosquitoes become infected by biting infected birds. After about a week, infected mosquitoes can pass the virus to other birds, people, horses, and other mammals. Humans, horses, and other mammals are “dead end” hosts, meaning they do not develop sufficiently high levels of virus in their bloodstream to transmit the virus to other biting mosquitoes. By monitoring WNV infection prevalence in mosquito vectors and incidence in non-human vertebrate hosts and comparing these indices to historical environmental and epidemiological surveillance data, conditions associated with increasing human risk can be detected 2-4 weeks in advance of human disease onset. This provides additional lead time for critical vector control interventions and public education programs to be put in place.

St. Louis encephalitis (SLE) is maintained in a similar bird-mosquito cycle as WNV and shares the same primary vectors. Most cases historically have occurred in eastern and central states, where episodic urban-centered outbreaks have recurred since the 1930s. However, in more recent years sporadic cases and outbreaks have occurred in the Southwest. In temperate areas of the United States, SLE cases occur primarily in the late summer or early fall. N.J. last reported a human case of SLE in 1978. According to CDC records, N.J. has had a total of 131 cases with the majority occurring in 1964 and 1975.

#### WNV & SLE mosquito species of concern

The principal mosquito vectors for WNV and SLE on the East Coast are members of the genus *Culex* (*Cx*), primarily *Cx. pipiens* and *Cx. restuans*. These species may be abundant in urban areas inhabiting artificial containers (e.g., birdbaths, discarded tires, buckets, clogged gutters) as well as catch basins and other standing, polluted water sources. Woodland pool habitat following the univoltine species emergence is also an abundant source of *Cx. restuans* in suburban and rural areas. Both species feed mainly on birds and occasionally on mammals, including humans. Peak feeding activity for these species occurs from dusk into the late evening. Consistently high temperatures and lower precipitation rates are factors that have been associated with higher mosquito infection and WNV human illness rates. Additionally, warmer winter temperature conditions may result in larger numbers of *Culex* species overwintering as adults, with resulting increases in early season *Culex* abundance.

*Culex salinarius* has been identified as an important enzootic and epidemic vector of WNV and SLE. *Culex salinarius* thrives in brackish and freshwater wetlands and feeds on amphibians, birds, and mammals. It

is well known for biting humans. *Aedes japonicus* may be involved in the transmission of both WNV and EEE. This species uses natural and artificial containers, such as tires and rock pools, as larval habitat. It feeds mainly on mammals and can be an aggressive human biter near the preferred larval habitat.

The role of *Ae. albopictus* in WNV transmission is not well understood, although positive results are reported nearly every year in N.J. *Ae. taeniorhynchus* and *An. quadrimaculatus* have been implicated in SLE transmission.

Mosquitoes become infected with WNV primarily through taking blood meals from infected birds. However, WNV may be passed from infected female mosquitoes to their eggs (i.e., vertical transmission), resulting in infected offspring. Vertical transmission is likely responsible for virus maintenance over the winter in northern parts of the country, but the extent of its contribution to virus amplification and human risk during the peak transmission season is not well understood.

In N.J., WNV has been detected in >25 species with the greatest number of positive species in 2002 (19), and a continued involvement of numerous species when WNV activity was high [2001 (18), 2003 (16), 2012 (10), 2018 (18)].

CMCAs should conduct surveillance for WNV (and SLE) by focusing on:

1. *Cx. pipiens*
2. *Cs. restuans*
3. *Cx. salinarius*
4. *Ae. taeniorhynchus* (for SLE; inefficient for WNV)
5. *An. quadrimaculatus* (for SLE)

Other species that might want to be considered for WNV surveillance include *Ae. albopictus*, *Ae. japonicus*, and *Cs. melanura*.

#### WNV & SLE Surveillance - Timing

Surveillance for WNV should be conducted throughout the mosquito surveillance season.

#### WNV & SLE Surveillance – Geography

WNV is found throughout N.J. and should be a primary focus of surveillance efforts in all counties.

#### WNV & SLE Trapping

The most used mosquito traps for WNV surveillance sample gravid mosquitoes seeking a place to lay eggs. Gravid traps are highly effective at collecting *Cx. pipiens* and *Cx. restuans*. The advantage of gravid traps is that gravid females have previously taken a blood meal, which greatly increases the likelihood of detecting virus. Gravid traps should be baited with an infusion (“stink water”) which may be comprised of hay, grass clippings, rabbit chow, or other materials that mimic the stagnant water in habitats where these species lay eggs. The different infusions vary in attractiveness. It is, therefore, advisable that infusion preparations are consistent within a surveillance program over time because variations may lead to changes in number and/or type of species captured. Maintaining a consistent effective recipe

and infusion mixing routine is recommended. One limitation of gravid traps is that they selectively capture mosquitoes in the *Cx. pipiens* complex, and therefore provide limited information on infection prevalence in other species within a region.

Several other traps such as New Jersey light traps or CO<sub>2</sub>-baited light traps may be used to collect mosquitoes for WNV monitoring depending on the species or mosquito reproductive state the county wishes to sample. They may also be more effective than gravid traps to detect SLE vectors, *Ae. taeniorhynchus* and *An. quadrimaculatus*. Carbon dioxide-baited CDC miniature light traps may be used to sample potential bridge-vector species including *Aedes*, *Psorophora*, and *Anopheles* species. The advantage of light traps is that they collect a wide range of mosquito species, which provides information about both primary and secondary vectors and a better understanding of the species composition in an area. A limitation of CO<sub>2</sub>-baited light traps is that the collections in certain locations and times may consist largely of unfed, nulliparous individuals, which greatly reduces the likelihood of detecting WNV and other arboviruses when only a subset of the trap collections are able to be submitted for testing. Light traps aren't recommended exclusively for WNV & SLE surveillance, however, because not all mosquito species are attracted to light traps and the numbers captured may not reflect the population size of a particular species.

