

GUIDANCE FOR SURVEILLANCE OF AND RESPONSE TO INVASIVE AEDES MOSQUITOES AND DENGUE, CHIKUNGUNYA, AND ZIKA IN CALIFORNIA California Department of Public Health



Updated June 2021



Guidance for Surveillance of and Response to Invasive Aedes Mosquitoes and Dengue, Chikungunya, and Zika in California

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This document was prepared by the California Department of Public Health, Division of Communicable Disease Control, with input from the Mosquito and Vector Control Association of California and the California Conference of Local Health Officers.

OBJECTIVE

This document was developed to guide local vector control agencies and health departments to prepare for, conduct surveillance of, and respond to the detection of invasive *Aedes* mosquitoes and human cases of dengue, chikungunya, Zika, or other exotic mosquito-borne viral infections potentially transmitted by these mosquitoes. Mosquito species of immediate concern are *Aedes aegypti*, also known as the "yellow fever mosquito," and *Aedes albopictus*, also known as the "Asian tiger mosquito," which have become established in over 300 cities within 22 central and southern California counties. Although locally acquired human infection with dengue, chikungunya, or Zika has not been detected in California to date, this is an ongoing concern in cities with invasive *Aedes* mosquitoes as travelers return and visitors come from areas with known disease transmission. A comprehensive local plan should be developed to address detection of invasive *Aedes* mosquitoes and potential transmission of exotic mosquito-borne viral infections.

CONTENT

The items below are hyperlinked; click to go to a section.

This document includes:

- Page 2: Introduction to *Ae. aegypti* and *Ae. albopictus* mosquitoes and the exotic viruses they can carry and transmit to humans.
- Page 3: Recommended actions for local agencies
 - Recommendations for local vector control agencies
 - Pre-detection of Aedes aegypti/albopictus
 - Post-detection of Aedes aegypti/albopictus
 - Detection of Aedes aegypti/albopictus positive for dengue, chikungunya, or Zika virus before local human infection is documented
 - Locally acquired human infection(s) identified
 - Recommendations for local health departments
 - Pre-detection of Aedes aegypti/albopictus
 - Post-detection of Aedes aegypti/albopictus
 - Detection of Aedes aegypti/albopictus positive for dengue, chikungunya, or Zika virus before local human infection is documented
 - Locally acquired human infection(s) identified
 - Role of CDPH

- Page 9: Discussion of recommended actions for local vector control agencies
 - Mosquito surveillance
 - Pre-detection versus post-detection mosquito surveillance
 - Detection and control response
 - Detection of dengue, chikungunya, or Zika virus in *Aedes* mosquitoes before locally acquired infection documented
 - **o** Locally acquired or travel-associated human arboviral infections
- Page 14: Discussion of recommended actions for local health departments
- Appendices
 - Page 18: **Appendix A** Examples of target-specific traps for invasive *Aedes*
 - Page 21: Appendix B Media release templates
 - Page 24: Appendix C Dengue surveillance case definition, reporting, and laboratory testing
 - Page 27: Appendix D Chikungunya surveillance case definition, reporting, and laboratory testing
 - Page 30: Appendix E Zika surveillance case definition, reporting, and laboratory testing
 - Page 32: Appendix F Procedures for processing mosquitoes for arbovirus detection
 - Page 34: **Appendix G** Additional resources

It is recommended that vector control and public health staff read all parts of this document to better understand the activities involved under different scenarios and to coordinate the appropriate activities at the local level.

INTRODUCTION

The discovery of *Aedes albopictus*, also known as the "Asian tiger mosquito," in 2011 in Los Angeles County, and of *Ae. aegypti*, also known as the "yellow fever mosquito," in 2013 in urban areas of Fresno, Madera, and San Mateo counties demonstrated that California was vulnerable to colonization by these highly invasive mosquitoes. By the end of 2020, detections of one or both species had been made in over 300 cities within 22 central and southern counties. Both species are vectors of exotic arthropod-borne viruses (arboviruses) including dengue, chikungunya, Zika, and yellow fever. Travel-associated human cases of dengue, chikungunya, and Zika have been reported in California, but none of these viruses are known to be transmitted locally by mosquitoes at present. Established invasive *Aedes* mosquito populations increase the potential for local transmission to occur.

Dengue is a viral disease characterized by fever, headache, joint and muscle pain, which can progress to bleeding and shock in some people. Dengue transmission is common in much of the tropics, and outbreaks have occurred in areas of the United States where *Ae. aegypti* and *Ae. albopictus* are established, including Florida, Texas, and Hawaii. Presumably, infected visitors or returned travelers to these areas imported dengue virus and served as sources for these outbreaks.

Chikungunya is another viral disease with fever and severe joint pain. Outbreaks had occurred in countries in Africa, Asia, Europe, and the Indian and Pacific Oceans. In late 2013, the first

local transmission of chikungunya virus in the Americas was identified in the Caribbean Islands, followed by rapid spread to other countries in South and Central America.

Zika is another viral disease with fever, rash, and joint pain, and, before 2015, outbreaks had occurred in areas of Africa, Southeast Asia, and the Pacific Islands. In May 2015, human cases were detected for the first time in Brazil, and Zika spread rapidly to other countries throughout the Americas. Zika was initially considered a mild disease, but there is a now an association between Zika infection during pregnancy and birth defects such as microcephaly; the development of abnormally small head and brain. In adults, Zika infection has been associated with Guillain-Barré syndrome, an autoimmune neurological disease. Zika virus can be sexually transmitted or acquired via blood transfusion, thus all blood products in California are screened for Zika virus.

The behavior and habitat preferences of *Ae. aegypti* and *Ae. albopictus* differ substantially from the indigenous *Culex* species that are the primary targets of control programs in California's urban areas. Adult *Ae. aegypti* and *Ae. albopictus* are active during the day, have short flight ranges, and females are aggressive and persistent biters of mammals, especially humans. What is most distinctive is their preference for small, artificial water-holding containers for laying eggs (oviposition) and larval development, hence they are known as "container-breeding" mosquitoes. Their close association with and dependence on humans to provide larval habitat, particularly within residential properties, results in a widespread but often patchy distribution, making effective surveillance and control a challenge. Detection and control are further complicated by eggs that resist desiccation and can remain viable for months on dry surfaces of containers.

It can be difficult to determine the source of *Ae. aegypti* and *Ae. albopictus* infestations, but transport of dormant eggs via imported tires and house plants has been associated with introductions of these mosquito species into California in the past. Individuals moving materials via planes, ships, cars, or other vehicles from infested areas to non-infested areas may also facilitate spread. It is important that local vector control agencies, health departments, and other agencies work collaboratively to raise public awareness of these mosquitoes and the mosquito-borne viruses they can carry, and develop proactive surveillance and response plans. Early detection and response is critical to protect public health and is essential if mosquito eradication is to remain an option in new localities. Once established, these mosquito species are very difficult to eliminate from urban residential areas.

RECOMMENDED ACTIONS FOR LOCAL AGENCIES

The recommended surveillance and response actions for vector control agencies and health departments depend on whether invasive *Aedes* mosquitoes have been detected locally, whether a locally captured *Aedes* mosquito has been found positive with dengue, chikungunya, or Zika virus, and whether human infections with dengue, chikungunya, or Zika have been acquired locally. Support services available to local agencies by the California Department of Public Health (CDPH) are listed at the end of this section.

Recommendations for Local Vector Control Agencies

Pre-Detection of Aedes aegypti/albopictus

- Identify local, state, and federal agencies and resources that can be consulted regarding identification, surveillance, and control of *Ae. aegypti* and *Ae. albopictus*.
- In coordination with the local health department, develop a response plan that can be implemented at the first detection of invasive mosquitoes. The plan should include preparedness for enhanced mosquito surveillance and control activities, protocols and responsibilities for sharing information about human cases of dengue, chikungunya, and Zika, working drafts of public relations materials, and agreements with neighboring health departments and vector control agencies to provide assistance if needed.
- Develop and implement an early detection plan for invasive mosquitoes.
 - Ensure staff are able to identify all life stages of *Ae. aegypti* and *Ae. albopictus*.
 - Notify CDPH of any mosquitoes tentatively identified as *Ae. aegypti* or *Ae. albopictus*; send specimens to confirm identification if needed.
 - Initiate an education and outreach program designed to educate and mobilize the public to report daytime-biting mosquitoes and eliminate larval sources.
 - Ensure receptionists are trained to ask appropriate questions to walk-in and callin customers relative to invasive mosquitoes and recognize when information given warrants a precautionary follow-up inspection or referral to the local health department.
 - Deploy strategically-placed, target-specific egg and adult mosquito surveillance traps. See Appendices A and G.

