Guidelines for *Aedes aegypti* and *Aedes albopictus* Surveillance and Insecticide Resistance Testing in the United States

Version 2, 11/9/16

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Intended Audience

These surveillance guidelines were developed for local, state, and territorial health departments and cities in the United States receiving Epidemiology and Laboratory Capacity cooperative agreement funding.

Part 1: Guidelines for Conducting Surveillance and for Reporting Presence, Absence, and Relative Abundance of *Aedes aegypti* and *Aedes albopictus*

Background

Aedes aegypti and *Aedes albopictus*, known vectors of Zika, dengue, and chikungunya viruses, are present in parts of the United States. They are the only known members of the *Aedes* sub-genus *Stegomyia*, the most important vectors of these viruses, known to occur in the continental United States. Maps on the CDC website (http://www.cdc.gov/zika/vector/range.html) show the areas where these mosquitoes are known or suspected to occur. *Ae. aegypti* is more common in the warmer, southern states. *Ae. albopictus* occurs over

a greater range and can extend into more temperate climates. Both species can, however, be introduced seasonally to areas with climates unlikely to allow overwintering. Such introductions may initiate and sustain temporary local transmission of arboviruses during the warm part of the year.

Surveillance Aims

- To improve knowledge of the distribution and local abundance of *Ae. aegypti* and *Ae. albopictus* in the United States.
- To produce and publically post regularly updated mosquito distribution maps.

This information should be used to guide vector surveillance and control activities targeting *Ae. aegypti*- and *Ae. albopictus*-borne arboviral diseases in the continental United States.

Surveillance Recommendations

A) Surveillance should be conducted in each county where *Ae. aegypti* or *Ae. albopictus* are likely to be found. As many locations as possible should be monitored in these counties (especially cities/towns). More locations in the surveillance program will increase the chances of detecting these mosquitoes, if they are present, thus providing information necessary for developing accurate distribution maps. Negative surveillance results are important information, and reporting of such negative results to CDC is strongly encouraged but might not provide definitive evidence for absence of these species within a county.

B) *Ae. aegypti* is adapted to human habitations; it is less likely to be found in rural areas, parkland, or other open or sparsely populated areas. *Ae. albopictus* can be found in a wider variety of habitats including wooded, forested, and rural areas away from population centers, as well as human habitations. The best locations for *Ae. aegypti* and *Ae. albopictus* surveillance are
   - Highly populated urban/suburban centers
   - Tire dumps, trash dump sites, junk yards, and cemeteries
   - Port cities, cities along rivers, and cities with high commercial traffic
   - Historic urban areas, which have many underground hibernation sites

C) Using higher numbers of traps per surveillance location increases the chances of detecting *Ae. aegypti* and *Ae. albopictus*. Methods should be used to survey for adults as well as immature stages and mosquito eggs. Of the methods available, the least expensive are sampling from containers and distributing ova collection cups. Traps that collect adults are relatively expensive and their usefulness might differ between regions; for example, in the desert of the Southwest, light traps are reported to efficiently sample *Ae. aegypti* (Monaghan et al. 2016). Collection specifics can be modified based on professional local experience with the targeted mosquito species, but collection should be systematic.
   - Adult *Ae. aegypti* and *Ae. albopictus* can be sampled by using
      i. **BG-Sentinel Trap®** (http://www.bg-sentinel.com/). This trap targets blood meal-seeking female mosquitoes. It can be used with a proprietary lure (BG-Lure®). Similar to BG-Sentinel traps, light traps target blood meal seeking females. Light traps have

been reported to be effective in the arid Southwest but less so elsewhere. Relative abundance can be reported as the number of mosquitoes collected per trap per day.

ii. **Autocidal Gravid Ovitrap (AGO)** (https://springstar.net/store/). This trap targets gravid female mosquitoes. Ovipositing females get stuck to the adhesive on the oviposition substrate. Relative abundance can be reported as the number of mosquitoes collected per trap per day.

iii. **Mechanical collections with aspirators**. Hand-held or backpack aspirators can be used to sample resting mosquitoes indoors or outdoors. Outdoors, these mosquitoes usually rest under vegetation or in shaded corners; indoors, they are often found in closets and other undisturbed, dark places. Relative abundance can be reported as number of mosquitoes collected per unit time of active sampling; for example the number of mosquitoes collected at a site in 15 minutes.

- Immature stages are sampled by examining water-holding containers for larvae and pupae. Because of the wide variety of water-holding containers in which the mosquitoes can be found, there is no standard equipment for sampling immature stages. When possible, the entire water content should be emptied onto a tray or a pan and the immature stages picked out using a dropper. Larger containers can be sampled by using dippers or utensils of various size (e.g., turkey basters, pipettes, or ladles). The entire contents may be poured through sieves to strain out immature mosquito stages. The aim is to collect a representative sample; the higher the number of locations inspected the more representative the sample.