#### WNV & SLE Surveillance Summary:

- Targeted species: *Cx. pipiens*, *Cx. restuans*, *Cx. salinarius*, *Ae. taeniorhynchus*, *An. quadrimaculatus*
- Timing: Throughout surveillance season
- Geography: All N.J. counties
- Traps: Gravid, CO<sub>2</sub>-baited light traps, BGS
- Volume: 10-15 pools/week
- Testing: WNV Panel

## Eastern Equine Encephalitis Surveillance

Eastern equine encephalitis virus (EEE), a mosquito-borne alphavirus, is one of the most severe arboviral diseases in North America. Human EEE cases are rare, with an average of 5-10 cases reported in the United States each year. Most cases of EEE have been reported from Atlantic and Gulf Coast states and occur primarily from late spring through early fall. Approximately 30% of people with EEE die and many survivors have ongoing neurologic problems. In 2019, 4 human cases of eastern equine encephalitis virus (EEE) were reported in N.J., from Atlantic, Middlesex, Somerset, and Union counties. 2019 was the most active season since the 1959 and 1968 epidemic years where 32 and 12 people respectively contracted the disease. Since 2000, N.J. has reported a total of 8 EEE cases, 3 cases in 2003, 1 case in 2016, and 4 cases in 2019. EEE infection is often fatal in unvaccinated horses as well, making them a useful sentinel for evaluating EEE human risk. The risk of EEE in humans varies by geographical area and is correlated with the location of necessary Atlantic white cedar and red maple swamp larval habitats supporting amplifying and bridge vector species. In N.J. this is typically in the southern part of the state, although human cases and arboviral activity in mosquitoes have more recently been detected in the north central part of the state as well.

### EEE mosquito species of concern

Eastern equine encephalitis virus is maintained in a cycle between *Cs. melanura* mosquitoes and avian hosts in freshwater hardwood swamps. Although infection with EEE can kill some birds, the virus does not kill large proportions of these species. The virus is circulated among the bird populations by *Cs. melanura* and under some circumstances *Cs. morsitans*, another bird-biting mosquito. The virus persists in birds over the winter, and has been suggested to overwinter in reptiles, but is not known to overwinter in mosquitoes in N.J. Initially, a relatively smaller proportion of birds and mosquitoes carry the virus; throughout the mosquito season, continuous transmission between mosquito vectors and bird reservoir hosts increases the proportion of infected birds and mosquitoes leading to an overall greater amount of virus present in the environment.

In the Northeast, the EEE enzootic foci are large swamps of mature Atlantic white cedar and red maple. To grow in the permanently wet swamps, tree roots spread out across the peat soils characteristic of these habitats. These root systems create dark air-filled voids, or crypts, that are flooded with water. These crypts are the preferred oviposition (egg-laying) sites for *Cs. melanura* and are where the larvae develop in the cool, dark, acidic waters. *Culiseta melanura* overwinters as larvae in these environmentally stable protected habitats. The amount of rainfall occurring during the summer and fall of the preceding year affects the survival of the larvae during the winter period and, in part, determines the population of adult mosquitoes successfully emerging the following year.

Depending on when virus circulation begins, the size of the *Culiseta* populations, weather conditions, and probably additional currently unidentified factors, this virus amplification cycle may eventually spill over and involve secondary, or "bridge" mosquito vectors that feed on both birds and mammals. In the Northeast, these bridge vectors include *Ae. vexans*, *Cq. perturbans*, *Cx. salinarius* and *Ae. canadensis*. *Coquillettidia perturbans* is considered to be the most important bridge vector in Massachusetts and is thought to be an inland driver of EEE in N.J. *Aedes sollicitans* plays an important role in coastal cases in N.J., although more data is needed to characterize its role in transmission. *Culex erraticus* is another possible bridge and endemic vector for transmission in the southeast U.S. *Culex pipiens* and *Cx. restuans* mosquitoes found positive for EEE are not considered to play a significant role in transmission of the

virus to humans or animals because research indicates that *Cx. pipiens* clears the virus within 4 days. EEE positive *Cx. pipiens/restuans* may, however, indicate the presence of virus circulating in the area.

*Aedes canadensis* develops in a wide variety of freshwater habitats, including temporary leaf-lined pools in wooded areas, roadside ditches, pools of water in open fields, and permanent swamps. They are univoltine and peak in the beginning of the season and potentially again in August following heavy rainfall events. They primarily feed on mammals, including humans and deer but have also been observed aggressively feeding on reptiles.

In NJ, EEE has been detected in 13 mosquito species: *Ae. canadensis*, *Ae. japonicus*, *Ae. sollicitans*, *Ae. triseriatus*, *Ae. vexans*, *An. bradleyi*, *An. punctipennis*, *Cq. perturbans*, *Cs. melanura*, *Cx. erraticus*, *Cx. pipiens*, *Cx. restuans*, *Cx. salinarius*.

CMCAs should conduct surveillance for EEE by focusing on:

- *Cs. melanura*
- *Cq. perturbans*
- *Cx. erraticus*

Other species that should be considered for EEE surveillance, especially during periods of high EEE activity, include *Ae. sollicitans*, *Ae. vexans*, *Ae. canadensis*, *Ae. triseriatus*, *An. quadramaculatus*, and *Cx. salinarius*.

#### EEE Surveillance - Timing

Surveillance for EEE should be conducted throughout the mosquito surveillance season. Note: resting boxes may need to be put out earlier than the start of the season for weathering. New or freshly painted boxes can repel mosquitoes. Surveillance for the three target vectors should be done throughout the mosquito surveillance season, and if positive, other species should be considered for surveillance. Surveillance for EEE in *Cs. melanura* will often go late into the year, given this species cold hardiness.