Post-Detection of Aedes aegypti/albopictus

- When *Ae. aegypti* or *Ae. albopictus* mosquito identification is confirmed, immediately notify the local health department, CDPH, and neighboring vector control agencies; request assistance if indicated.
- In coordination with the local health department, distribute public relations materials, including a media release, describing the discovery of invasive mosquitoes and the disease risks they present. Reassure the public that the risks are low if no locally acquired human infection has been confirmed, and request that the public contact the local vector control agency regarding daytime-biting mosquitoes. See media release template, Appendix B.
- Discuss with CDPH observations and findings of confirmed mosquitoes, potential infestation areas, and possible introduction and movement pathways.
- Enhance egg and adult (e.g., ovi- and adult traps) and larval (e.g., door-to-door) mosquito surveillance to delineate the infested areas.
- Initiate a door-to-door campaign in urban areas surrounding the point(s) of discovery to:
 - Distribute public education materials urging the public to empty or discard small containers of standing water and take personal prevention measures to reduce mosquito bites.
 - Gain permission to conduct larval surveillance on selected residential or commercial properties and, if a desirable location, to place ovi- and adult mosquito traps; educate property owners regarding habitat reduction.
 - If necessary, apply EPA-registered chemical products to control immature and adult mosquitoes on selected properties.

- When indicated, initiate chemical control of immature and adult mosquitoes using EPAregistered products. Define areas of control based on surveillance data, including presence, relative abundance, and distribution of invasive *Aedes* within the urban environment. Products can be applied on foot and with vehicle-mounted sprayers. Depending on the extent of the infestation, local topography, and environmental conditions, aerial applications also can be considered, especially if there is local arbovirus transmission.
- Send pools of female mosquitoes (≤50 mosquitoes per pool) to the DART Lab at UC Davis for arboviral testing. See Appendix F.
- If notified by the local health department of any travel-associated case of dengue, chikungunya, or Zika infection who might have been viremic while being in an invasive *Aedes*-infested area:
 - Request from the local health department the case-patient's residential address and any additional information on other areas the patient may have visited while potentially viremic.
 - Ensure patient confidentiality by protecting any personal identifiers including name, address, or other personal information.
 - Ensure staff are trained regarding state laws that govern the use of confidential information.
 - Enhance mosquito surveillance and control and public outreach in the identified area(s).
 - Send pools of female mosquitoes (≤50 mosquitoes per pool) to DART for arboviral testing. See Appendix F.

Detection of Aedes aegypti/albopictus positive for dengue, chikungunya, or Zika virus before local human infection is documented

- Immediately notify the local health department, CDPH, and neighboring vector control agencies.
- Work collaboratively with the local health department and CDPH to issue a joint media release, with careful wording to raise awareness of an increased threat potential, but at the same time acknowledging that no locally acquired human case has yet been confirmed. See media release template, Appendix B.
- Enhance public outreach and mosquito surveillance and control in and around the area from where infected mosquitoes were collected as well as in the vicinities of any diagnosed human cases within nearby invasive *Aedes*-infested areas.
- Assume that the finding is indicative of potential local transmission and implement all applicable steps listed in *Locally Acquired Human Infection(s) Identified*.

Locally Acquired Human Infection(s) Identified

- Work collaboratively with the local health department and CDPH to issue a joint media release ensuring patient confidentiality. See media release template, Appendix B.
- In coordination with the local health department, immediately implement enhanced mosquito surveillance and control (physical habitat removal and chemical control of larvae and adults) within a 150 meter radius of the case-patient's residence (maintaining patient confidentiality), and similarly in other locations where exposure to invasive *Aedes* mosquitoes may have occurred. Distribute public relations materials to raise

awareness about invasive *Aedes* mosquitoes, the viruses they can transmit, symptoms of disease, and use of personal protective measures.

- Continue to closely monitor for presence of invasive *Aedes* mosquitoes within the identified areas of concern for 45 days (three extrinsic viral incubation periods in mosquitoes; see "Human Disease Surveillance, page 14"), and implement additional control measures if indicated.
- Send pools of female mosquitoes (≤50 mosquitoes per pool) to DART for arboviral testing. Continue to engage the public in detecting and reporting daytime-biting mosquitoes, reducing larval habitats on their properties, and taking personal protective measures to prevent mosquito bites.
- Escalate and expand all activities in the event of widespread local transmission.

Recommendations for Local Health Departments

Pre-Detection of Aedes aegypti/albopictus

- Identify local, state, and federal agencies and resources that can be consulted regarding human surveillance and laboratory confirmation for suspected cases of dengue, chikungunya, and Zika infections.
- In coordination with the local vector control agency, prepare a public relations response plan that can be implemented at the first detection of invasive *Aedes* mosquitoes. A similar plan should be prepared for the first detection of locally acquired human infections with dengue, chikungunya, or Zika virus. Where no local vector control agencies exist, coordination should be with CDPH. The plans should include a media release and other relevant public relations materials.
- Continue to report to CDPH via the California Reportable Disease Information Exchange (CalREDIE) or, for non-participating jurisdictions, by fax or secure email any suspect, probable, or confirmed cases of dengue, chikungunya, and Zika virus infections; ensure report includes patient(s) symptom onset date and travel history.
 - If the case-patient(s) had <u>not</u> traveled to an area known to have active transmission of these viruses, immediately alert CDPH and the local vector control agency that the disease may have been locally acquired (which suggests that invasive *Aedes* mosquitoes may be present in the area but not yet detected).

Post-Detection of Aedes aegypti/albopictus

- Collaborate with the local vector control agency in issuing a media release describing the discovery of invasive mosquitoes and the disease risks they present. Reassure the public that risks are low if no locally acquired human infection has been confirmed, and request that the public contact the local vector control agency regarding any daytime-biting mosquitoes. See media release template, Appendix B.
- Enhance surveillance for human cases of dengue, chikungunya, and Zika by following up as soon as possible with all suspect, probable, and confirmed case-patients for their travel history and by entering all patient information into CalREDIE. Immediately notify CDPH of any patient who had <u>not</u> traveled to an area where active transmission of their infection is ongoing.
- Notify the local vector control agency of any suspect, probable, or confirmed cases of dengue, chikungunya, or Zika infection. Timely notification is critical to enhance

mosquito surveillance and control in the vicinity of the case-patient's residence, particularly within a 150-meter radius, to minimize the potential for arbovirus transmission.

- Advise the local vector control agency of their responsibility to maintain patient confidentiality. The information disclosed to local vector control should be limited to that needed to investigate and control virus transmission by mosquitoes.
- Educate the local medical community on signs and symptoms of dengue, chikungunya, and Zika infection (see Appendices C, D, E, and G) and remind healthcare providers to report suspect cases. Dengue, yellow fever, chikungunya and Zika virus infection are all reportable in California.
 - Provide and disseminate educational materials from CDPH or the US Centers for Disease Control and Prevention (CDC). See Appendix G.
 - Provide information on testing suspect patients for infection.
- Assess your local public health laboratory's capacity to test for dengue, chikungunya or Zika viruses. If no capacity exists, specimens can be sent to commercial laboratories or the CDPH-Viral and Rickettsial Disease Laboratory (VRDL) for testing. See Appendices C, D, and E.

Detection of Aedes aegypti/albopictus positive for dengue, chikungunya, or Zika virus before local human infection is documented

- Work collaboratively with CDPH and the local vector control agency to issue a joint media release, with careful wording to raise awareness of an increased threat potential, but at the same time acknowledging that no locally acquired human case has yet been confirmed. See media release template, Appendix B.
- Immediately begin enhancing surveillance for potential local human cases, starting at the area where positive mosquitoes were collected.
- Notify the medical community, including hospitals and laboratories, to look for all diagnosed and suspected cases of dengue, chikungunya, and Zika infections, regardless of recent travel history, and to report them as soon as possible. Focus on cases in and around areas where infected mosquitoes were collected.
- Work collaboratively with CDPH and the local vector control agency to issue a joint media release, with careful wording to raise awareness of an increased threat potential, but at the same time acknowledging that no locally acquired human case has yet been confirmed. See media release template, Appendix B.
- Notify the local vector control agency of any suspect, probable, or confirmed cases of dengue, chikungunya, or Zika infection. Timely notification is critical to enhance mosquito surveillance and control in the vicinity of the case-patient's residence, particularly within a 150-meter radius, to minimize the potential for arbovirus transmission.
- Assume that the finding is indicative of potential local transmission and implement all applicable steps in *Locally Acquired Human Infection(s) Identified.*

Locally Acquired Human Infection(s) Identified

• Work collaboratively with CDPH and the local vector control agency to issue a joint media release ensuring patient confidentiality. See media release template, Appendix B.

- Conduct epidemiologic investigation and enhanced surveillance where the case-patient spent the most time during the 2 weeks prior to onset of illness, e.g., home, neighborhood, and work place.
- Work with the local vector control agency to enhance mosquito surveillance and control in the vicinity of each case-patient's residence (maintaining patient confidentiality), neighborhood, and similarly in other locations where exposure to invasive Aedes mosquitoes may have occurred, and to distribute public relations materials to raise awareness about invasive Aedes mosquitoes, the viruses they can transmit, symptoms of disease, and use of personal protective measures.
- Advise patients to take all steps to avoid mosquito bites to minimize the risk of infecting mosquitoes and furthering local transmission.
- Enhance surveillance for additional locally acquired human cases by notifying the local medical community, including hospitals and laboratories, to look for and encourage testing of all suspected dengue, chikungunya, and Zika infections, regardless of recent travel history, and to report them as soon as possible; discuss the issuance of a California Health Alert Network (CAHAN) notification with CDPH.
- Once local human transmission is documented, follow up promptly on all suspect cases of dengue, chikungunya, and Zika infections as potentially locally acquired and notify CDPH via CalREDIE or by telephone.
- Notify the local vector control agency of any suspect, probable, or confirmed cases of dengue, chikungunya, or Zika infection. Timely notification is critical to enhance mosquito surveillance and control in the vicinity of the case-patient's residence, particularly within a 150-meter radius, to minimize the potential for arbovirus transmission.
- Engage the public in detecting and reporting daytime-biting mosquito activity to the local vector control agency, reducing mosquito larval habitats on their property, and protecting themselves from mosquito bites.
- Escalate and expand all activities in the event of widespread local transmission.