  With experience, the third and fourth instar larvae can be identified microscopically (Farajollahi and Price 2013), but the easiest, albeit slower, means of identifying immature stages is to rear them to adults. Care should be taken to ensure data on original collection sites are not lost in the rearing process.

  From the data collected, larval and pupal indices are calculated. Commonly used indices include the House Index (HI), the Container Index (CI), the Breteau Index (BI), and pupal surveys to estimate the number of pupae per house, per person, or per hectare. Further descriptions of these indices can be found at https://www.cdc.gov/chikungunya/resources/vector-control.html.

- Egg sampling is conducted using ovitraps (ovicups). Ovitraps are small black containers made of glass or plastic, which contain water or hay infusion and a substrate (typically a strip of paper, such as coffee filter or germination paper) on which females lay their eggs. Ovitraps are simple, inexpensive and easy to deploy. CDC uses black plastic 22 oz. souvenir cups (giacona.com, product MTC22T) and Anchor Paper (http://www.anchorpaper.com/index.php/seed-solutions/germination-papers/#heavyweightpaper, product SD 7606) as oviposition substrates. Anchor Paper is cut into 10 by 3 inch pieces and placed inside the cups. Eggs should be reared out in the laboratory and identified to species as adults to ensure definitive species identification. Data should be reported as number of eggs per trap per day. More information can be found at https://www.cdc.gov/chikungunya/resources/vector-control.html.

- Some state/county health departments and mosquito abatement districts are already using light traps and CDC gravid traps to survey for the *Culex* vectors of West Nile virus. Setting traps specifically for *Ae. aegypti* and *Ae. albopictus* at some of these locations, if they include

suitable collection environments, may reduce sampling time and effort. To avoid interference between types of traps

- Where light traps are being operated, only ovitraps and AGO traps should be deployed.
- Where CDC gravid traps are installed, only BG Sentinel traps should be used.

D) Surveillance should begin when daytime maximum outdoor temperatures consistently reach 50°F (10°C) and continue as long as these temperatures are maintained. BG-Sentinel traps should be checked daily, AGO traps should be checked once or twice a week, and ovitraps at least every 3 days. Sampling multiple days a week is recommended to compensate for weather variations that may affect sampling efficiency. In areas with seasonal mosquito activity, surveillance may be discontinued when daytime maximum outdoor temperatures consistently drop below 50°F or after the first frost. For areas where daytime maximum outdoor temperatures do not drop below 50°F, surveillance should be conducted year-round. The same sites for trapping should, as far as possible, be used throughout the season and between years to provide a basis for comparison over time. It might be necessary to move traps or increase the number of traps used in sectors where an outbreak is occurring or control efforts must be monitored.

E) Recording data. Accurate records are essential. A unique identification number should be assigned to each collection. Additionally, the following minimum information should be recorded:

- Collection location
    i. City/town and county
    ii. Neighborhood/address
    iii. GPS coordinates
- Habitat type
- Life stage targeted
- Collection method
- Collection date
- Presence of eggs, larvae or pupae of *Ae. aegypti* or *Ae. albopictus*
- Number of *Ae. aegypti* or *Ae. albopictus* adults, separately by sex

Reporting to CDC

CDC has launched the MosquitoNET online portal to collect data for mosquito presence and abundance, and for insecticide resistance testing. The MosquitoNET portal can be accessed at http://www.cdc.gov/zika/vector/for-professionals.html. Following initial set-up of a user account, monthly data reporting is required.

CDC Use of Reported Data

The data will be used to regularly update distribution maps of *Ae. aegypti* and *Ae. albopictus* in the United States. The maps will be posted on a CDC website accessible to the general public. The data will be used to guide surveillance and control efforts against *Ae. aegypti* and *Ae. albopictus* and their associated arboviruses.

For further questions on mosquito surveillance, contact: CDCMosqSurveillance@cdc.gov

References

Surveillance and Control of *Aedes aegypti* and *Aedes albopictus* in the United States http://www.cdc.gov/chikungunya/pdfs/surveillance-and-control-of-aedes-aegypti-and-aedes-albopictus-us.pdf

Daytime Traps for *Aedes aegypti* and *Aedes albopictus*. http://johnwhock.com/products/mosquito-sandfly-traps/

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Part 2: Insecticide Resistance Testing for Known Mosquito Vectors of Zika, Dengue, and Chikungunya Viruses

Background

Insecticides to control larval and adult stages of *Aedes aegypti* and *Aedes albopictus* are important components of an integrated mosquito management (IMM) program. With frequent use, insecticide resistance (IR) may develop, particularly with insecticides used for adult control. Systematic resistance testing is an important tool for choosing the types of insecticides that will be most effective. Recent insecticide resistance testing for *Ae. aegypti* in Puerto Rico provides an excellent example of how systematically generated data on spatial patterns of resistance to specific insecticides can be displayed as maps (http://www.cdc.gov/zika/vector/insecticideresistancetesting.html) and used to guide emergency control efforts.

