Surveillance and Control of *Aedes aegypti* and *Aedes albopictus* in the United States

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Contents

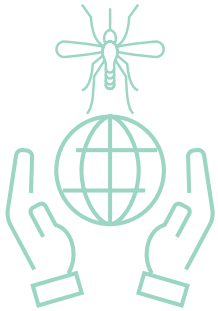
Cover photos

Top photo: *Aedes aegypti,* courtesy of James Gathany/CDC.

Bottom photo: *Ae. albopictus,* courtesy of James Gathany/CDC.

Intended Audience

Vector control professionals



Objectives

The primary objective of this document is to provide guidance for *Aedes aegypti* and *Ae. albopictus* surveillance and control in response to the risk of introduction of dengue, chikungunya, Zika, and yellow fever viruses in the United States and its territories. This document is intended for state and local public health officials and vector control specialists.

Overview

In the United States, mosquitoes transmit a variety of arboviruses (arthropod-borne viruses). This document is limited to arboviruses transmitted by *Ae. aegypti* and *Ae. albopictus*, the principal vectors of dengue (DENV-1, DENV-2, DENV-3, DENV-4), chikungunya (CHIKV), yellow fever (YFV), and Zika (ZIKV) viruses. Of the above seven arboviruses, CHIKV, DENV, YFV, and ZIKV have caused outbreaks within the United States and its territories in the past 110 years. Whereas dengue viruses are endemic to Puerto Rico, in other territories including American Samoa, Guam, Northern Mariana Islands, and the U.S. Virgin Islands, only sporadic outbreaks of DENV have occurred. Most recently, focal outbreaks of locally transmitted DENV have occurred in the continental United States including Florida, Hawaii, and Texas.

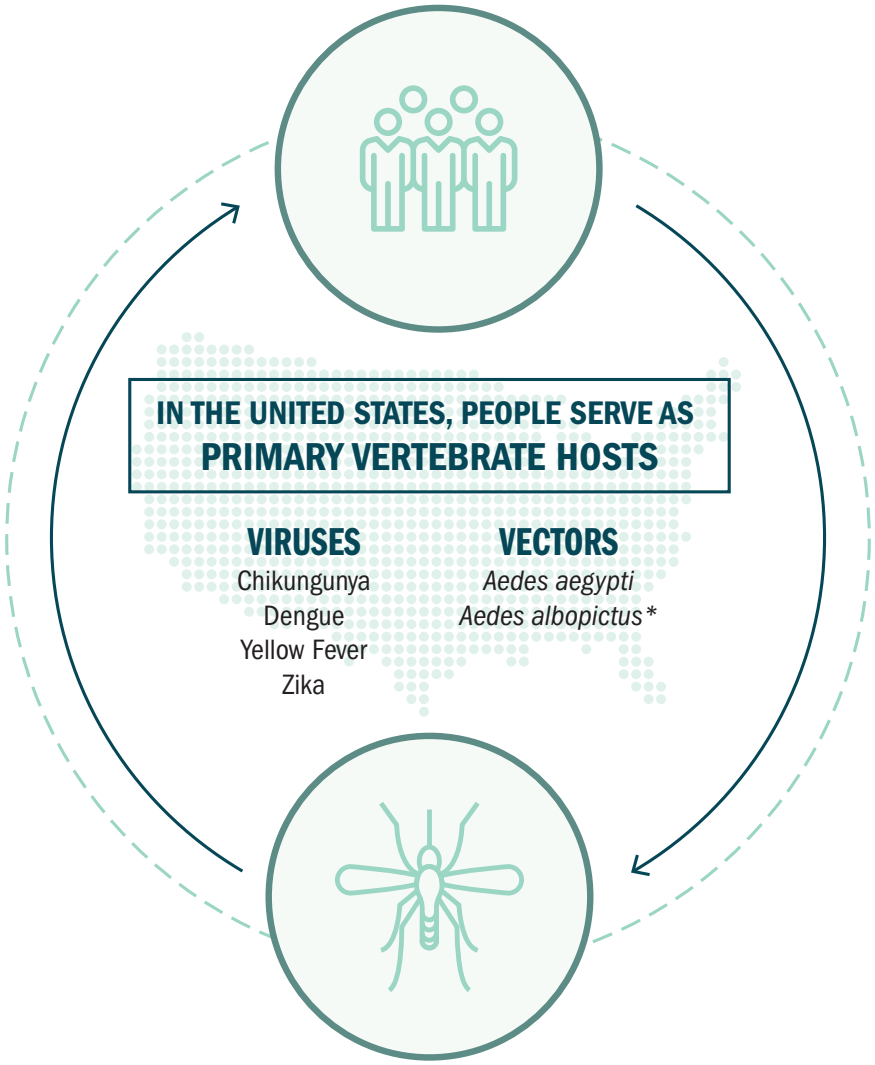
In 2014, 12 cases of locally acquired CHIKV infections were reported in Florida, and in 2015, 1 case of locally acquired CHIKV was reported in Texas. YFV, once common in the United States, has not caused locally transmitted outbreaks since 1905. However, it circulates in tropical forests of Latin America and infected travelers periodically return to the United States. In 2015, ZIKV outbreaks were, for the first time, reported in the Western Hemisphere, with local transmission occurring in Central and South America, the Caribbean, and Mexico. In 2016, local transmission of ZIKV was first reported in the United States. ZIKV transmission increased throughout the region, which increased the incidence of infection in returning travelers and contributed to local transmission in the United States.