#### EEE Surveillance – Geography

While EEE has been concentrated in the southern part of the state, it has been detected in northern counties. EEE surveillance should be targeted in all areas of the state. Note: If no EEE has been detected and there are no *Cs. melanura* populations in a county, CMCAs may want to rely on *Culex* species submitted for WNV testing to also capture possible EEE activity.

#### EEE Trapping

Long-term EEE monitoring using resting box locations have been established in Atlantic (Corbin City), Burlington (Green Bank/Bass River), Camden (Winslow), Cape May (Dennisville) Gloucester (Glassboro), Monmouth (Turkey Swamp), and Salem (Centerton) counties. These sites were established to monitor *Cs. melanura* populations to provide advance warning of EEE activity cycling before the virus spills over into potential bridge vectors like *Aedes sollicitans*. In addition to the State Mosquito Control Commission supported vector surveillance efforts, several county mosquito control agencies have volunteered to

establish monitoring locations to collect additional samples from resting boxes and deliver them to PHEL or the Cape May County Department of Mosquito Control laboratories for arboviral testing.

Resting boxes are generally used to sample *Cs. melanura* and *Cx. erraticus*. Other species (*Ae. vexans*, *Cq. perturbans*, *Cx. salinarius*, *Ae. canadensis*) can be sampled through typical trapping methods such as gravid or un-baited light traps (data from the CDC EEE surveillance program evaluation found un-baited light traps were more effective in attracting *Cs. melanura* compared to those baited with CO<sub>2</sub>). To maximize *Cs. melanura* collections, county programs are encouraged to use at least one un-baited light trap in addition to resting boxes at sites targeting *Cs. melanura*. These can be placed to sample ponds with emergent vegetation (*Cq. perturbans*), flooded riverine areas (*Ae. canadensis*), flooded flatlands (*Ae. vexans*) and freshwater impoundments with varying amounts of saltwater (*Cx. salinarius*).

#### EEE Surveillance Summary:

- Targeted species: *Cs. melanura*, *Cq. perturbans*, *Cx. erraticus*
- Timing: Throughout surveillance season
- Geography: All N.J. counties (see note above)
- Traps: Resting boxes with un-baited light trap(s) (preferred), gravid traps
- Volume: 2-5 pools/week (apart from state resting box sites)
- Testing: WNV Panel

## Jamestown Canyon Surveillance

Human JCV cases are rare, with 166 cases reported between 2004-2018 in the U.S., mostly in the midwestern and northeastern states. Only two human cases have been reported in N.J. in 2015 and 2021, both in Sussex County. White-tailed deer are recognized as a principal amplification host based on experimental infections and presence of antibodies. With increasing reforestation and increases in deer population, this arbovirus may be an increasing concern. It has been isolated from 26 mosquito species, including many *Aedes* species, and can be vertically transmitted.

Long-term surveillance in Connecticut (1997-2017) detected JCV in 92% of the surveillance sites equally distributed in urban, suburban, and rural locales, with no significant association with land cover categories and no significant relationship with deer abundance.

### JCV mosquito species of concern

Long-term surveillance in Connecticut detected JCV in 25 mosquito species, most *Aedes* and *Anopheles*. Five species have been implicated as being of highest importance based on the number of years, sites, and virus detections. These include *Ae. aurifer*, *Ae. abserratus*, *Ae. canadensis*, *Ae. cantator*, and *An. punctipennis*. *Aedes canadensis* (Heard, et al, 1991) and *An. punctipennis* have been shown to be competent vectors in laboratory experiments. In Connecticut most virus isolations have been detected most often in *Ae. canadensis* and detected between mid-June and mid-July, consistent with vertical transmission. Long-term maintenance of local populations of JCV suggests overwintering and persistence of virus.

NJ started testing mosquitoes for JCV in 2019. Between 2019-2020, JCV has been identified in 11 pools in seven counties: Bergen, Burlington, Cumberland, Middlesex, Monmouth, Salem, and Sussex, and in seven species: *Ae. abserratus*, *Ae. cantator*, *Ae. taeniorhynchus*, *An. crucians*, *An. punctipennis*, *An. quadrimaculatus*, *Cq. perturbans*. In 2019 pools were detected from MMWR weeks 23-27 (weeks ending June 8- September 14) and in 2020 from MMWR weeks 23-32 (weeks ending June 6 - August 8).

*Aedes canadensis* develop in a variety of freshwater habitats, including temporary leaf-lined pools in wooded areas, roadside ditches, pools of water in open fields and permanent swamps. They are univoltine and peak in the beginning of the season and potentially again in August following heavy rainfall events. They primarily feed on mammals, including humans and deer.

It isn't known which species are responsible for JCV transmission in NJ. Some species of interest may include:

- Species of importance in CT: *Ae. aurifer*, *Ae. abserratus*, *Ae. canadensis*, *Ae. cantator*, and *An. punctipennis*.
- Positive pools in NJ: *Aedes abserratus*, *Aedes cantator*, *Aedes taeniorhynchus*, *Anopheles crucians*, *Anopheles punctipennis*, *Anopheles quadrimaculatus*, *Coquillettia perturbans*
- Referenced in literature: *Ae. communis*, *Ae. punctor*, *Ae. stimulans*, *Cs. inornata*

### JCV Surveillance - Timing

Surveillance for JCV should be initiated early in the season (late April/early May) and continue through mosquito surveillance season. Because early season species may be collected before the start of the mosquito surveillance season, samples should be frozen and sent to PHEL once routine shipments begin.

### JCV Surveillance – Geography

Initial surveillance data has detected JCV sporadically in several regions of the state. Surveillance should be conducted statewide to better characterize the distribution of this emerging arbovirus.

### JCV Trapping

Although a variety of trap types have been used in JCV surveillance, CO<sub>2</sub>-baited light traps have proven most useful for collecting the greatest variety of *Aedes* species implicated in Jamestown Canyon virus. Traps should be set in areas where the targeted species larval habitat is nearby.