Role of CDPH

Services available to support local agencies during pre and post-detection response actions include:

- Development of public education materials (e.g., fact sheets, flyers, door hangers) and local media releases.
- Identification of potential invasion pathways and geographic origin of invasive mosquito populations which may prompt an intervention response.
- Consultation and assistance regarding:
 - Mosquito identification, surveillance techniques, control options, and allocation of limited resources.
 - Human arbovirus infection symptoms and diagnosis.
 - Human arbovirus case testing and evaluation.
 - Response to outbreak of human disease.
- Interpretation of laboratory-based insecticide resistance testing for Aedes aegypti.
- Facilitation of collaboration and communication among agencies in affected and neighboring counties.

- Providing fact sheets and information for clinicians, including "Information for Clinicians: *Aedes aegypti* and *Aedes albopictus* Mosquitoes in California and Reporting Patients with Suspected Dengue or Chikungunya to Public Health" (see Appendix G).
- Providing epidemiological information on cases of dengue, chikungunya, Zika, and other mosquito-borne viral infections in California.
- Issuing statewide media releases.
- Coordinate and lead the regional or statewide public health response including surveillance, investigation, and control in the event of widespread local transmission involving multiple jurisdictions.
- Providing back up and/or surge diagnostic laboratory testing of clinical specimens to determine possible dengue, chikungunya, Zika, and other mosquito-borne viral infections and providing technical support for laboratory testing as needed.

DISCUSSION OF RECOMMENDED ACTIONS FOR LOCAL VECTOR CONTROL AGENCIES

Mosquito Surveillance

Detection of invasive *Aedes* in urban environments often occurs after adult mosquito populations have increased to numbers that motivate people to complain to their local vector control agency. Standard surveillance traps used in California and elsewhere in the United States (e.g., New Jersey light, CO₂, and gravid) may not capture adult *Ae. aegypti* and *Ae. albopictus* unless the traps are located near a breeding location, or until adult mosquito populations are relatively abundant or widespread. A number of target-specific attraction and capture devices have been developed specifically for the detection of eggs and adults of these anthropophilic, container-breeding mosquitoes. For simplicity, these devices will be referred to as ovitraps and adult traps. See Appendix A and "Traps and collection methods for *Aedes aegypti* and *Aedes albopictus* surveillance and control" in Appendix G for a description and discussion of several of these traps.

Effective surveillance for *Ae. aegypti* and *Ae. albopictus* requires the careful selection and placement of ovitraps and adult traps, larval surveys in unconventional areas, and a much greater level of interaction with the public. The success of any or all of these activities depends on understanding the ecology and behavior of these container-breeding mosquitoes to maximize the potential for detection. Field and laboratory staff should be able to identify egg, larval, pupal, and adult stages of these mosquito species and always consider the possibility of specimens being collected during routine surveillance operations. Currently available target-specific traps have limited success in collecting egg or adult specimens, especially when adult mosquito populations are low or patchy in an environment, but the likelihood of trap capture success can be improved by increasing the number of traps. At present, there are no established guidelines on the number of traps (of any type) necessary for a comprehensive *Ae. aegypti* or *Ae. albopictus* surveillance program.

Pre-Detection versus Post-Detection Mosquito Surveillance

Surveillance strategies will vary depending on whether invasive *Aedes* mosquitoes have been detected.

Pre-Detection of Aedes aegypti/albopictus

Container-breeding mosquitoes such as *Ae. aegypti* and *Ae. albopictus* are notoriously difficult to control once they become established in residential areas. The best chance for eradicating these mosquitoes is early detection, before the population has a chance to become abundant and widespread. Local agencies should develop and implement an early detection plan for invasive mosquitoes that employs the use of strategically placed ovi- and adult traps and an outreach program designed to educate and mobilize the public to report daytime-biting mosquitoes. Soliciting public participation is critical because residents are most likely to observe unusual mosquito activity on their own properties where there may be large numbers of water-holding containers to support larval development.

The mosquito surveillance database maintained by the local vector control agency should be reviewed and, if necessary, modified to include data on invasive *Aedes* mosquitoes. Data should be maintained locally in a standardized format that allows for easy comparisons of data over time and among geographic locations. All data on invasive *Aedes* collection efforts, including traps or door-to-door surveys that did not find mosquitoes, should be reported in the <u>Vectorborne Disease Surveillance Gateway (VectorSurv)</u> (https://gateway.vectorsurv.org/). To avoid redundant entry, agencies with in-house data systems may exchange data automatically with the VectorSurv Gateway database using web services. *Aedes* surveillance data will be uploaded monthly to CDC's MosquitoNET. For questions or suggestions, contact the VectorSurv Gateway development team (help@vectorsurv.org).

The potential routes of invasive *Aedes* mosquito introduction into a given area need to be considered and a portion of the early detection efforts focused on these areas. Past records suggest that commercial importation of certain goods (e.g., live plants, used tires) can facilitate introductions; however, individual residents and visitors to urban areas can also unintentionally introduce invasive mosquitoes. The type and number of sites selected for surveillance will be determined by the local agency but should include both commercial and residential areas where ample habitat exists for larvae such as cemeteries, plant nurseries, and any other known properties with an abundance of potential water-holding containers.

- **Traps.** Placement of traps should be carefully considered to maximize the likelihood of detection. There is currently no established formula for determining the best traps to use, the ideal number of traps, or trap placement for any given area. However, the known advantages, disadvantages, and performance of different trap types (Appendices A and G) suggest that using more than one trap type and using as many as economically feasible should increase the chance of detecting invasive mosquitoes. Trap inspections and maintenance can be extended to approximately one-week intervals to optimize and make best use of resources.
- **Public Education and Outreach.** Educating the public about invasive mosquitoes and instructing people to report any suspicious sightings or daytime biting annoyance is crucial for early detection. The outreach program should include educational materials that are culturally and linguistically appropriate to fit the diversity of the local community and target residential, commercial, and industrial sectors. The program can include written and electronic materials available at the agency headquarters and website, flyers for distribution to homes and businesses, roadway billboards, ads on public transportation vehicles, workshops, and oral presentations. Information can also

be provided to the media to prompt news coverage. Public education and outreach activities have the dual benefit of increasing the chances of early detection while also increasing the visibility of local vector control services.

Post-Detection of Aedes aegypti/albopictus

The surveillance approach following the discovery of invasive *Aedes* mosquitoes should become much more aggressive and rigorous to provide a comprehensive assessment of population size, geographical spread, and control effectiveness. Rapid surveillance of larger areas can be accomplished by focusing on presence versus absence of invasive mosquitoes, i.e., no need to identify more than one specimen of an invasive species per property. Additionally, focused surveillance near the residences and in the areas where viremic patients with travel-associated dengue, chikungunya, or Zika infection could have been exposed to *Ae. aegypti* or *Ae. albopictus* may be useful in detecting infected mosquitoes before any locally acquired human infection has been identified.

- **Traps.** The number and variety of traps should be increased relative to pre-detection levels and placed in the areas surrounding the site(s) of discovery to assess the abundance and distribution of invading mosquitoes. Additional traps should be placed outward from identified infestation areas to determine the geographical extent of the population. It should not be assumed that the index location (first site where invasive species were discovered) is the initial site of introduction. To aid in these assessments, inspection intervals should be increased to every 1-3 days.
- **Public Education and Outreach.** All aspects of the education and outreach program should be intensified throughout the jurisdictional area of the agency, but particularly in the urban areas surrounding the point(s) of discovery and other known infested areas. Door-to-door campaigns should be initiated immediately to inform and educate individual property owners and their on-site residents about the invading mosquitoes, how they can minimize habitat on their property, and encourage people to report daytime-biting mosquitoes. The door-to-door campaign will also provide an opportunity for larval and adult (i.e., host-seeking adult females landing on inspectors) surveillance on the property, providing additional information on mosquito abundance and spread.