Though none of these arboviruses continuously circulate in the continental United States, local outbreaks have and will continue to occur as a result of virus importation by infected, viremic travelers. Any viremic travelers visiting or returning to parts of the United States with established populations of *Ae. aegypti* or *Ae. albopictus* mosquitoes could initiate local virus transmission.

Transmission Cycle

CHIKV, DENV, YFV, and ZIKV are maintained in enzootic transmission cycles in forested areas of Africa, Asia, or South America (see Figure 1). YFV is only endemic in Africa and South America. However, in urban and suburban areas, these arboviruses are transmitted between people by *Aedes* mosquitoes in the subgenus *Stegomyia* especially *Ae. aegypti* (the main vector worldwide) and potentially *Ae. albopictus*.

Figure 1. In the United States and U.S. territories, people serve as the primary vertebrate hosts for *Ae. aegypti* and *Ae. albopictus* mosquitoes spreading chikungunya, dengue, yellow fever, or Zika virus.



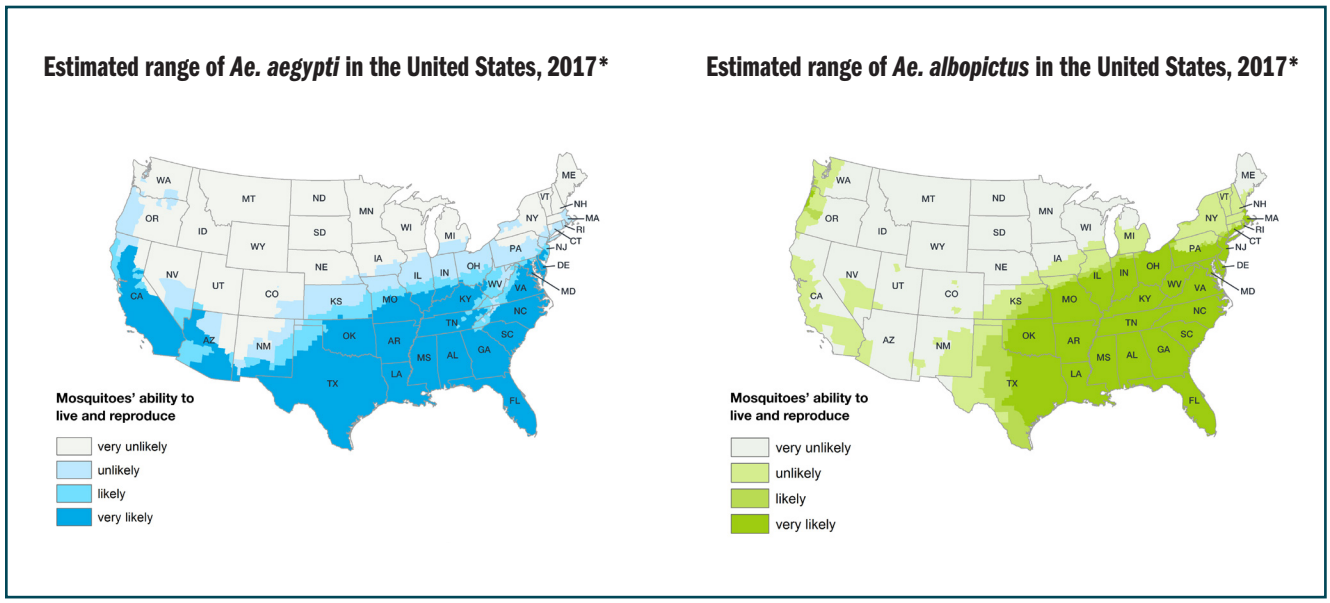
*Unproven vector of yellow fever virus

Global Distribution

Ae. aegypti most likely originated in Africa; since then, the mosquito has been transported globally throughout the tropical, subtropical, and parts of the temperate world, through global trade and shipping activities (Powell and Tabachnick 2013). *Ae. aegypti* mosquitoes have a high vectorial capacity (effectiveness of virus transmission in nature) for CHIKV, DENV, YFV, and ZIKV.

Ae. albopictus originated in Asia. Like *Ae. aegypti*, *Ae. albopictus* has been transported globally throughout the tropical, subtropical, and temperate world, primarily through international trade in used tires (Reiter and Sprenger 1987; Hawley 1988). *Ae. albopictus* has adapted to survive in a broader temperature range and at cooler temperatures, which enables them to persist in more temperate climates. These mosquitoes live in close proximity to people, but less so than *Ae. aegypti*.

Figure 2. Estimated potential range maps for *Ae. aegypti* and *Ae. albopictus* in the contiguous United States.