Testing Aims

To better enable local, state, and national programs to effectively respond to epidemics of Zika, chikungunya, and dengue viruses through control of *Ae. aegypti and Ae. albopictus*. An additional aim is to improve our knowledge of evolving geographical patterns of insecticide resistance and devise programs for managing it.

CDC Testing Recommendations

A) Testing strategy
- Testing of mosquito populations should occur at least once a year to monitor for changes in insecticide susceptibility.
- Testing should be done before selection of insecticide products to be used in the next mosquito season.
- Testing should be done in every municipality where mosquito control is conducted.
- It is important to determine which public health insecticides have been used in an area during the past 5 years. Testing by using technical grade active ingredients should be done for all adulticides that are currently or were recently used locally. In some areas, the ancillary effects of agricultural insecticides might contribute to resistance. It is also useful to test active ingredients not currently used but commercially available, in order to be prepared should an alternative insecticide be needed. Adulticide active ingredients currently registered for use in the United States are malathion, naled, chlorpyrifos, permethrin, sumethrin (d-phenothrin), prallethrin, deltamethrin, etofenprox, and pyrethrins.
- To ensure that local resistance has not developed in mosquitoes due to use of pesticides for reasons other than mosquito control, a quick resistance assessment should be done before emergency adulticiding, even in areas where no mosquito control was conducted during the past 5 years.
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B) Testing methods
- The CDC bottle bioassay (http://www.cdc.gov/parasites/education_training/lab/bottlebioassay.html) should be used for testing.
- The primary goal is to determine local susceptibility of *Ae. aegypti* and *Ae. albopictus* to the main classes of insecticides. By 2017 or earlier, it should be a goal to also establish

mechanisms of resistance (http://www.irac-online.org/about/resistance/mechanisms/). Knowledge of resistance mechanisms can more precisely guide choice of alternate chemicals.

- Resistance mechanisms can be determined by using a variety of assays. At a minimum, the CDC bottle bioassay can be used to assess resistance mechanisms by adding enzyme inhibitors for the 3 classes of metabolic enzymes that can be overexpressed, resulting in resistance. These inhibitors counteract overexpression of enzymes and can be used to determine which enzyme class is responsible for the observed resistance (see: using these inhibitors [http://www.cdc.gov/parasites/education_training/lab/bottlebioassay.html]). Additional testing to measure expression of resistance enzymes (microplate assays) and molecular tests can also be performed (McAllister et al. 2012, Scott and McAllister 2012).

C) General notes on mosquitoes used for testing

- Approximately 125 mosquitoes per chemical per site are needed to run a test. Results will be most accurate if adults collected the same day from the same site are used. Because of their relative scarcity, this may not be practical in all cases.
- Pooling of test mosquitoes from a large geographic area should be avoided if at all possible because the overall results for the larger area can mask local insecticide resistance.
- If necessary, field collected females allowed to oviposit in the laboratory can be used to generate mosquitoes for testing. Insecticide resistance assays should be conducted with the first generation brood if possible, because successive blood feeding of mosquitoes to generate large specimen numbers for testing can either mask or overestimate the level of resistance in a population.
- Mosquitoes used for testing should be collected from the same locality each year.

Specific notes on collection of mosquitoes for testing

- Mosquitoes may be obtained as part of routine surveillance activities by adding additional traps at existing surveillance locations (see Part 1).
- The BG Sentinel trap or ovicups can be used to collect live *Ae. aegypti* and *Ae. albopictus* specimens. This may result in collection of a mixture of mosquito species, and some effort will be required to separate the species for testing.
- Adult mosquitoes that have been collected in traps should be allowed to recover for 12-24 hours before testing to allow for those damaged by trapping, e.g., from fan blades, to die.