Detection and Control Response

The initial discovery of mosquitoes tentatively identified as *Ae. aegypti* or *Ae. albopictus* should be immediately reported to CDPH. Mosquito specimens should be sent to CDPH to confirm identification (if needed) and the local agency should communicate any observations and findings, potential infestation areas, and possible introduction pathways to allow a better assessment of the situation. Upon species confirmation, the local vector control agency should initiate their response plan beginning with the notification of the local health department, neighboring vector control agencies, and other agencies as appropriate. Public relations materials regarding the discovery should be released at this time, either independently or as joint efforts with other local agencies according to previously established plans. Materials should include a media release urging the public to eliminate sources of standing water on their property and report any daytime-biting mosquitoes to the local vector control agency. The successful control of invasive mosquitoes is dependent on a number of factors, especially if eradication is the objective. Consider the following:

- Proactive planning and preparation are critical following the discovery of *Ae. aegypti* or *Ae. albopictus* to ensure a rapid and smooth transition from routine vector control activities to the targeted surveillance and control of an invasive mosquito species.
- Agreements previously made with neighboring local agencies can be of great assistance in conducting certain aspects of the mosquito surveillance and control response, especially with regard to door-to-door campaigns and ground-based application of insecticides.
- Public education and outreach programs and door-to-door surveillance activities not only provide important information on abundance and spread of invasive mosquitoes, but also aid control in urban environments by reducing potential larval habitats.
- A combination of physical, biological, and chemical control approaches should be used against immature and adult invasive mosquitoes. For thorough implementation, these control activities frequently require the collaboration and cooperation of residential property owners established during education, outreach, and door-to-door campaigns.
- In addition to containers (e.g., jars, pots, bird baths, rain barrels), relatively small subsurface habitats (e.g., catch basins, dry wells, yard drains, storm water treatment devices, public utility vaults), surface pools (e.g., neglected ponds, water-holding surface depressions in lawns), and vegetation (e.g., tree holes, bromeliad leaf axils) are sometimes utilized as larval habitat by invasive *Aedes* mosquitoes.
- Thorough and effective mosquito surveillance is the key to successful control. Information obtained from post-detection surveillance should be used to guide control activities.

Data collected from combined surveillance activities that provide reliable information on presence, relative abundance, and distribution of invasive mosquitoes within the urban environment should be carefully recorded and mapped and used to continually focus and refocus resources and control efforts. The greatest emphasis of the control program should center on educating and mobilizing the public to implement physical controls to eliminate opportunities for immature mosquito development on private properties. Eradication should be the initial objective, and therefore it is crucial that local agency staff gain access for inspection of every property in an affected area, including vacant properties and properties with uncooperative owners/residents. A single neglected property can provide the habitat necessary for invasive mosquito production thus allowing rapid re-invasion and counteracting all previous and ongoing control efforts. Coordination with the local code enforcement agency may be helpful in ensuring access to properties.

EPA-registered biological and chemical control products labeled for larval and adult mosquitoes in California can be used against invasive mosquitoes but may require the use of equipment and application techniques not normally employed for the control of indigenous species. Insecticides should be applied in accordance with surveillance data that confirms the presence, abundance, and relative distribution of invasive mosquitoes. If available, pesticide resistance profiles of local *Ae. aegypti* or *Ae. albopictus* populations should be considered to inform the selection of adulticide products. Treatment options are outlined below; none should be expected to provide long-term control of invasive mosquitoes without simultaneous removal of aquatic habitats suitable for larval development. In addition, the structural complexity of the urban environments where invasive mosquitoes thrive may preclude effective insecticide penetration of broadcast sprays to all targeted areas even when treatment conditions appear ideal.

- Formulations of larvicidal products containing active ingredients such as methoprene and *Bacillus thuringiensis var. israelensis* can be broadcast into urban environments using spray equipment calibrated to produce larger droplet sizes than typical adulticide applications.
- Residual adulticide sprays can be applied to vegetation and other surfaces of individual properties where adult mosquitoes might take refuge or rest.
- Ultra-low volume (ULV) adulticides can be used to knock-down the adult population over larger areas using truck-mounted foggers when environmental conditions are appropriate.
- Aerial adulticide applications may be considered over urban areas too large to treat efficiently and effectively using ground-based equipment, especially under conditions of human disease outbreak or if adult mosquito numbers require rapid knockdown.

Detection of dengue, chikungunya, or Zika virus in *Aedes* mosquitoes before locally acquired infection documented

The detection of an exotic arbovirus in captured *Ae. aegypti* or *Ae. albopictus* mosquitoes implies that a person returned from a region endemic for dengue, chikungunya, or Zika virus while still viremic and was bitten locally by *Aedes*. It is possible that additional human cases remain undetected or asymptomatic, and that the virus is circulating in the environment at a low level. Surveillance and control should be rapidly amplified to reduce risk of transmission to local residents and visitors as described in the subsequent section. The local health department should immediately be notified to enhance human case finding.

Locally Acquired or Travel-associated Human Arboviral Infections

Local health departments and CDPH continuously monitor suspect, probable, and confirmed human cases of dengue, chikungunya, and Zika infections and establish the patients' travel history to determine whether a person likely acquired the infection from recent travel to an area with ongoing disease transmission or locally. When local transmission is suspected, the local health department should promptly notify local vector control of such cases. The mosquito surveillance and control response should be intensified in areas where potentially viremic persons may have been bitten by Ae. aegypti or Ae. albopictus mosquitoes to minimize the potential for local disease transmission. If additional locally acquired human cases are subsequently identified, a more aggressive response should be planned in consultation with CDPH, in coordination with the local health department, and other appropriate agencies. The response will further escalate in the event of widespread local transmission, and regional coordination may be necessary. If multiple jurisdictions are involved, CDPH may coordinate and lead the regional public health response including surveillance, investigation, and control. The local health department will follow up rapidly with all suspect, probable, and confirmed cases of dengue, chikungunya, and Zika infections, whether travel-associated or locally acquired, viremic or not, and share appropriate information with local vector control. Activities triggered by human infections should include enhanced mosquito surveillance and control in areas where potentially infected persons may have come into contact with invasive mosquitoes, collection

and submission of female mosquito samples to DART to be tested for dengue, chikungunya, and Zika, and consideration of more aggressive mosquito control including aerial spraying.

DISCUSSION OF RECOMMENDED ACTIONS FOR LOCAL HEALTH DEPARTMENTS

Human Disease Surveillance

To date, none of the exotic arboviruses carried and transmitted by *Ae. aegypti* and *Ae. albopictus* are known to be circulating among mosquitoes in California and the risk of these viruses being introduced into established populations of invasive *Aedes* from infected visitors and returning travelers is currently considered to be low; however, a single viremic person with dengue, chikungunya, or Zika who is subsequently bitten by a female *Ae. aegypti* or *Ae. albopictus* could start local disease transmission within a community. There are several conditions and a sequence of events that would need to be in place for local transmission of dengue, chikungunya, or Zika to occur.

These include:

- An infected and viremic individual would need to return to a locality in California where there are *Ae. aegypti* and/or *Ae. albopictus* mosquitoes. The viremic period is typically 1-2 days before until 3-4 days after symptom onset for dengue, 4-6 days after symptom onset for chikungunya, and 3-5 days after symptom onset for Zika. Some people are asymptomatic. If the infected person returned more than a week after onset of illness, then transmission of virus from this person is less likely.
- 2. A female mosquito would need to bite the infected person while this person is viremic.
- 3. The mosquito would need to live approximately 10-11 days after taking a virus-infected blood meal to allow for the virus to multiply and migrate to the salivary glands (extrinsic incubation period); most mosquitoes live <14 days, but this is dependent on many environmental and ecological factors.
- 4. The infected mosquito would need to bite one or more susceptible persons who become infected and then viremic, but may or may not become symptomatic. Both *Ae. aegypti* and *Ae. albopictus* typically take multiple blood meals during each gonotrophic cycle (blood ingestion and egg development cycle; 2-7 day intervals) and therefore an infectious female may bite multiple people over a short period of time.
- 5. This cycle would need to be repeated for sustained transmission to occur.

Note that detection of locally acquired human infection with dengue, chikungunya, or Zika virus may occur prior to the discovery of invasive *Aedes* mosquitoes in a given area.

Pre-Detection of Aedes aegypti/albopictus

Detection and reporting of suspect, probable, or confirmed human infections with dengue, chikungunya, or Zika viruses is critical to monitor the possible points of introduction of these pathogens into California and the spread of disease in the event of an outbreak. All infections, regardless of status (i.e., suspect, probable, or confirmed), should be reported using the real-time, secure web-based California Reportable Disease Information Exchange (CalREDIE) system maintained by CDPH. Non-participating jurisdictions should report all infections by submitting the appropriate paper case report form by secure email or fax immediately after the investigation is complete. Dengue, yellow fever, chikungunya, and Zika virus infection are all

reportable in California. The surveillance case definitions and laboratory testing for dengue (i.e., dengue and severe dengue), chikungunya, and Zika are summarized in Appendices C, D, and E, respectively. Appendix G contains resources for more information on dengue, chikungunya, Zika, and invasive *Aedes* mosquitoes.

Reports associated with human arboviral infections should include information regarding symptom onset date and travel history to elucidate if infections were acquired outside of California or locally. If the case-patient had no travel history to areas endemic for the disease within the incubation period and for Zika cases, no sexual contact with a returned traveler, CDPH and the local vector control agency should be contacted immediately. The local health department should ensure that patient confidentiality is maintained regarding sharing of personal identifiers (e.g., name, address, laboratory test results). The absence of travel suggests that the infection may have been acquired locally even if the person resides in an area not known to be infested with *Ae. aegypti* and *Ae. albopictus.* Invasive mosquitoes can be elusive in the environment and can be associated with relatively small habitats (e.g., residential backyards). The local vector control agency should conduct a follow-up investigation of the general area surrounding the case-patient's residence to determine if invasive *Aedes* mosquitoes are present, but previously undetected.