*These maps **DO NOT** show:

- Exact locations or numbers of mosquitoes living in an area
- Risk or likelihood that these mosquitoes will spread viruses

These maps show:

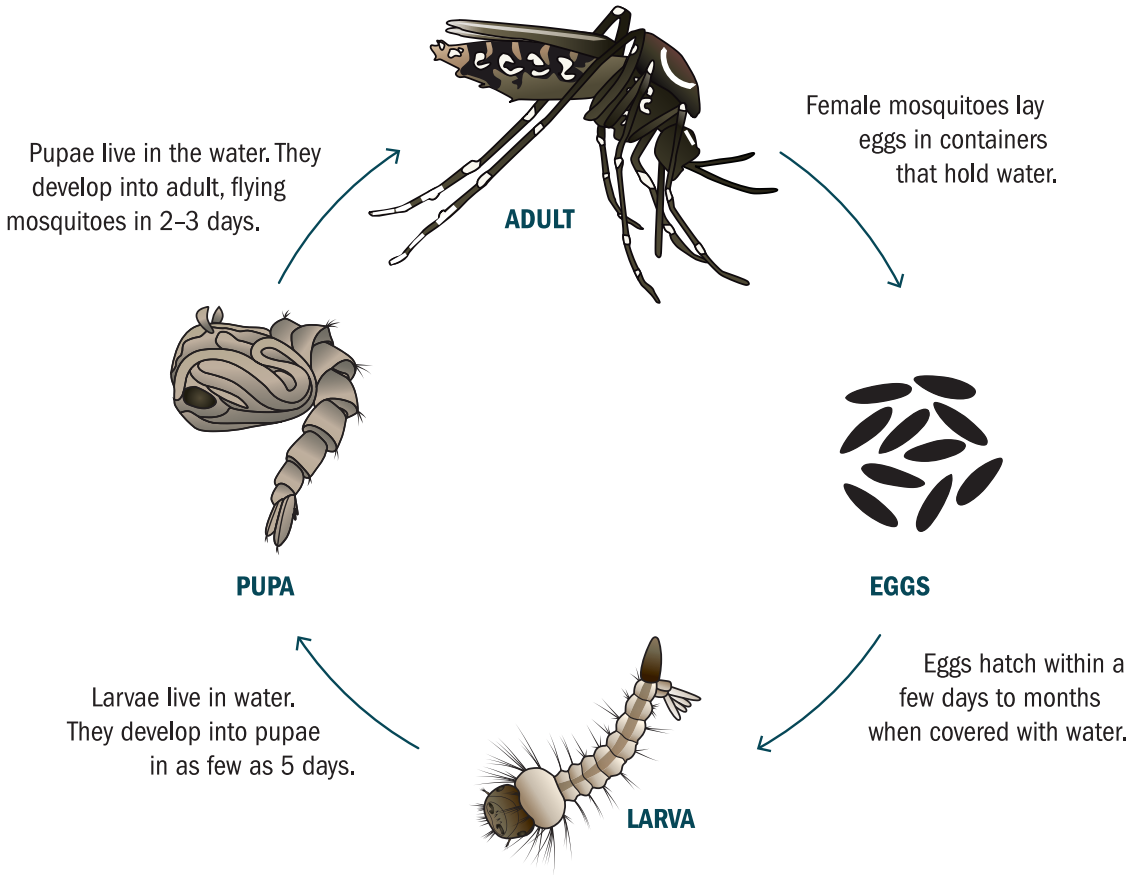
- CDC's best estimate of the potential range of *Ae. aegypti* and *Ae. albopictus* in the contiguous United States
- Areas where mosquitoes are or have been previously found

For more information on these maps, see Johnson et al 2017.

Life Cycle

Ae. aegypti and *Ae. albopictus* use natural and artificial water-holding containers (e.g., treeholes, used tires, plastic containers, clogged gutters) to lay their eggs. After hatching, larvae grow and develop into pupae and subsequently into a terrestrial, flying adult mosquito (Figure 3).

Figure 3. *Ae. aegypti* and *Ae. albopictus* life cycle.



Prevention and Control

The prevention or reduction of transmission of CHIKV, DENV, and ZIKV (there is a safe and efficacious vaccine against YFV) is completely dependent on the control of mosquito vectors and limiting person-mosquito contact. Mosquito surveillance is a key component of any local integrated vector management program. The goal of mosquito-based surveillance is to quantify human risk by determining local vector presence and abundance. The principal functions of CHIKV, DENV, and ZIKV mosquito-based surveillance programs are to:

- Determine presence or absence of *Ae. aegypti* and *Ae. albopictus* in a geographic area.
- Identify what types of containers are producing the most mosquitoes for targeting vector control efforts.
- Develop detailed maps to track larval sites if *Ae. aegypti* or *Ae. albopictus* are detected in an area.
- Collect mosquito population data and identify geographic areas of high abundance (high-risk).
- Monitor the effectiveness of vector control efforts.
    - Identify primary/secondary mosquito vectors
    - Establish thresholds at which humans get infected

Arbovirus transmission ecology varies regionally and surveillance practices vary among programs (e.g., number and type of traps, frequency of sampling, etc.) based on available funding, resources, and trained staff. However, in order to quickly identify and mitigate a mosquito-borne disease outbreak, establishing and maintaining a local vector surveillance program is critical.