D) The following information on mosquitoes used should be recorded:

- Collection identification number
- Sample origin location(s)
    i. City/town and county
    ii. Neighborhood/address
    iii. GPS coordinates
- Collection date
- Life stage collected
- Species and generation being tested

E) The following bottle assay information should be recorded for reporting to CDC:

- Active ingredient and, if used, inhibitor

- Concentration(s) (µg/bottle)
- Time between bottle preparation and testing
- Number of mosquitoes tested
- Diagnostic time and total test time
- Percent mortality at diagnostic time and, if applicable, end of test
- Time 100% mortality achieved

Reporting to CDC

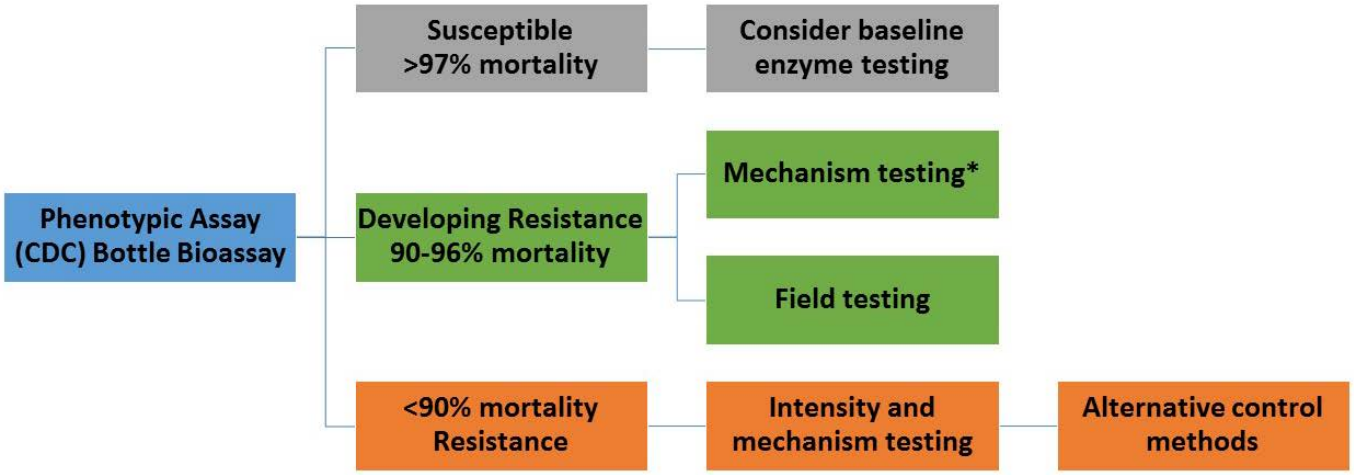
CDC has launched the MosquitoNET online portal to collect data for mosquito presence and abundance, and for insecticide resistance testing. The MosquitoNET portal can be accessed at: http://www.cdc.gov/zika/vector/for-professionals.html. Following initial set-up of a user account, monthly data reporting is required.

CDC Use of Reported Data

The data will be used to develop and regularly update maps for resistance to insecticides in *Ae. aegypti* and *Ae. albopictus* in the United States. The maps will be posted on a CDC website, still under development, accessible to the general public. The data will be used to guide the choice of insecticides for emergency control efforts against *Ae. aegypti* and *Ae. albopictus* and their associated arboviruses, and to inform insecticide resistance management planning.

For further questions on insecticide resistance, contact: CDCInsectResistance@cdc.gov

Addendum 1: Overview of insecticide Resistance Testing Algorithm



*Mechanism testing options: enzymes, molecular assays, bottle bioassay with inhibitors

Addendum 2: Extended Background on Insecticide Resistance

With the use of insecticides comes the possibility that insecticide resistance (IR) may develop, particularly with insecticides used for adult control. Currently, two classes of insecticides are used on adult mosquitoes, organophosphates and pyrethroids. These two classes are also used for agriculture, urban pest control,

homeowners and mosquito control for over 40 years. If insecticides are used in a program, then routine testing for IR should also be conducted to assure that an effective product is being used.

IR is a genetic change in response to selection by toxicants that may impair control in the field. (Sawicki, 1987). This definition allows for recognizing the development of resistance and altering control techniques to mitigate it before the use of a particular chemical is lost. The most direct way to test for IR is to perform a phenotypic assay, known as the CDC bottle bioassay (Brogdon and McAllister, 1998), which measures the amount of time it takes for an insecticide to get to the target site and act on that site. A target site is the tissue that an insecticide interacts with to cause mortality. There are other less direct ways to measure resistance, which include assessing relative changes in doses required to kill 50% of a population (sometimes difficult to interpret), or comparing responses of caged susceptible colonies and field collected mosquitoes (labor intensive and dependent on stringent environmental conditions that one has no control over). In addition, both tests require relatively large numbers of mosquitoes compared to the CDC bottle bioassay. For these reasons, the CDC bottle bioassay is the preferred method of performing insecticide resistance testing.

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