A public relations response plan should be prepared to include a media release to be implemented if invasive *Aedes* mosquitoes are detected within the jurisdiction of the local health department. A similar response plan should be prepared in the event that local transmission of dengue, chikungunya, or Zika virus is confirmed. Both plans should include: 1) a local health advisory to the medical community to increase awareness of exotic mosquitoborne viral infections in humans (the advisory should specify whether a locally acquired human case has been detected and recommendations should be tailored accordingly) and 2) a request for the public to report daytime-biting mosquitoes, minimize habitat suitable for invasive *Aedes* mosquitoes, protect themselves from mosquito bites, and recognize common symptoms of dengue, chikungunya, and Zika disease. Coordination with the local vector control agency, or CDPH where no local vector control agency exists, ensures that messages and materials distributed to the public and to the media remain consistent. Response plans can be administered independently or jointly with the local vector control agency.

Post-Detection of Aedes aegypti/albopictus

Once *Ae. aegypti* and *Ae. albopictus* mosquitoes are established in urban environments, visitors and returned travelers infected with dengue, chikungunya, or Zika virus may infect local *Aedes* mosquitoes if they are bitten while viremic. The previously established public relations response plan regarding the discovery of invasive *Aedes* mosquitoes should be initiated. Local health departments should ensure that the local medical community is educated about the exotic arboviral disease risks associated with *Ae. aegypti* and *Ae. albopictus*, signs and symptoms of these diseases in humans, human specimen collection for laboratory confirmation and clinical diagnosis, proper patient treatment, and disease reporting.

The local vector control agency should be notified of any suspect, probable, or confirmed cases of dengue, chikungunya, or Zika identified from areas known to be infested with *Ae. aegypti* or *Ae. albopictus*, particularly if evidence suggests that the person may have been exposed to mosquitoes during the viremic period. Patient confidentially should be maintained.

Detection of dengue, chikungunya, or Zika virus in invasive Aedes mosquitoes before locally acquired human infection is documented

If *Ae. aegypti* or *Ae. albopictus* mosquitoes collected by a local vector control agency test positive for dengue, chikungunya, or Zika virus before any human case of locally acquired infection has been documented, this suggests that an infected individual returned from a region endemic for these diseases while still viremic and was bitten locally by *Aedes* mosquitoes. In addition, the presence of locally infected mosquitoes suggests that the virus may be circulating in the environment at a low level and increases the threat for locally acquired human infection.

The public and medical community should be notified via a press release, with careful wording to highlight the increased risk of exotic arboviral infection while acknowledging that no locally acquired infection has been confirmed. The public should be advised to use mosquito bite prevention measures, and the medical community encouraged to consider these conditions in patients with compatible illness or travel history and report promptly all suspect, probable, or confirmed cases of dengue, chikungunya, or Zika infection. The local health department should coordinate with the local vector control agency and CDPH on following up to human cases subsequently diagnosed in the vicinity of the positive mosquitoes to determine the extent of virus circulation in the environment.

Locally Acquired Human Infection(s) Identified

The discovery of one or more human infections of dengue, chikungunya, or Zika virus suspected to have been locally acquired should be addressed aggressively and immediately. To identify additional cases in an area where the locally acquired case may have been exposed to infected mosquitoes, an epidemiologic investigation and enhanced surveillance should be implemented to cover the areas where the case-patient has spent the most time within the 2 weeks leading to onset of illness, e.g., home, neighborhood, and work place. Local vector control agencies and CDPH should be notified to ensure that mosquito surveillance and control is enhanced around the residence and any areas the identified case-patient may have been exposed to biting mosquitoes during their viremic period. Patients should be advised to take all steps to prevent mosquito bites to reduce the risk of spread to local mosquito populations. Zika case-patients should be advised to take measures to avoid sexual transmission to partners. The previously developed public relations response plan should be initiated. Additional response efforts could include: facilitated testing of suspect cases and enhanced case finding, additional coordination between local and state public health epidemiologists and public health laboratorians, enhanced coordination and communication with clinical diagnostic laboratories, outreach and education to healthcare providers on the diagnosis and clinical management of dengue, chikungunya, and Zika viruses, and an enhanced media campaign to the public. All activities should escalate in the event of widespread local transmission, and, if multiple jurisdictions are involved, CDPH may coordinate and lead the regional public health response including surveillance, investigation, and control. Neighboring jurisdictions, states, and the CDC should also be notified, depending on the extent of disease transmission.

The implications of local transmission of exotic mosquito-borne viruses are many and require the greatest level of response. Close and rapid interagency communication with CDPH and the local vector control agency is critical to ensure rapid suppression of invasive *Aedes* mosquitoes to break the human-mosquito-human disease cycle and prevent outbreaks of dengue, chikungunya, or Zika.

APPENDIX A

Examples of Target-Specific Traps for Invasive Container-Breeding Mosquitoes such as Aedes aegypti and Aedes albopictus

Ovitrap

Advantages	Disadvantages
 Inexpensive Easy to deploy, inspect, and refresh Inspection intervals can be up to 7 days 	 Requires that eggs be reared in the laboratory to confirm species identification Ovitraps can support mosquito production if left in the environment for more than 7 days or if misplaced on properties Success may be influenced by availability of competing container habitats May be tipped over or flooded by rainfall or other environmental factors

The ovitrap is the most basic surveillance tool for *Ae. aegypti* and *Ae. albopictus* in the urban environment. In general, an ovitrap consists of a small dark-colored container (e.g., 24-32 oz black plastic cup) partially filled with water or mild attractant infusion and with an oviposition medium (e.g., wood tongue depressor, germination paper, construction paper). Female mosquitoes seeking an egg-laying site may choose to deposit some eggs on the oviposition medium provided in the cup. Almost any small container can be used as an ovitrap, but studies have found black-colored containers to have superior performance.

Limitations

- Detection success may be directly dependent on the number of ovitraps deployed. For example, a city block with one ovitrap per property may increase the likelihood of detecting presence of *Ae. aegypti* and *Ae. albopictus* than the same city block with only one deployed ovitrap.
- Does not provide any information on the abundance of adults in the environment; only evidence of the presence of at least one adult female.

CDC-AGO (Autocidal Gravid Ovitrap)

Advantages	Disadvantages
 Inexpensive Easy to deploy, inspect, and refresh Inspection intervals can be lengthened; the trap will function for more than 8 weeks without need for maintenance The design prevents access to standing water, thus will not support mosquito production if left unattended Removes egg-laying females from the environment Allows immediate identification of captured adults Can provide some information on the relative abundance of adults in a given environment. 	 Larger, bulkier, and heavier than standard ovitraps More visible in the environment Adults trapped by the adhesive may be difficult to dislodge for identification and may not be suitable for testing for viruses or pesticide resistance Glue paper maintenance frequency may vary depending on air-borne debris and relative humidity of trap site

Several variants of "lethal ovitraps" similar in concept to the CDC-AGO have been developed. The concept behind these traps is to lure oviposition-site-seeking females to a container from which they cannot escape or where they come into contact with a lethal dose of insecticide. The AGO Trap is made from modified 1 gallon and 5 gallon black plastic utility buckets partially filled with a hay-based infusion. Female mosquitoes seeking an egg-laying site can enter partway into the bucket through an opening but are blocked from accessing the water by a screen. An adhesive on the surface of the entrance captures mosquitoes on contact.

Limitations

• Detection success may be directly dependent on the number of AGO traps deployed in a given area.

Biogents (BG) Sentinel Adult Trap

Advantages	Disadvantages
 Target-specific trap developed for capture of adult <i>Ae. aegypti</i> and <i>Ae.</i> <i>albopictus</i>. Few non-target species are attracted to these traps unless other lures such as CO₂ are added to the system. Does not require CO₂ to attract <i>Ae.</i> <i>aegypti</i> and <i>Ae. albopictus</i> Can be plugged into available 110V outlets for continuous operation if desired to increase inspection intervals Captures both males and females Allows immediate identification of captured adults Can provide some information on the relative abundance of adults in a given environment Trapped live mosquitoes can be tested for arbovirus if traps are serviced frequently (e.g., every 1-2 nights) 	 Expensive Selection of suitable deployment areas safe from theft, vandalism, and weather/environmental damage can be time-consuming Battery packs discharge rapidly, usually in less than 3 days Trapped mosquitoes can escape from the net bag if the power supply is disconnected or discharged, or if the fan motor fails. Note* Newer models provide a trap-door to minimize escape in the event of a fan failure Ants and other predators may damage or remove mosquitoes from trap

The "BG Trap" is an adult trap that preferentially attracts *Ae. aegypti* and *Ae. albopictus* and is currently considered the most effective commercially available adult trap for these two species. Both males and females may be attracted to the trap and are captured by a suction fan into a small net bag. The design is versatile in that commercially available lures can be incorporated into the body of the trap to improve attractiveness.

Limitations

• Detection success may be directly dependent on the number of BG Traps deployed in a given area.

APPENDIX B

Media Release Templates

Example Vector Control Agency (VCA) / Local Health Department joint press release subsequent to first detection of an invasive Aedes mosquito

Aedes aegypti Mosquito found in [City, County] (Substitute Aedes albopictus for Aedes aegypti as appropriate.)