Whereas mosquito-based surveillance is the preferred method for monitoring or predicting West Nile virus outbreaks, it is not the preferred method for monitoring or predicting CHIKV, DENV, YFV, or ZIKV outbreaks. For these arboviruses, it is more efficient to detect cases in people. In the United States, CHIKV, DENV, and ZIKV are nationally notifiable conditions. Healthcare providers are therefore required to report any confirmed or suspected cases to local and state health departments. In turn, health departments should immediately notify state or local vector control districts or authorities. Timely identification and response to mosquito-borne disease outbreaks like CHIKV, DENV, YFV, and ZIKV require constant communication between healthcare providers, local and state public health departments, and vector control specialists.

Effective vector-based CHIKV, DENV, YFV, and ZIKV prevention involves initiating control measures such as source reduction (container elimination) and larvicide treatments before the beginning of the mosquito season, and adult reduction measures such as adulticide treatments following detection of human arbovirus activity. Containment, a combination of procedures to prevent CHIKV, DENV, YFV, and ZIKV from spreading, may be initiated whenever a suspected/confirmed imported or locally acquired case is detected. During outbreaks a combination of containment and large-scale vector control may be used to minimize vector-human contact.

Vector Surveillance and Control Recommendations

Before mosquito season

Water-holding containers, like tires, can be removed or treated with larvicide.

- Conduct public mosquito education campaigns focusing on reducing or eliminating larval habitats for the *Ae. aegypti* and *Ae. albopictus* vectors.
- Conduct surveys to determine abundance, distribution, and type of containers; large numbers of containers may translate into high mosquito abundance and high risk.
- Initiate a community wide source reduction campaign—the goal of the campaign is to motivate the community to remove and dispose of any water-holding containers.
- Cover, dump, modify, or treat large water-holding containers with long-lasting larvicide.
- Reduce adult mosquito resting sites by keeping vegetation trimmed and tall grass cut.
- Develop mosquito education materials about *Ae. aegypti* and *Ae. albopictus* and personal protection measures.

Beginning of mosquito season

- Continue public education campaigns focusing on reducing or eliminating larval habitats for *Ae. aegypti* and *Ae. albopictus* vectors.
- Continue to distribute mosquito education materials about *Ae. aegypti* and *Ae. albopictus* and personal protection measures.
- Initiate *Ae. aegypti* and *Ae. albopictus* community-wide surveys to:
  - Determine presence or absence
  - Estimate relative abundance
  - Determine distribution
  - Develop detailed vector distribution maps
  - Evaluate the efficacy of source reduction and larvicide treatment.
- Continue/maintain community source reduction efforts.
- Initiate adult sampling to identify or confirm areas of high adult mosquito abundance.
- Initiate preventive adult control to reduce adult populations targeting areas of high mosquito abundance.
- Concentrate control efforts around places with high mosquito density.

Single or several suspected/confirmed imported/locally acquired cases

- Begin public mosquito containment education campaigns aimed at preventing or minimizing contact between vectors and suspected or confirmed human cases, especially during the first week of illness when an infected person is viremic and can infect mosquitoes, thereby possibly triggering or contributing to a local outbreak.
  - Educate the public to continually dispose of water-holding containers to eliminate larval habitats. Or, if funding allows, host a community volunteer/waste disposal program to help facilitate removal of larval habitats.
  - Treat with long-lasting larvicide any water-holding containers that cannot be dumped, covered, discarded, or otherwise modified.
  - Eliminate larval habitats within 100-200 yards/meters around a case's home.

- Initiate community source reduction, adult mosquito, and case containment initiatives to minimize the spread of infected mosquitoes.
- Educate the public about reported cases of disease and urge them to use:
  - Insect repellents
  - Window and door screens to prevent mosquitoes from entering the house
  - Air conditioning

Adult mosquito control

- Treat the outdoors within 100–200 yards/meters around a case's home with adulticide.
- Provide outdoor residual and spatial insecticide treatments; repeat as necessary to reduce vector abundance.
- Initiate/maintain adult sampling to estimate adult mosquito abundance and evaluate effectiveness of insecticide treatments.

Outbreak; clusters of suspected or confirmed cases

- Divide the outbreak area into operational management areas where control measures can be effectively applied within a few days; repeat as needed to reduce mosquito density.
- Conduct door-to-door inspections and mosquito control in an area-wide fashion (reach >90% coverage of the control area within a week).
- Identify and treat, modify, or remove mosquito-producing containers.
- Organize area/community clean-up campaigns targeting disposable containers (source reduction), including large junk objects that accumulate water (broken washing machines, refrigerators, toilets) in buildings, public areas, etc.
- Combine outdoor spatial and residual spraying with source reduction and larviciding (including residual spraying of container surfaces and adjacent mosquito resting areas). Remember to treat storm drains, roof gutters, and other often overlooked cryptic water sources.