### JCV Surveillance Summary:

- Targeted species: Early season *Aedes* species and others as listed above
- Timing: Late April/early May and throughout the season
- Geography: All N.J. counties
- Traps: CO<sub>2</sub> baited light traps
- Volume: 2-4 pools/week
- Testing: WNV Panel

## La Crosse Surveillance

In the United States, an average of 68 La Crosse encephalitis virus (LAC) neuroinvasive disease cases are reported each year, although this number is thought to greatly underestimate the number of total infections, with mild and asymptomatic infections not included. Severe disease occurs most often in children under the age of 16. LAC disease cases occur primarily from late spring through early fall. Historically, most cases of LAC neuroinvasive disease were reported from the upper Midwestern states (Ohio, Wisconsin, Minnesota, Indiana, Illinois, and Iowa). Recently, more cases have been reported from northeastern, mid-Atlantic and southeastern states (North Carolina, Tennessee, West Virginia, Georgia, Virginia, Kentucky, and Rhode Island). N.J. hasn't reported a human LAC case in at least 20 years (but reported 3 cases between 1964 and 2000). LAC is maintained in a cycle between *Ae. triseriatus* (the eastern tree hole mosquito) and vertebrate hosts (especially small mammals such as chipmunks and squirrels) in deciduous forest habitats (i.e., forests with trees that lose their leaves each year).

### LAC mosquito species of concern

*Aedes triseriatus* is an aggressive daytime-biting mosquito, especially in or near infested woods. True to its nickname, *Ae. triseriatus* normally lays its eggs in pools of water accumulated in tree holes, but it will also lay eggs in man-made water holding containers, particularly discarded tires. LAC is transmitted vertically, and the virus can survive in dormant eggs through the winter and develop into infected, flying mosquitoes in the spring.

Vertical transmission is a significant mechanism to maintain LAC in nature and horizontal transmission between mosquito vectors and sciurid rodents (i.e., chipmunks, squirrels) allows LAC to persist in the environment and to maintain its ability to amplify successfully in vertebrate hosts. Vector competence studies have identified the capacity for other *Aedes* species to serve as vectors of LAC: *Ae. albopictus*, *Ae. aegypti* and *Ae. japonicus*. Virus isolation from field-collected *Ae. albopictus* mosquitoes has confirmed their potential to serve as vectors in the field. Although vector competence research revealed poor virus replication in *Ae. canadensis*, field research has shown that they may serve as accessory vectors of LAC. Laboratory studies in Appalachian states have shown that *Culex* species (*Cx. restuans*, *Cx. pipiens*) may play a role in transmission in endemic areas as well.

In 2019, one mosquito pool (*Ae. triseriatus*) collected in Passaic County tested positive for La Crosse virus at PHEL. This was the first positive pool detected in the state since 2014. In 2014, 2 mosquito pools collected from the Joint Base MDL (Burlington County) by the Department of the Airforce tested positive for LACV (*Ae. triseriatus* and *Ae. albopictus*).

CMCAs should conduct surveillance for LAC by focusing on:

- *Ae. triseriatus*

Other species that might want to be considered for LAC surveillance include *Ae. albopictus*, *Ae. canadensis*, *Ae. hendersoni*, *Ae. japonicus*.

### LAC Surveillance - Timing

Surveillance for LAC should be initiated early in the season (late April/early May) and continue throughout mosquito surveillance season.

### LAC Surveillance – Geography

Surveillance data is lacking and should be conducted statewide.

### LAC Trapping

The primary vector, *Ae. triseriatus*, is not usually caught in high numbers using conventional trapping methods. Studies going back to the 1960s have used a variety of trapping methods as well as trapping types to maximize catches. It has been reported that *Ae. triseriatus* are not particularly attracted to light traps and may even be reluctant to enter traps baited with light, so the light is often removed from CO<sub>2</sub>-baited traps to increase catches. In recent studies, BGS traps baited with CO<sub>2</sub> caught higher numbers of *Ae. triseriatus* compared to CO<sub>2</sub>-baited CDC or gravid traps. Since these traps are set on the ground, this could be partially due to *Ae. triseriatus*'s preference to host-seek low to the ground. BGS traps baited with CO<sub>2</sub> have the added advantage of also collecting ample numbers of possible secondary vectors of LAC, *Ae. albopictus* and *Ae. japonicus*. Since BGS traps are expensive and cumbersome, many programs do not use them; therefore, CDC traps baited with CO<sub>2</sub> with or without light can also be set low to the ground to maximize trap catches of *Ae. triseriatus*. The addition of the BG-lure to either the BGS or CDC trap may increase catch as well, but these lures are relatively expensive and their attractiveness to *Ae. triseriatus* is not well proven. Recent research has shown R-1-octen-3-ol with ammonium bicarbonate to work as an effective lure for *Ae. triseriatus*, but these lures are not commercially available.

Adults are often found in wooded, shaded areas where suitable egg-laying habitat can be found. Since human cases of La Crosse peak in August and September, mosquito surveillance for this disease should begin in late May or June, when *Ae. triseriatus* populations are building.

### LAC Surveillance Summary:

- Targeted species: *Ae. triseriatus*
- Timing: Late April/early May through end of surveillance season
- Geography: All N.J. Counties
- Traps: CO<sub>2</sub>-baited BGS, CO<sub>2</sub>- baited light traps (set low to the ground)
- Volume: 1-2 pools/week
- Testing: LAC Panel

## Exotic Aedes Species Surveillance

Several travel-associated arboviral human cases are reported in N.J., including chikungunya, dengue, and Zika. Most human cases in N.J. occur in Bergen, Hudson, Passaic, Middlesex, Essex, and Mercer counties, which correlates with concentrations of population groups that travel to countries with endemic activity. These arboviruses are transmitted primarily by exotic *Aedes* species, namely *Ae. aegypti*, which is not established in N.J. and *Ae. albopictus*, which is also a competent vector and found throughout the state. In response to the Zika outbreak in 2016-17, mosquito surveillance shifted to include *Ae. albopictus* in addition to species implicated in WNV and EEE transmission. While there are locally acquired infections reported in Florida and Texas, the primary surveillance goals in N.J. are to detect new introductions of *Ae. aegypti*, to understand the distribution of *Ae. albopictus*, and to detect travel associated *Aedes* infections that might be imported into N.J. via human infection post-travel.