[City]. -The [VCA] has detected *Aedes aegypti* mosquitoes at/in [area]. The first detection was on [date]. [VCA] is working with the [City, County] Department of Health to evaluate the extent of the infestation and will aggressively target problem areas to prevent its spread.

Aedes aegypti is not native to California; however, it is a common mosquito in some urban areas of the southeastern United States and Arizona. Elsewhere in California, Aedes aegypti have been found in [list counties]. Aedes aegypti has the potential to transmit several viruses including dengue, chikungunya, Zika, and yellow fever. These viruses are not currently found in California. Aedes aegypti is a small (about ¼ inch) black and white mosquito that bites aggressively during the day.

"Our goal is to control and eliminate this mosquito population." said [VCA Manager]. "We are doing everything to help ensure this mosquito does not become established in our communities."

The [VCA] has expanded surveillance efforts for this type of mosquito. [Text example: The District has deployed a variety of traps for adult mosquitoes and mosquito eggs surrounding the location where *Aedes aegypti* was found. Additionally, District staff are conducting door-to-door inspections of properties for mosquito breeding and standing water at homes near *Aedes aegypti* detections].

[Insert if relevant - This mosquito was previously found in [area or county] in [year] near [place], but was successfully eradicated by the [VCA] and did not become established here].

The public can play a critical role in helping to control the spread of this mosquito population. *Aedes aegypti* lays its eggs just above the water line in small containers and vessels that hold water, such as dishes under potted plants, bird baths and feeders, ornamental fountains, tin cans, children's toys, or discarded tires. It's important for residents to look around their yard and outside their home and dump out even the smallest amount of standing water. Clean and scrub bird baths and pet watering dishes weekly and dump the water from overflow dishes under potted plants.

[County] Health Officer [Name] reminds people to do the following to reduce the chances of being bitten by mosquitoes:

- Apply repellents containing EPA registered ingredients such as DEET, picaridin, oil of lemon eucalyptus, or IR3535 to exposed skin and/or clothing (as directed on the product label).
- Wear long sleeve shirts, long pants, socks and shoes when mosquitoes are most active.

• Be sure window and door screens are in good repair to prevent mosquitoes from entering your home.

Residents experiencing mosquito bites during the day should report them immediately to [VCA contact info]

If you are sick with fever, headache, and joint or muscle pain after returning from an area where dengue, chikungunya, or Zika occurs, contact your doctor and stay indoors as much as possible to avoid mosquito bites and help prevent possible spread of the virus.

Additional information on Aedes [species] can be found at:

[Local health department website] [VCA website] <u>California Department of Public Health</u> (https://www.cdph.ca.gov/Programs/CID/DCDC/Pages/Aedes-aegypti-and-Aedes-albopictusmosquitoes.aspx) Example Local Health Department [LHD] press release subsequent to first detection of a locally acquired human case of dengue. If this template is used for another locally acquired exotic mosquito-borne disease, such as chikungunya or Zika, please edit the paragraph describing symptoms.

--First Confirmed Locally Acquired Dengue Case in [County]

[City/County] Today, the [County] Health Department announced that the first locally acquired human dengue case has been confirmed in a [county] resident. [if applicable: To date, (number) locally acquired dengue cases have been previously detected in California].

Dengue (pronounced den' gee) is caused by a virus that is transmitted to humans by the bite of an infected *Aedes aegypti* or *Aedes albopictus* mosquito. *Aedes* mosquitoes have been found in [cities] in [county]. Dengue virus cannot be transmitted from person-to-person. Symptoms of dengue may include high fever, severe headache, pain behind the eyes, joint pain, and rash. Health care providers should contact the [County] Health Department if they suspect an individual may have dengue or another mosquito-borne illness.

The [Vector Control Agency - VCA] and the [LHD] are enhancing surveillance, prevention, and mosquito control efforts. Residents should take basic precautions to protect themselves from mosquitoes by following the Department of Health recommendations. [County] Health Officer [Name] reminds people to do the following to reduce their chances of being bitten by mosquitoes and to help prevent spread of the virus:

- Apply repellents containing EPA registered ingredients such as DEET, picaridin, oil of lemon eucalyptus, or IR3535 to exposed skin and/or clothing (as directed on the product label).
- Wear long sleeve shirts, long pants, socks and shoes when mosquitoes are most active.
- Be sure window and door screens are in good repair to prevent mosquitoes from entering your home. [and/or use air conditioning keeping windows and doors closed.]
- Residents experiencing mosquito bites during the day should report them to [VCA contact info] and should contact their health care provider if they have dengue-like symptoms.

If you are sick with fever and joint pain contact your doctor and stay indoors as much as possible to avoid mosquito bites and help prevent possible spread of the virus.

Additional information on dengue and *Aedes* [aegypti or albopictus] can be found at:

[Local health department website] [VCA website] <u>California Department of Public Health</u> (https://www.cdph.ca.gov/Programs/CID/DCDC/Pages/Aedes-aegypti-and-Aedes-albopictusmosquitoes.aspx)

APPENDIX C

Dengue Surveillance Case Definition, Reporting, and Laboratory Testing

Clinical Description (Dengue, Severe Dengue)

Dengue: Dengue is most commonly an acute febrile illness defined by the presence of fever and one or more of the following, nausea/vomiting, rash, aches and pains (headache, retro-orbital or ocular pain, joint pain, muscle pain), leukopenia, positive tourniquet test, or any warning signs of severe dengue (persistent vomiting, extravascular fluid accumulation (e.g., pleural or pericardial effusion, ascites), mucosal bleeding at any site, liver enlargement >2 centimeters, or increasing hematocrit concurrent with rapid decrease in platelet count).

Severe Dengue is characterized by all of the following:

- Severe plasma leakage evidenced by hypovolemic shock and/or extravascular fluid accumulation (e.g., pleural or pericardial effusion, ascites) with respiratory distress. A high hematocrit value for patient age and sex offers further evidence of plasma leakage.
- Severe bleeding from the gastrointestinal tract (e.g., hematemesis, melena) or vagina (menorrhagia) as defined by requirement for medical intervention including intravenous fluid resuscitation or blood transfusion.
- Severe organ involvement, including any of the following: elevated liver transaminases (aspartate aminotransferase (AST) or alanine aminotransferase (ALT) ≥1,000 per liter (U/L)), impaired level of consciousness and/or diagnosis of encephalitis, encephalopathy, or meningitis, or heart or other organ involvement including myocarditis, cholecystitis, and pancreatitis.

Laboratory Criteria for Classification

Confirmatory: Any one of the following:

- Isolation of dengue virus from or demonstration of specific arboviral antigen or genomic sequences in serum, plasma, blood, cerebrospinal fluid (CSF), or other body fluid or tissue by cell culture, reverse-transcriptase polymerase chain reaction (RT-PCR) test, immunofluorescence or immunohistochemistry.
- Detection in serum or plasma of DENV NS1 antigen by a validated immunoassay.
- Seroconversion from negative for dengue virus-specific serum immunoglobulin M (IgM) antibody in an acute phase (≤ 5 days after symptom onset) specimen to positive for dengue-specific serum IgM antibodies in a convalescent-phase specimen collected ≥ 5 days after symptom onset.
- Seroconversion or demonstration of a ≥ 4-fold rise in reciprocal immunoglobulin G (IgG) antibody titer to dengue virus antigens in serum samples collected >2 weeks apart, AND confirmed by a neutralization test (e.g., plaque reduction neutralization test) with a >4fold higher end point titer as compared to other flaviviruses tested.

Presumptive/Probable:

• A positive dengue-specific IgM antibody test, on a single acute or convalescent phase serum specimen.

Suspect:

• The absence of IgM anti-DENV by validated immunoassay in a serum or CSF specimen collected <5 days after illness onset and in which molecular diagnostic testing was not performed in a patient with an epidemiologic linkage.

<u>Exposure</u>

- Travel to a dengue endemic country or presence at location with ongoing outbreak within previous two weeks of dengue-like illness, OR
- Association in time and place with a confirmed or probable dengue case.

Case Classification

Suspected: A clinically compatible case of dengue, or severe dengue with an epidemiologic linkage

Probable: A clinically compatible case of dengue, or severe dengue with laboratory results indicative of probable infection

Confirmed: A clinically compatible case of dengue, or severe dengue with confirmatory laboratory results

Dengue Reporting

All infections, regardless of status (i.e., suspect, probable, or confirmed) should be reported using the real-time, secure web-based California Reportable Disease Information Exchange (CalREDIE) system maintained by CDPH. Non-participating jurisdictions should report all dengue by submitting the paper dengue case report form by secure email or fax immediately after the investigation is complete. For cases in which no travel history is indicated or local transmission is suspected, CDPH should be notified immediately by telephone.