Specimen Collection and Types of Traps

Ovitraps

Ovicups are a type of ovitrap used to attract gravid females looking for a place to lay their eggs.

Ovitraps are small metal, glass or plastic containers, usually dark in color, containing water and a substrate (wood, seed germination paper, cloth, plant gel) where female mosquitoes lay their eggs. Ovitraps can be used to detect the presence of gravid *Ae. aegypti*, *Ae. albopictus* and a wide variety of other gravid females of container *Aedes* mosquitoes (Fay and Eliason 1966; Mackay et al. 2013; Reiter et al. 1991). Ovitraps take advantage of the fact that gravid *Ae. aegypti* and *Ae. albopictus* females lay their eggs in artificial containers. Adequate sampling requires regular (weekly) trapping at fixed sites, representative of the habitat types, present in the community. Ovitraps should not be deployed in the field for more than a week at a time because they could become larval sites and may begin producing adult mosquitoes, however, some ovitraps are specifically designed not to produce mosquitoes (Chan et al. 1977; Barrera et al. 2013).

Ovitraps have several advantages, including being inexpensive, easily deployed, and not invasive. A small number of ovitraps is usually enough to determine vector presence; less than 100 ovitraps can reliably estimate abundance in a large urban neighborhood (Mogi et al. 1990). Typically, one ovitrap is placed per city block. Lastly, ovitrap data is easy to analyze; it is usually expressed as the percentage of positive ovitraps (ovitraps with eggs). The mean number of eggs per ovitrap can be used to estimate adult mosquito abundance.

Interpreting ovitrap data may require caution, because ovitraps compete with naturally occurring larval habitats and the estimates from oviposition surveys may not accurately reflect the abundance of gravid females under some conditions. For example, oviposition indices may be skewed after source reduction campaigns when gravid females find fewer suitable habitats and lay a larger proportion of eggs in the ovitraps confounding the evaluation of control efforts (Focks 2003). Some degree of training in microscopy may be needed for accurate egg counting especially when there is debris on the oviposition surfaces. Lastly, the collected eggs need to be hatched and reared out in the laboratory and the larvae or adults identified to species, which requires trained personnel.

Immature stage (larvae and pupae) surveys

Because of a wide variety in type, size, and shapes of water-holding containers, there is no standard equipment for sampling the immature stages of mosquitoes that lay eggs in containers. If the container is large enough, such as a 55-gallon barrel, a dipper or net may be used. However, the common containers are small cans, tires etc., and usually the entire contents are emptied onto a tray or a pan and the immature stages picked out using a dropper. The immature stages are usually reared out in the lab and identified to species.

Adult mosquito trapping

Ae. aegypti and *Ae. albopictus* are not efficiently captured by the most commonly used mosquito traps, such as the CDC miniature light trap, CDC gravid trap, or the New Jersey light trap. Several fan-operated traps are designed to capture *Ae. aegypti* adults, which take advantage of the propensity of this species to be attracted to dark objects (Fay 1968; Fay and Prince 1970; Freier and Francy 1991; Wilton and Kloter 1985). The Fay-Prince trap has been the most widely used, but it is heavy and bulky, making it difficult to use in sufficient numbers to obtain reliable estimates of mosquito abundance. Currently the most commonly used adult traps for *Ae. aegypti* and *Ae. albopictus* are BG-Sentinel traps, and a variety of gravid traps such the CDC-Autocidal Gravid Ovitrap (CDC-AGO) (Mackay et al. 2013; Barrera et al. 2014a, b).

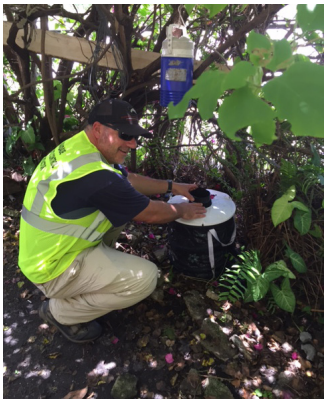
BG-Sentinel trap: The BG-Sentinel traps use a combination of attractive visual and olfactory cues. They have the advantage of being collapsible and light. BG-Sentinel traps are more effective in capturing *Ae. aegypti* than CDC backpack aspirators, and also collect adult females in all physiological states (Maciel-de-Freitas et al. 2006; Williams et al. 2006;

Ball and Ritchie 2010). These traps are also effective for collecting *Ae. albopictus* (Meeraus et al. 2008; Bhalala and Arias 2009; Farajollahi et al. 2009; Obenauer et al. 2010). The efficiency of BG-Sentinel traps can be increased by baiting them with lures (e.g., CO2, BG-Lure®).

Gravid female traps: : A number of recently developed traps use similar principles of attraction as ovitraps; that is, to attract and capture gravid females. These traps use either funnels (Gomes et al. 2007, Eiras et al. 2014) or sticky boards (Mackay et al. 2013; Chadee et al. 2010; Barrera et al. 2013) to prevent captured mosquitoes from escaping. The advantage of gravid traps is that they are considerably cheaper and easier to operate compared to BG-Sentinel traps.