### Aedes mosquito species of concern

A couple of *Ae. aegypti* mosquitoes have been detected in NJ, but established populations have not been identified. CMCA's that do identify *Ae. aegypti* should notify SMCC.

*Aedes albopictus* (the Asian tiger mosquito) is a container-inhabiting species which lays its eggs in any water-containing receptacle in urban, suburban, rural, and forested areas. The primary immature habitats of this species are artificial containers such as tires, flowerpots, cemetery urns/vases, buckets, tin cans, rain gutters, ornamental ponds, and drums. Larvae are also found in natural containers such as tree holes, bamboo pots and leaf axils. It is a very aggressive daytime biter with peak activity generally occurring during the early morning and late afternoon, feeding on a variety of hosts including humans, domestic and wild animals, and birds. Its generalized feeding behavior contributes to its vector potential. In the lab, northeastern strains of *Ae. albopictus* were shown to be potential vectors of Zika virus and dengue virus 2 and highly competent vectors of chikungunya virus. *Aedes albopictus* has been detected in all NJ counties, but abundance levels in each county is not known.

### Surveillance

All counties should conduct targeted surveillance for *Ae. albopictus* to monitor abundance trends over time using a minimum of 1-2 BGS traps. Abundance data should be reported in JerseySurv.

In addition, limited adult trapping programs for exotic species such as *Ae. aegypti* is advisable in areas with the highest likelihood of introduction such as airports, seaports, and any areas known to receive bulk goods from overseas. All ID staff should be trained to identify exotic species in case they occur in any of the routine surveillance efforts.

In the event *Ae. aegypti* is detected, trapping should be immediately increased radially from the point of detection to determine if it is a single introduction or to measure the geographic extent of an established population. Extensive larval surveillance and control should also occur in the area to determine the potential source of the introduction and limit the spread of the species. The CMCA should also alert the SMCC of the detection so that assistance can be provided and coordinated across counties if needed.

## Pathogen Testing

It should be noted that even in areas of the US with endemic human transmission, detection of these diseases in the mosquito population is extremely rare. Therefore, testing resources should be prioritized for:

- Response to imported human cases, particularly clusters of imported cases, OR
- Response to human cases with no international travel history, AND
- When *Ae. albopictus* is detected near the residence of human cases.

In the event of locally acquired human cases of disease, the SMCC & NJDOH would provide additional surveillance and/or control guidance.

## Exotic Aedes Species Surveillance - Timing

Surveillance for exotic *Aedes* species should be conducted throughout the mosquito surveillance season and in response to human case notification when indicated.

## Exotic Aedes Species Surveillance - Geography

Routine surveillance for exotic *Aedes* species should be conducted statewide, with additional surveillance conducted based on emerging needs.

## Exotic Aedes Species Surveillance Trapping

BGS, CDC trap

## Exotic Aedes Surveillance Summary:

- Targeted species: *Ae. albopictus*, *Ae. aegypti*
- Timing: Throughout surveillance season, and/or in response to human cases
- Geography: Statewide surveillance; focused testing only
- Traps: BGS
- Surveillance: 1-2 traps/week
- Testing Volume: No minimum; in response to human case notifications, or local needs
- Testing: Exotic Aedes Panel

## Appendix A: Mosquito Species of Interest and Habitat

***Aedes abserratus*** is an early season univoltine species that is most common in northwestern New Jersey, but that can be found in any area with suitable larval habitat, including freshwater bogs and marshes. It is primarily a mammal-biting mosquito that feeds on humans and can be a nuisance after dark. However, because of its early season activity and spotty distribution, it is generally a minor pest species in New Jersey. This species may be involved in the transmission of Jamestown Canyon virus.

***Aedes aegypti*** is a container-inhabiting species that is well-adapted to urban or domesticated habitats in the southern USA. It is the vector of Zika, dengue, chikungunya, and yellow fever viruses. Although individual mosquitoes have occasionally been found, there are no reports of established populations in New Jersey. *Ae. aegypti* has also been implicated in LAC transmission.

***Aedes albopictus*** (the Asian tiger mosquito) is a container-inhabiting species which lays its eggs in any water-containing receptacle in urban, suburban, rural, and forested areas. The primary immature habitats of this species are artificial containers such as tires, flowerpots, cemetery urns/vases, buckets, tin cans, rain gutters, ornamental ponds, and drums. Larvae are also found in natural containers such as tree holes, bamboo pots and leaf axils. It is a very aggressive daytime biter with peak activity generally occurring during the early morning and late afternoon, feeding on a variety of hosts including humans, domestic and wild animals, and birds. The role of *Ae. albopictus* in WNV transmission is not well understood, although positive results are reported nearly every year in N.J. *Ae. albopictus* is a vector of Chikungunya, Dengue, and Zika and may be associated with LAC transmission as well.

***Aedes aurifer*** is an early season species with a single generation of larvae that are present between April and May. The species is more common in the northern half of the state but are locally abundant as far south as Atlantic County. Larval habitat includes freshwater swamps, bogs, or woodland pools that intergrade with a freshwater swamp source and have emergent vegetation. The species will readily feed on humans, but it is a minor pest in New Jersey because of its limited numbers and early season activity. *Ae. aurifer* may be associated with JCV transmission.