Dengue Laboratory Testing

Dengue viruses are members of the Flaviviridae and have sufficient antigenic similarity to Zika virus, yellow fever virus, Japanese encephalitis virus, and West Nile virus that previous infection with or vaccination against another flavivirus may raise cross-reactive serum antibodies. After a primary infection with a heterologous flavivirus, subsequent antibody testing by ELISA may produce false positive results for a different flavivirus. The plaque reduction neutralization test (PRNT) may resolve cross-reactive serum antibodies and identify the infecting virus; however, in individuals with multiple previous flavivirus infections PRNT may not differentiate among different flaviviruses. This demonstrates the complexity inherent in serological diagnosis and differentiation in populations living in regions where more than one flavivirus co-circulates. However, only a small proportion of the US population has evidence of previous flavivirus infection (or vaccination) so that cross-reactive flavivirus antibodies should not be a significant limitation to dengue diagnosis among most US travelers. Among US residents, most testing for dengue is done through private clinical laboratories using IgM or IgG detection techniques.

Serologic (IgG and IgM) and molecular assays to detect evidence of dengue virus infection are available through commercial laboratories, from many local public health laboratories, as well as from the California Department of Public Health, Viral and Rickettsial Disease Laboratory (VRDL). Testing may include:

- EIA or IFA for IgM and IgG antibodies. Serologic assays do not distinguish among dengue serotypes and may be cross-reactive with other flaviviruses. When a positive detection is made for dengue, VRDL can perform a plaque reduction neutralization assay (PRNT) to attempt to distinguish between dengue and other exotic (e.g., Zika virus) or endemic flaviviruses (i.e., West Nile virus, St. Louis encephalitis virus).
- Real-time RT-PCR (rRT-PCR) for acute serum, plasma or blood specimens. Some rRT-PCR tests will discriminate among the four dengue serotypes. Blood for rRT-PCR should be collected within 8 days of symptom onset.

Acute samples that test positive for dengue-virus specific antibodies at commercial laboratories should prompt ordering of a convalescent testing. Both acute and convalescent samples should be forwarded to VRDL for confirmatory testing.

Samples may be submitted to VRDL using the "General Purpose Specimen Submittal Form" available at the <u>VRDL Specimen Shipping Guidelines and Current Specimen Submittal Forms</u> <u>webpage</u> (https://www.cdph.ca.gov/Programs/CID/DCDC/Pages/VRDL Specimen Submittal Forms.aspx)

Detailed instructions on sample submission can be found in the VRDL "Guidelines to Viral and Rickettsial Disease Laboratory Services" available at <u>VRDL's webpage</u> (https://www.cdph.ca.gov/Programs/CID/DCDC/Pages/VRDL.aspx).

VRDL Contact information

Main Telephone Number	(510) 307-8585
Fax Number	(510) 307-8599

Shipping Address (for hand delivery or private carriers): Viral and Rickettsial Disease Laboratory Attn: Specimen Receiving 850 Marina Bay Parkway Richmond, CA 94804

Reference testing is available from CDC's Dengue Branch, Division of Vector-Borne Infectious Diseases, National Center for Infectious Diseases, 1324 Calle Cañada, San Juan, PR 00920-3860, telephone 787-706-2399, fax 787-706-2496.

To arrange for reference testing at CDC, contact your local public health department.

APPENDIX D

Chikungunya Surveillance Case Definition, Reporting, and Laboratory Testing

Clinical Description (Chikungunya Fever)

Chikungunya Fever: Chikungunya fever is characterized by ALL of the following:

- Fever or chills as reported by the patient or a health-care provider.
- Arthralgia or arthritis involving two or more joints.
- Absence of a more likely clinical explanation.

Chikungunya is most often characterized by acute onset of fever (typically >39°C [102°F]) and polyarthralgia. Joint symptoms are usually bilateral and symmetric, and can be severe and debilitating. Other symptoms may include headache, myalgia, arthritis, conjunctivitis, nausea/vomiting, or maculopapular rash. Clinical laboratory findings can include lymphopenia, thrombocytopenia, elevated creatinine, and elevated hepatic transaminases.

Acute symptoms typically resolve within 7–10 days. Rare complications include uveitis, retinitis, myocarditis, hepatitis, nephritis, bullous skin lesions, hemorrhage, meningoencephalitis, myelitis, Guillain-Barré syndrome, and cranial nerve palsies. Persons at risk for severe disease include neonates exposed intrapartum, older adults (e.g., > 65 years), and persons with underlying medical conditions (e.g., hypertension, diabetes, or cardiovascular disease). Some patients might have relapse of rheumatologic symptoms (e.g., polyarthralgia, polyarthritis, tenosynovitis) in the months following acute illness. Studies report variable proportions of patients with persistent joint pains for months to years. Mortality is rare and occurs mostly in older adults. The majority of people infected with chikungunya virus become symptomatic. The incubation period is typically 3–7 days (range, 1–12 days).

Laboratory Criteria for Classification

Confirmatory: A clinically compatible case as reported by the patient or healthcare provider, absence of a more likely explanation and one or more of the following laboratory criteria:

- Isolation of chikungunya virus from, or demonstration of specific viral antigen or nucleic acid in, serum, plasma, blood, or other body fluid or tissue, by cell culture, polymerase chain reaction (PCR) test, immunofluorescence or immunohistochemistry OR
- Demonstration of a
 <u>></u> 4-fold rise in virus-specific antibody titers in paired acute and convalescent serum samples, OR
- Demonstration of virus-specific IgM antibodies in serum with confirmatory virus-specific neutralizing antibodies in the same or later specimen.
- For neuroinvasive cases, demonstration of virus-specific IgM antibodies in CSF along with a negative result for other endemic arbovirus-specific IgM antibodies in the same specimen.

Probable:

• A clinically compatible case as reported by the patient or healthcare provider, absence of a more likely explanation, and virus-specific IgM antibodies in serum or CSF but with no other testing.

Suspected:

• A clinically compatible case with acute onset of fever and severe arthralgia or arthritis not explained by other medical conditions, and who resides or has visited epidemic or endemic areas within 2 weeks before the onset of symptoms.

Not a Case:

 A suspected case with negative virus-specific IgM or neutralizing antibodies in serum collected <u>></u>8 days after illness onset or evidence of a more likely explanation for their illness.

Chikungunya Reporting

All infections, regardless of status (i.e., suspect, probable, or confirmed) should be reported using the real-time, secure web-based California Reportable Disease Information Exchange (CalREDIE) system maintained by CDPH. Non-participating jurisdictions should report chikungunya infections by submitting the paper chikungunya case report form by secure email or fax immediately after the investigation is complete. For cases in which no travel history is indicated or local transmission is suspected, CDPH should be notified immediately by telephone.

Chikungunya Laboratory Testing

Serologic (IgG and IgM) and molecular assays to detect evidence of chikungunya virus infection are available through commercial laboratories, from many local public health laboratories, as well as from the California Department of Public Health, Viral and Rickettsial Disease Laboratory (VRDL). Testing may include:

- EIA or IFA for IgM and IgG antibodies. Serologic assays may be cross-reactive with other alphaviruses. When a positive detection is made for chikungunya, VRDL can perform a plaque reduction neutralization assay (PRNT) to attempt to distinguish between chikungunya and other endemic alphaviruses (i.e., western equine encephalitis virus).
- Real-time RT-PCR (rRT-PCR) for acute serum, plasma or blood specimens. Blood for rRT-PCR should be collected within 8 days of symptom onset.

Acute samples that test positive for chikungunya-virus specific antibodies at commercial laboratories should prompt ordering of a convalescent testing. Both acute and convalescent samples should be forwarded to VRDL for confirmatory testing.

Samples may be submitted to VRDL using the "General Purpose Specimen Submittal Form" available at the <u>VRDL Specimen Shipping Guidelines and Current Specimen Submittal Forms</u> webpage

(https://www.cdph.ca.gov/Programs/CID/DCDC/Pages/VRDL_Specimen_Submittal_Forms.aspx)

Detailed instructions on sample submission can be found in the VRDL "Guidelines to Viral and Rickettsial Disease Laboratory Services" available at <u>VRDL's webpage</u> (https://www.cdph.ca.gov/Programs/CID/DCDC/Pages/VRDL.aspx).

VRDL Contact information

Main Telephone Number	(510) 307-8585
Fax Number	(510) 307-8599

Shipping Address (for hand delivery or private carriers): Viral and Rickettsial Disease Laboratory Attn: Specimen Receiving 850 Marina Bay Parkway Richmond, CA 94804

APPENDIX E

Zika Surveillance Case Definition, Reporting, and Laboratory Testing

Clinical Description (Zika virus disease)

Zika is most often characterized by acute onset of fever with maculopapular rash, arthralgia, or conjunctivitis. Other commonly reported symptoms include myalgia and headache. Clinical illness is usually mild with symptoms lasting for several days to a week. Severe disease requiring hospitalization is uncommon and case fatality is low. However, there have been cases of Guillain-Barré syndrome reported in patients following suspected Zika virus infection and increased cases of microcephaly among newborns in areas with ongoing Zika outbreaks. Due to concerns of microcephaly associated with maternal Zika virus infection, fetuses and infants of women infected with Zika virus during pregnancy should be evaluated for possible congenital infection and neurologic abnormalities in the months following diagnosis. The majority of people infected with Zika virus are asymptomatic. The incubation period is typically 3–7 days.