Mechanical aspirators: Several aspirator devices may be used to collect resting mosquitoes. Collecting resting mosquitoes provides a good representation of vector population structure since un-fed, gravid, and blood-fed females (as well as males) may be collected (Service 1992). Since resting populations typically provide representative samples of the population, they will also provide more representative information on population infection rates. Handheld or backpack mechanical aspirators can be used to remove mosquitoes from natural resting harborage or artificial resting structures (e.g., wooden resting boxes, red boxes, fiber pots and other similar containers) (Service 1992). Aspirators are particularly useful for collecting *Ae. aegypti* indoors. The data obtained from this collecting technique provide more representative data on mosquito abundance per unit area (e.g., per house, master bedroom, etc.). Sampling indoors can be standardized such as aspirating for 15 minutes per house, etc., but frequently there are large variations in number of mosquitoes collected per house, therefore, this technique requires sampling large numbers of houses in short periods of time (e.g., 100-200 houses per neighborhood). Due to the wide variety of resting sites and the low density of resting mosquitoes in most locations, sampling resting populations especially outdoors is difficult to standardize, labor intensive, requires trained personnel, and sufficient sample sizes are often difficult to obtain.

Landing–biting counts: This is one of the oldest and most effective, but labor-intensive techniques used to detect, capture, and quantify host-seeking biting mosquito vectors such as *Ae. aegypti* and *Ae. albopictus*. However, due to potential health risks to field staff, especially in areas with ongoing arbovirus transmission, CDC does not recommend this technique. Another limitation of this collection method is the inherent variation among collectors both in attracting and collecting specimens. A tent trap has been recently developed, which can provide protection to collectors from mosquito bites (Casas-Martinez et al. 2013).



BG-Sentinel Traps target female mosquitoes looking for a blood meal.

Autocidal gravid ovitraps captures gravid female mosquitoes.

Large, handheld mechanical aspirator removes mosquitoes from natural resting harborage or artificial resting structures.

Mosquito-based Surveillance Indicators

Data derived from mosquito surveillance primarily estimates mosquito abundance; estimates are used to indicate levels of risk. The indices derived from those data vary in information content, ability to be compared over time and space, and association with arbovirus transmission levels and levels of human risk. The indicators that are commonly used can be broadly divided into 1) immature stage (larvae and pupae) survey indices, 2) eggs per ovitrap per week, 3) female mosquitoes per sticky gravid trap per week, and 4) adult infection rates (IR).

Immature stage survey indices

Larval surveys (*Stegomyia* indices): Larval surveys usually involve identifying all or most of the immature mosquitoes found in every container (or a representative sample of containers) in the target area, home(s) community, neighborhood etc. Every water-holding container is inspected and categorized as positive (contains larvae/pupae) or negative otherwise (no larvae/pupae). The second and less used method is single-larva surveys where only a single larva is identified from each container (Sheppard 1969). The container indices below are computed from survey data.

- House Index (HI; percentage of houses with at least one positive container)
- Container Index (CI; percentage of all containers with water that are larva/pupa positive), and
- Breteau Index (BI; number of positive containers per 100 houses [Connor et al. 1923; WHO 2009]).

Mosquito thresholds for CHIKV, DENV, YFV, and ZIKV transmission using larval indices should be determined by each local vector control program for each location; state or national wide thresholds should be used with caution. It was proposed that a House Index of 5% (Soper 1967), a Container Index of 10% (Connor et al. 1923), or a Breteau Index of 5 (Brown 1977) prevented YFV transmission, and that HI of 1% suppressed DENV transmission (Pontes et al. 2000). Such thresholds may not apply to all locations and to all arboviruses. A recent study in Taiwan reported the following container *Aedes* threshold values for DENV transmission: BI= 1.2, CI= 1.8%, and HI= 1% (Chang et al. 2015).

Pupal surveys: Pupal surveys (pupae per house, per person, per hectare) are based on the assumption that pupal productivity is a better estimate of the adult population than the traditional indices (HI, CI, and BI) or larval counts (Focks 2003). Pupal surveys can also identify the types of containers that produce the majority of adult mosquitoes; these data can help vector control programs identify target containers for enhanced surveillance and control (Focks and Chadee 1997; Nathan and Focks 2006). Pupal surveys usually involve sampling large numbers of houses and containers to obtain reliable estimates (Reuben et al. 1978; Barrera et al. 2006a, b). However, several methods have been developed to guide sample size requirements for pupal surveys (Alexander et al. 2006; Barrera et al. 2006a, b; Barrera 2009).

As with larval surveys, pupal surveys to determine CHIKV, DENV, YFV, and ZIKV transmission thresholds (pupal abundance indices) should be determined by each local vector control program for each location. Currently, there is no information on pupal indices on CHIKV and ZIKV transmission, however, some models show that it takes between 0.5 and 1.5 *Ae. aegypti* pupae per person to sustain DENV transmission at 28˚C in a human population with 0–67% immunity (Focks et al. 2000).