***Aedes canadensis*** develops in a wide variety of freshwater habitats, including temporary leaf-lined pools in wooded areas, roadside ditches, pools of water in open fields, and permanent swamps. They are univoltine and peak in the beginning of the season and potentially again in August following heavy rainfall events. They primarily feed on mammals, including humans and deer but have also been observed aggressively feeding on reptiles. *Ae. canadensis* is thought to be a bridge vector for EEE and may be involved in transmission of JCV and LAC.

***Aedes cantator*** is a multivoltine salt marsh species that occurs in greatest numbers during the spring. Larvae are present along the upland edge of salt marsh habitats and generally peak in mid-May. As the season advances, larvae appear in lesser numbers and become mixed with *Aedes sollicitans*, although they can generally be collected well into the fall. Inland populations are often found in floodwater areas that receive runoff from highways that are salted during the winter for ice removal. *Aedes cantator* is a significant pest because high population numbers may be present in coastal areas. *Ae. cantator* may also be involved in JCV transmission.

***Aedes communis*** is a true snow pool species and is one of the earliest mosquitoes to appear in New Jersey. Larvae are present in March and begin pupating during the 3rd week of April. The mosquito is found in mountainous habitat in New Jersey where few homes exist and is not generally a significant pest. Jamestown Canyon virus has been isolated from larvae, suggesting *Ae. communis* may play a role in the maintenance cycle of this virus.

***Aedes hendersoni*** is a multivoltine freshwater species closely related to *Aedes triseriatus* in both morphology and larval habitat. Their ranges overlap in the northeastern USA. *Aedes hendersoni* is a natural container inhabiting mosquito which can be found using deep rotted holes in maple trees higher up in the canopy. Because this species can be difficult to separate from *Aedes triseriatus*, a very common mosquito in New Jersey, reports of this species tend to be rare. *Aedes hendersoni* has been reported to feed predominantly on small mammals including tree squirrels and racoons. Its role in transmission of LAC remains unclear.

***Aedes japonicus*** uses natural and artificial containers, such as tires and rock pools, as larval habitat. It feeds mainly on mammals and can be an aggressive human biter near the preferred larval habitat. *Aedes japonicus* may be involved in the transmission of WNV, EEE, and LAC.

***Aedes punctor*** is a univoltine northern *Aedes* species with a typical northern life cycle type. Eggs are deposited singly in leaf lined depressions with moist earth at higher elevations that will become flooded with water in the spring. Larvae occur most often in heavily wooded areas early in the spring in temporary pools of cool acidic water. Adults, which are long lived, are most abundant in May and can occasionally be collected through July and into August in cold heavily shaded dense forest habitats. *Aedes punctor* primarily feeds on large mammals and can be a significant pest feeding during daylight hours and after dark. *Ae. punctor* may also be involved in JCV transmission.

***Aedes sollicitans*** is a major pest species in many areas on the east coast. Besides being a serious nuisance, the eastern saltmarsh mosquito is an important vector of Eastern equine encephalitis, Venezuelan equine encephalitis, and dog heartworm. *Aedes sollicitans* larvae develop in pools and puddles that are produced by high tides and/or heavy rainfalls. Since these aquatic systems tend to dry up quickly, emergence of adult *Ae. sollicitans* can occur in as little 4 to 5 days following egg hatch. Females obtain blood primarily from mammals and to a lesser extent from birds. In New Jersey *Ae. sollicitans* adults are most abundant in the summer into early fall. *Ae. sollicitans* plays an important role in coastal cases in N.J., although more data is needed to characterize its role in EEE transmission.

***Aedes stimulans*** is an early season univoltine species that is widely distributed in New Jersey but is more common in the northern third of the State. Larvae are primarily found in shaded woodland pools with a lining of leaf litter. *Aedes stimulans* is an important early season pest because females may enter residential areas in high numbers in search of a blood meal. *Aedes stimulans* is exceptionally long lived and can be collected in light traps into the month of August. Because of its longevity, the species is considered a probable vector of dog heartworm in northern New Jersey and may be involved in JCV transmission.

***Aedes taeniorhynchus*** is an aggressive human biter that is most common along the southern coasts from North Carolina to the Caribbean. It has been shown to transmit eastern equine encephalitis and St. Louis encephalitis in the laboratory. It is present throughout coastal New Jersey, but usually less common than *Ae. sollicitans*. Like *Ae. sollicitans*, larval habitat includes grassy salt marshes associated

with spike grass (*Distichlis spicata*) and salt meadow hay (*Spartina patens*). This mosquito will feed on birds as well as mammals.

***Aedes triseriatus*** is an aggressive daytime-biting mosquito, especially in or near infested woods. True to its nickname, *Ae. triseriatus* normally lays its eggs in pools of water accumulated in tree holes, but it will also lay eggs in man-made water holding containers, particularly discarded tires. It is the primary vector of La Crosse virus, which is transmitted vertically; La Crosse virus can survive in dormant eggs through the winter and develop into infected, flying mosquitoes in the spring. *Ae. triseriatus* may play a role in EEE transmission.

***Aedes vexans*** is a multivoltine mosquito that is found in a wide variety of freshwater habitats, often following rains. Females will feed during the day but are most active at dusk. In New Jersey, emergence of *Ae. vexans* usually begins in mid-May and populations often reach nuisance levels early in June. Adults may be present into October when autumn temperatures remain warm. Transmission of St. Louis encephalitis has been demonstrated in the laboratory. *Ae. vexans* is thought to be a bridge vector for EEE.

***Anopheles crucians*** is part of a complex of *Anopheles* species including *An. bradleyi*, *An. georgianus*, and several others that are nearly indistinguishable as adults. *Anopheles crucians* is the freshwater species found in a variety of aquatic habitats including ponds, swamps and marshes containing acidic water. The mosquito overwinters as a larva in New Jersey. Because these adult mosquitoes are difficult to separate from the salt tolerant species *An. bradleyi*, which is also found in New Jersey, adult samples are often speciated by their proximity to known larval habitat. Adults are reported to feed on a wide variety of large and small mammals including humans. They have also been reported to feed on chickens. Members of the *An. crucians* complex were important vectors of malaria. *Anopheles crucians* is considered a potential vector for EEE, SLE, and WNV based on isolations from this species.