Eggs per ovitrap per week. Although no specific threshold values have been established for each arbovirus, absence of severe DENV cases in Thailand was noted when the densities of *Ae. aegypti* eggs per ovitrap per week was less than two (Mogi et al. 1990). Also, although using a different ovitrap, DENV transmission occurred in Taiwan when the density of eggs per house (2 ovitraps/house) was around two (Wu et al. 2013).

Female adults per sticky trap per week. Sticky gravid traps used for *Ae. aegypti* surveillance during a DENV outbreak in Australia indicated that a density of two or more females per trap per week was associated with DENV cases, whereas a density of less than one female per trap per week was a safe level (Ritchie et al. 2004). A recent study showed lack of local CHIKV transmission when the density of *Ae. aegypti* was less than two per sticky AGO trap per week in Puerto Rico (Lorenzi 2016; Barrera 2017).

Adult infection rates

In the past, *Ae. aegypti* and *Ae. albopictus* surveillance has relied heavily on immature indices because until recently it has been difficult to monitor adult mosquito abundance. However, the BG-Sentinel trap and a variety of gravid traps make it possible to accurately estimate adult mosquito abundance and to track infected mosquitoes. Tracking adult infected mosquitoes may help establish entomological infection rate thresholds for human disease risk for CHIKV, DENV, YFV, and ZIKV transmission similar to work performed for West Nile, St. Louis, and Eastern equine encephalitis viruses (CDC 2013).

The infection indices used are the same as those used for other arboviruses: Minimum Infection Rate (MIR), Maximum Likelihood Estimates of the Infection Rate (MLE), and Vector Index (VI) (CDC 2013). However, adult mosquito infection rates cannot be used to predict outbreaks in CHIKV, DENV, YFV, and ZIKV surveillance programs because of the very limited data on infection rates and prevalence of human infections. Data obtained in DENV surveillance programs show that, in some cases, an elevation in mosquito infection rates precede outbreaks or increased transmission (Chow et al. 1998; Mendez et al. 2006) but not in others (Chen et al. 2010). These mixed results make it difficult to establish threshold mosquito infection rates for human infections and outbreaks for DENV. However, these studies used different mosquito collection methods. There is a chance that data obtained from BG-Sentinel traps and gravid traps may improve abundance and infection rate estimates and provide timely risk assessment.

Handling of Field-Collected Adult Mosquitoes

Because virologic surveillance relies on identifying CHIKV, DENV, YFV, and ZIKV in the collected mosquitoes through detection of viral proteins, viral RNA, or live virus, efforts should be made to handle and process the specimens in a way that minimizes exposure to conditions (e.g., heat, successive freeze-thaw cycles) that would degrade the virus. Research has shown that CHIKV, DENV, and ZIKV RNA could be detected by RT-PCR in dead mosquitoes exposed in sticky cards or dried at ambient temperature for several weeks (Bangs et al. 2001; Mavale et al. 2012; Burkhalter and Savage 2017).

- Optimally, a cold chain should be maintained from the time mosquitoes are removed from the traps to the time they are delivered to the processing laboratory, and through any short-term storage and processing.
- Transport mosquitoes from the field in a cooler either with cold packs or on dry ice. Sort and identify the mosquitoes to species on a chill-table or tray of ice if available.
- If arbovirus screening is not done immediately after mosquito identification and pooling, the pooled samples should be stored frozen, optimally at -70°C, but temperatures below freezing may suffice for short-term storage.

Mosquitoes are generally tested in pools no greater than 50 and only female mosquitoes are tested in routine arbovirus surveillance programs. Arboviruses can be detected in mosquito pools by using RT-PCR assays (Lanciotti et al. 1992; Lanciotti et al. 2007; Lanciotti et al. 2008; Laurent et al. 2007; Ummul Haninah et al. 2010; Santiago et al. 2013; Savage et al. 2015; Chow VTK et al. 1998; Shu et al. 2003; Chien et al. 2006; Santos et al. 2008; Chen et al. 2010; Balm et al. 2012; Faye et al. 2013; Dash et al. 2012).

Limitations to Mosquito-Based Surveillance

- Currently available information on adult infection rates and larval/pupal indices may not predict risk for human infection.
- Larval/pupal surveys may miss cryptic, often overlooked habitats (e.g. gutters, broken septic tanks, sprinkler heads/assemblies, storm drains, etc.) and fail to provide accurate data on the relative abundance of the vector species.
- Larval/pupal indices may not correlate with adult mosquito abundance.
- Developing useful thresholds requires consistent effort to assure the surveillance indices and their association to human risk is comparable over time. Mosquito surveillance and human disease incidence data collected over several transmission seasons is required to produce useful predictive indicators. However, this is challenging to obtain with only sporadic arboviral outbreaks.

Vector Control

General guidelines for the diagnosis, treatment, prevention, and control of CHIKV and DENV have been published (PAHO 2011; WHO 2009).