***Anopheles punctipennis*** is a common multivoltine freshwater species that can survive in a wide variety of semi-permanent to permanent water larval habitats. These would include both natural and artificial containers, ponds, pools in intermittent streams, rainwater filled puddles, deep tire ruts, flooded pastures and roadside ditches sometimes containing a significant amount of organic matter. This mosquito feeds primarily on mammals, both small and large, but will occasionally feed on birds. It has been reported to be an important vector of human malaria and may also be associated with JCV and EEE virus transmission.

***Anopheles quadrimaculatus*** was an important vector of malaria in the southeastern United States and today is a major host of the nematode that causes dog heartworm. The species is widespread in New Jersey and is a significant pest of humans and livestock. Larvae are found in a wide variety of clean water habitats, including reservoirs, lakes, rivers, and swamps. *An. quadrimaculatus* is an important vector of human malaria and have been implicated in SLE and may be involved in EEE transmission.

***Coquillettidia perturbans*** is a significant nuisance mosquito in New Jersey and is thought to be involved in transmission of eastern equine encephalitis virus in New Jersey. Larvae use a modified air tube to attach themselves to the roots and stems of aquatic plants where they remain throughout development. *Cq. perturbans* is thought to be a bridge vector for EEE.

***Culex pipiens*** may be abundant in urban areas inhabiting artificial containers (e.g., birdbaths, discarded tires, buckets, clogged gutters) as well as catch basins and other standing, polluted water sources. It

feeds mainly on birds and occasionally on mammals, including humans. Peak feeding activity occurs from dusk into the late evening. Warmer winter temperature conditions may result in larger numbers of *Culex* species overwintering as adults, with resulting increases in early season *Culex* abundance. *Cx pipiens* is a primary vector of WNV and SLE.

***Culex restuans*** may be abundant in urban areas inhabiting artificial containers (e.g., birdbaths, discarded tires, buckets, clogged gutters) as well as catch basins and other standing, polluted water sources. Woodland pool habitat following the univoltine species emergence is also an abundant source of *Cx. restuans* in suburban and rural areas. They feed mainly on birds and occasionally on mammals, including humans. Peak feeding activity occurs from dusk into the late evening. Warmer winter temperature conditions may result in larger numbers of *Culex* species overwintering as adults, with resulting increases in early season *Culex* abundance. *Cx restuans* is a primary vector of WNV and SLE.

***Culex salinarius*** thrives in brackish and freshwater wetlands and feeds on amphibians, birds, and mammals. It is well known for biting humans. *Culex salinarius* has been identified as an important enzootic and epidemic vector of WNV and SLE and may be implicated in transmission of EEE.

***Culiseta inornata*** is a multivoltine freshwater species in the *Culex pipiens* life cycle type. Larvae can be collected from a wide variety of freshwater habitats including foul, deoxygenated water, woodland pools, snow melt pools and roadside ditches with emergent vegetation. Dredge spoil habitats also support populations of this species in New Jersey. Females will feed readily on large and small mammals and have been reported to feed on domestic chickens. Several arboviruses, including WNV, have been isolated from this species and it should be considered a potential vector.

***Culiseta melanura*** live in large swamps of mature Atlantic white cedar and red maple. To grow in the permanently wet swamps, tree roots spread out across the peat soils characteristic of these habitats. These root systems create dark air-filled voids, or crypts, that are flooded with water. These crypts are the preferred oviposition (egg-laying) sites for *Cs. melanura* and are where the larvae develop in the cool, dark, acidic waters. *Cs. melanura* overwinters as larvae in these environmentally stable protected habitats. The amount of rainfall occurring during the summer and fall of the preceding year affects the survival of the larvae during the winter period and, in part, determines the population of adult mosquitoes successfully emerging the following year. Eastern equine encephalitis (EEE) virus is maintained in a cycle between *Cs. melanura* mosquitoes and avian hosts in freshwater hardwood swamps.

## Appendix B: Trap Considerations

There are many different trap types available for mosquito surveillance. Each trap type comes with its own built-in bias which is largely based on the choice of attractant used. Examples would include traps that use light, color, CO<sub>2</sub>, a gravid trap infusion, and or a specific lure. The micro habitat where the trap is placed is also an extremely important factor to consider with respect to the mosquitoes that may be collected (e.g., a trap placed in a shaded forested habitat). Attractants aside, placing the trap on the ground, at chest height or up in the canopy will often produce significantly different results. Other variables within trap types include different “brands” of traps that are available on the market and that use a specific attractant.

Significant differences in trap collections can occur between these various designs. The differing components used to build the trap (batteries, motors, fans, tubes, collection vessels) introduce a seemingly endless source of variation. Combining attractants is yet another significant source of variability with respect to accurately reporting repeatable routine surveillance efforts. Examples here might include a BGS trap (contrasting color is one design source of attractant) with the addition of CO<sub>2</sub>. This is now a CO<sub>2</sub> baited trap without a light source sitting on the ground. Should a lure also be added the variables continue to increase and should be documented. JerseySurv allows the user to document and track attractants used with the various traps available (see table below).

Within the mosquito control community, there have been ongoing efforts to build a better trap (more efficient, less expensive, better fit for the program) to meet local needs. Perhaps the most important consideration for a mosquito surveillance program is to decide on a trap type for a specific purpose; ensure that traps are performing consistently through regular calibration checks and routine maintenance; and sustain a consistent approach over time. Should changes be incorporated into a trapping program, documenting these changes at the time they are made is very important and extremely helpful when analyzing historical data trends.