Control of immature stages

An important step in *Ae. aegypti* and *Ae. albopictus* control operations is identifying the types and abundance of containers producing mosquitoes and their productivity. Different containers require specific control measures that depend on the nature of the container and how it is used. Five general types of containers produce *Ae. aegypti* and *Ae. albopictus*:

- Phytotelmata (treeholes, leaf axils, etc.)
- Non-essential or disposable containers (food and drink containers, tires, broken appliances)
- Useful containers (water-storage vessels, potted plants and trivets, animal drinking pans, paint trays, toys, pails, septic tanks)
- Cavities in structures (fence poles, bricks, uneven floors and roofs, roof gutters, air-conditioner trays)
- Outdoor underground structures (storms drains, water meters, public wells, septic tanks)

Commonly used control methods

Environmental sanitation: This is the permanent elimination of containers producing *Ae. aegypti* and *Ae. albopictus* such as establishing reliable supplies of piped water, municipal refuse recycling programs (glass, metal, and plastic), used-tire recycling operations, replacing septic tanks with sewerage, etc.

Larvicides: This is the use of chemicals or biological agents to kill or prevent development of mosquito immature stages. A number of agents can be used to control mosquito production in containers:

- Biological larvicides: These include products containing *Bacillus thuringiensis* var. *israelensis* (Bti), spinosad, and Insect Growth Regulators (IGR's) such as juvenile hormone analogs (methoprene, pyriproxyfen) and chitin synthesis inhibitors (Diflubenzuron, Novaluron). Biological larvicides have little or no impact on non-target organisms and do not accumulate in the environment.
- Monomolecular films and oils. These products spread on the water surface forming a thin film that causes suffocation of immature mosquitoes by preventing gas exchange.

Evaluation of the effectiveness of pre-adult mosquito control may be accomplished by comparing the presence/absence and abundance of immature stages in treated containers before and after treatment or by comparing treated and untreated areas (Chadee 2009).

Larvicides can be used to treat standing water that cannot be covered, dumped, or removed.

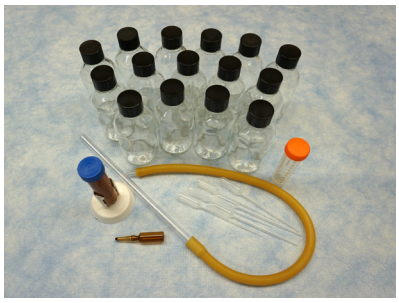
Biological control: A variety of aquatic predators may be used especially in large containers. These include carnivorous copepods and larvivorous fish *(Gambusia affinis).* However, biological control may not be practical especially since *Ae. aegypti* and *Ae. albopictus* often develop in small containers that may completely dry out between rainfall events.

Control of adult mosquitoes

Chemical control:

- Chemical control of adult mosquitoes includes space spraying, residual spraying, barrier spraying, and using attractive toxic baits.
- Barrier spraying of residual insecticides on external walls of houses and vegetation has been effectively used to reduce exposure to exophilic mosquito species (Anderson et al. 1991; Perich et al. 1993l Cilek 2008), including *Ae. albopictus* (Trout et al. 2007).
- Residual insecticides are used on surfaces that adult mosquitoes frequently visit and land on, such as walls and ceilings, discarded containers, vegetation, curtains, covers for water-storage vessels, lethal ovitrap oviposition strips, etc. There is evidence that indoor residual spraying (IRS) is particularly effective for controlling *Ae. aegypti* (Chadee 1990; Vazquezp-Prokopec et al. 2010) primarily due to its indoor resting behavior. However, there are concerns about continuous insecticide exposure for the residents. In the continental United States, many houses are air conditioned or have screening preventing *Ae. aegypti* from establishing itself indoors. In such structures, the need for indoor residual spraying is not necessary.
- Space spraying of insecticides is carried out by backpack, truck- or air-craft mounted equipment.

Using insecticides to control mosquitoes should always include insecticide resistance monitoring and management. Insecticide resistance has been demonstrated in almost every class of insecticide, including microbial pesticides and IGRs (Brogdon and McAllister 1998a). Insecticide resistance, which is an inheritable trait, usually leads to significant reduction in the susceptibility of insect populations which renders insecticide treatments ineffective. Insecticide resistance may be monitored using bioassays in larvae and adult mosquitoes (WHO 2009; Brogdon and McAllister 1998b).



The CDC bottle bioassay kit is used to determine whether a particular insecticide is effective against local mosquito species.

Physical control (non-insecticidal mosquito traps): Gravid female mosquitoes can be lured to traps baited with an oviposition medium and captured using sticky glue while attempting to lay eggs (CDC Autocidal Gravid Ovitrap, AGO trap; Barrera et al. 2014a, b; Mackay et al. 2013). The use of three AGO traps per home in more than 85% of houses in neighborhoods in southern Puerto Rico has shown sustained and effective reductions of *Ae. aegypti* populations (80%).

Personal protection

Insect repellents: CDC recommends the use of products containing active ingredients that have been registered by the U.S. Environmental Protection Agency (EPA) for use as insect repellents applied to skin and clothing. EPA registration of insect repellent active ingredients indicates the materials have been reviewed and approved for efficacy and human safety when applied according to the instructions on the label. For more details, see https://www.cdc.gov/zika/prevention/prevent-mosquito-bites.html.

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