<b>Trap Name</b>	<b>JerseySurv Acronym</b>	<b>Available Attractants</b>
American Biophysics Corporation (ABC) trap	CO2-ABC	Light, CO2, Lure
Backpack aspirator	BACKPACK	None
BG Sentinel	BGSENT	CO2, BG-Lure, BG-Sweetscent, Octenol
Biogents Counter Trap	BGCOUNTER	CO2, Lure
Biogents Gravid Aedes Trap	BGGAT	Infusion
Biogents Pro - CDC-style	BGPRO-CDC	Light, CO2, Lure
Biogents Pro - EVS-style	BGPRO-EVS	Light, CO2, Lure
Biogents Pro - Sentinel-style	BGPRO-SENT	Light, CO2, Lure
Bird baited trap	BBT	Avian host
Carbon dioxide baited trap	CO2	CO2
CDC Autocidal Gravid Ovitrap (counts)	CDCAGO	Infusion
CDC Autocidal Gravid Ovitrap (presence/absence)	CDCAGO-PRES	Infusion
CDC Fay-Prince Trap	FAYPRINCE	Motion, Light, CO2
CDC miniature light trap	CO2-CDC	Light, CO2, Lure
CDC-style Gravid Trap	GRVD-CDC	Infusion
Collection Bottle Rotator Trap	CO2-ROTAT	Light, CO2, Lure
EVS (Encephalitis Vector Survey) trap	CO2-EVS	Light, CO2, Lure
Frommer Updraft Gravid Trap	GRVD-FROMM	Infusion
Gravid trap	GRVD	Infusion
Hand or another manual aspirator	ASPIRATOR	Human, None
Human Landing Counts	LANDING	Human, None
Mammal baited trap	MBT	Mammalian host
Modified CDC Autocidal Gravid Ovitrap (counts)	MODAGO	Colors, Infusion
Mosquito magnet trap	MMT	CO2, Lure
Nasci Backpack Aspirator	BACKPACK-N	None
New Jersey light trap	NJLT	Light, CO2, Lure
Omni-Directional Fay-Prince Trap	OMNIFAY	Light, CO2, Lure
Prokopack Aspirator	PROKOPACK	None
Reiter-Cummings Gravid Trap	GRVD-RC	Infusion
Resting box collection	REST	Colors
Sweep Net	SWEEPNET	Human, None
UV Light Trap	UVLT	Light, CO2, Lure

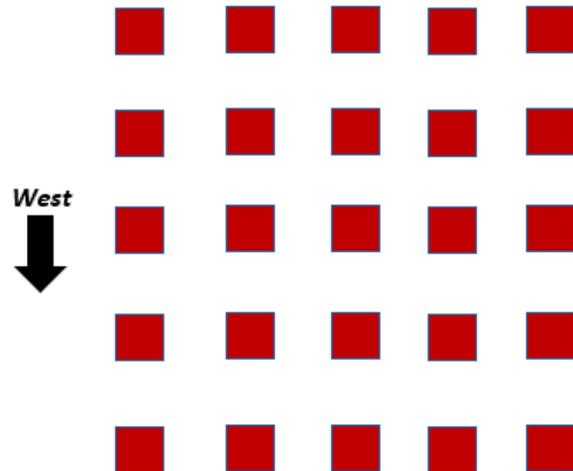
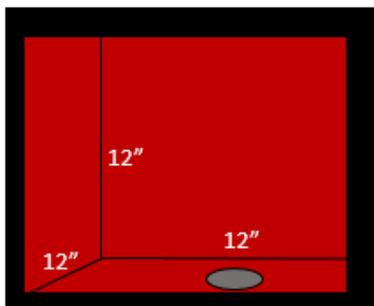
## Appendix C: New Jersey Resting Boxes & Collection Methods Protocol

For full instructions, please refer to the original document from which this guide was abridged:

Crans, Wayne J. (1995). Resting Boxes as Mosquito Surveillance Tools. *Proceedings of the 82<sup>nd</sup> Annual Meeting of the NJ Mosquito Control Association, Inc.* pp. 53-57. <https://vectorbio.rutgers.edu/outreach/restbox.htm>

*All State resting box sites MUST be collected using this methodology. It is strongly encouraged that County-run sites also be collected in this manner to provide state-wide consistency in data comparison.*

**Description:** Resting Boxes are 1 cubic foot wooden boxes which are painted black on the outside and red on the inside. The boxes should be placed in groups of 25 or 50 in thick forested areas with high canopy, adjacent to (not within) *Culiseta melanura* habitat. Place the boxes with the open-end facing West. The screened hole should be on the ground to increase humidity. Boxes should be 10 feet from one another, in a line or grid design.



If space does not allow for a grid, lines are acceptable.

**Collections:** Collections should be made between the hours of 10am and 2pm. Items to have on hand:

- Catch cover – this is to cover the end of the box & may be made of cloth, wood, or plexiglass.
- Spray – anesthetic (T.E.A., starter fluid, chloroform, etc.).
- Aspirator – mechanical or mouth (forceps may also be used).

**Method:**

1. Cover the opening of the box with the catch cover (wood, plexiglass, cloth) and tip the box to expose the screened hole.
2. Spray a few short bursts of the anesthetic into the screened hole.
3. Move to the next box and repeat (up to 3 boxes for knockdown).
4. Go back to the 1<sup>st</sup> box, remove the catch cover, and collect all the mosquitoes using the aspirator/forceps.
5. Move to the 3<sup>rd</sup> box and repeat steps 1 & 2.
6. Go back to box 2 and collect the mosquitoes.
7. Repeat until all boxes have been collected.

**JerseySurv data entry:**

- Number of nights should always be 1 (the mosquitoes are not trapped but are free to come and go; even if you haven't collected in 7 days, the number of nights will still be 1).
- Number of traps: this number depends on the program. Most state-run RB sites are in groups of 25 or 50. This number should reflect the number of boxes that you collected from during that trap collection. For example, if you collected from 25 boxes this morning, you'd enter 25 in the "number of traps" in the abundance portal.

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