Laboratory Criteria for Classification

Confirmatory: A clinically compatible case, or a person who does not meet clinical criteria but has an epidemiologic linkage, AND one or more of the following laboratory criteria:

- Detection of ZIKV by culture, viral antigen or viral RNA in serum, blood, CSF, tissue, or other specimen (e.g. amniotic fluid, urine, semen, saliva); OR
- Positive ZIKV IgM antibody test of serum or CSF with positive ZIKV neutralizing antibody titers and negative neutralizing antibody titers against dengue or other flaviviruses endemic to the region where exposure occurred.

Probable: A clinically compatible case, or a person who does not meet clinical criteria but has an epidemiologic linkage, AND

- Positive ZIKV IgM antibody test of serum or CSF with:
 - Positive neutralizing antibody titers against ZIKV and dengue or other flaviviruses endemic to the region where exposure occurred; OR
 - Negative dengue virus IgM antibody test and no neutralizing antibody testing performed.

Zika Reporting

All infections, regardless of status (i.e., probable or confirmed) should be reported using the real-time, secure web-based California Reportable Disease Information Exchange (CalREDIE) system maintained by CDPH on a daily basis. Non-participating jurisdictions should report Zika infections by submitting the paper Zika case report form by secure email or fax immediately after the investigation is complete. For cases in which no travel history is indicated or local transmission is suspected, CDPH should be notified immediately by telephone.

Zika Laboratory Testing

Serologic (IgM) and molecular assays to detect evidence of Zika virus infection are available through commercial laboratories, from many local public health laboratories, as well as from the California Department of Public Health, Viral and Rickettsial Disease Laboratory (VRDL).

Any diagnostic test performed for the detection of Zika virus in clinical samples must be granted Emergency Use Authorization (EUA) by the U.S. Food and Drug Administration. A list of current EUA-approved Zika virus assays can be found on their <u>Emergency Use Authorizations webpage</u> (https://www.fda.gov/MedicalDevices/Safety/EmergencySituations/ucm161496.htm).

Testing may include:

- EIA, MAC ELISA or immunochromatographic assay to detect IgM antibodies. Serologic assays may be cross-reactive with other flaviviruses. When a positive detection is made in a Zika virus IgM assay, VRDL can perform a plaque reduction neutralization assay (PRNT) to attempt to distinguish between Zika virus and other exotic (e.g., dengue virus) or endemic (i.e., West Nile virus, St. Louis encephalitis virus) flaviviruses.
- Depending upon the specific assay used, real-time RT-PCR may be performed on serum, whole blood, urine, CSF, or other fluid specimens.

Samples may be submitted to VRDL using the "General Purpose Specimen Submittal Form" available at the <u>VRDL Specimen Shipping Guidelines and Current Specimen Submittal Forms</u> webpage

(https://www.cdph.ca.gov/Programs/CID/DCDC/Pages/VRDL_Specimen_Submittal_Forms.aspx)

Detailed instructions on sample submission and the VRDL "Zika Testing Guidance" are available on the <u>VRDL Zika Virus Information website</u> (https://www.cdph.ca.gov/Programs/CID/DCDC/Pages/Zika_VRDL.aspx), or under the "Disease-Specific Information" on the <u>VRDL homepage</u> (https://www.cdph.ca.gov/Programs/CID/DCDC/Pages/VRDL.aspx).

VRDL Contact information Main Telephone Number (510) 307-8585 Fax Number (510) 307-8599

Shipping Address (for hand delivery or private carriers): Viral and Rickettsial Disease Laboratory Attn: Specimen Receiving 850 Marina Bay Parkway Richmond, CA 94804

APPENDIX F

Procedures for Processing Mosquitoes for Arbovirus Detection

- Collect mosquitoes alive and return them immediately to the laboratory. Collections should be kept humid during transport with moist toweling to prevent desiccation. Females held overnight or longer before processing should be offered 5-10 percent sucrose.
- 2. Anesthetize mosquitoes by cold, carbon dioxide, or triethylamine (TEA). TEA is recommended because specimens are permanently immobilized with minimal mortality and with no loss of virus titer. TEA should be used either outdoors or under a chemical hood. Collections can be anesthetized outdoors using a few drops of TEA, the specimens transferred to Petri dishes, and then taken into the laboratory for processing. If refrigerated and kept humid, mosquitoes will remain alive in covered Petri dishes for one or two days without additional anesthesia. If mosquitoes are frozen before processing, counting and sorting to species must be done on a chill table to prevent virus loss.
- 3. Sort mosquito collections to species under a dissecting microscope at 10X to ensure correct identification and to make sure that extraneous mosquito body parts (i.e., legs, wings) or other small insects such as chironomids or *Culicoides* are not inadvertently included in the pools. Avoiding sample contamination is extremely important because diagnostic testing involves highly sensitive RT-PCR that can detect even very small guantities of virus. Include dead and dried mosquitoes in counts for abundance purposes but exclude them from samples for virus testing. Pools are comprised of 1 to 50 females of each mosquito species from each collection site counted into individual polystyrene vials with snap caps (SPEX Sample Prep #3116) containing two 5mm glass beads. Vials with pools should be labeled sequentially starting with #1 each year after the agency code; e.g., KERN-1-20 to indicate pool #1 for the Kern agency for the year 2020. The same number series should be maintained for all pools, including both invasive Aedes and Culex species. Data on each pool should be entered online in electronic format through the California Vectorborne Disease Surveillance Gateway (https://gateway.vectorsurv.org/). Pools to be tested for chikungunya, dengue, and Zika viruses can be marked for the additional testing in the VectorSurv Gateway at the time pools are submitted. POOLS MUST BE ACCOMPANIED BY "MOSQUITO POOLS SUBMITTED FORM MBVS-3" AND CAN ONLY BE TESTED FROM SITES WITH DOCUMENTED LOCATIONS. Surveillance sites should be registered online at https://gateway.vectorsurv.org/. Pools from unregistered sites (e.g., from door-to-door collections or single-use trap locations) should be assigned the site code "000000" and the exact location should be recorded for each pool using the Gateway's online map.
- 4. Freeze pools immediately at -80C either on dry ice in an insulated container or in an ultralow temperature freezer. Pools should be shipped overnight frozen on dry ice to the Davis Arbovirus Research and Training (DART) laboratory for testing by real-time multiplex RT-qPCR. Agencies will receive an automated email notification when samples are received and when results have been entered into the VectorSurv Gateway; additionally, positive pools will be reported weekly in the California Arbovirus Surveillance Bulletin. Each pool is screened for WNV, SLEV, and WEEV, and if testing for chikungunya, dengue, and Zika viruses is also desired, this should be indicated by checking the box for "CDZ Testing" when preparing the online pool submission form in the VectorSurv Gateway. Pools can be

tested for other *Aedes*-borne viruses such as yellow fever on request. Care must be taken not to allow pools to defrost during storage or shipment, because each freeze-thaw cycle may result in a decrease in viral titer; all virus will be lost if the specimens sit at room temperature for extended periods.

Address shipments to: Ying Fang University of California One Shields Ave Vet Med: PMI (Room 3336 Vet Med 3A) Davis, CA 95616

APPENDIX G

Additional Resources

Peer-Reviewed Documents for Vector Control

Bonizzoni, M., G. Gasperi, X. Chen, and A.A. James. 2013. The invasive mosquito species *Aedes albopictus*: current knowledge and future perspectives. Trends Parasitol. 29(9): 460-468.

Crepeau, T.N, S.P Healy, K. Bartlett-Healy, I. Unlu, A. Farajollahi, and D.M Fonseca. 2013. Effects of Biogents Sentinel trap field placement on capture rates of adult Asian tiger mosquitoes, *Aedes albopictus*. PLoS ONE 8(3): e60524. doi:10.1371/journal.pone.0060524 http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0060524

Eisen, L., and C.G. Moore. 2013. *Aedes (Stegomyia) aegypti* in the continental United States: a vector at the cool margin of its geographic range. J. Med. Entomol. 50(3): 467-478. https://academic.oup.com/jme/article/50/3/467/889454

Farajollahi, A., and D.C. Price. 2013. A rapid identification guide for larvae of the most common North American container-inhabiting *Aedes* species of medical importance. J. Am. Mosq. Control Assoc. 29(3): 203-221.

Farajollahi, A, S.P. Healy, I. Unlu, R. Gaugler, and D.M. Fonseca. 2012. Effectiveness of ultra-low volume nighttime applications of an adulticide against diurnal *Aedes albopictus*, a critical vector of dengue and chikungunya viruses. PLoS ONE 7(11): e49181. doi:10.1371/journal.pone.0049181 http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0049181

Fonseca, D.M., I. Unlu, T. Crepeau, A. Farajollahi, S.P. Healy, K. Bartlett-Healy, D. Strickman, R. Gaugler, G. Hamilton, D. Kline, and G.G. Clark. 2012. Area-wide management of *Aedes albopictus*. Part 2: Gauging the efficacy of traditional integrated pest control measures against urban container mosquitoes. Pest Management Science. 69(12): 1351-1361. Doi 10.1002/ps.3511. http://onlinelibrary.wiley.com/doi/10.1002/ps.3511/pdf

Hawley, W.A. 1988. The biology of Aedes albopictus. J. Am. Mosq. Control Assoc. 4: 1-39.

Mackay, A.J., M. Amador, and R. Barrera. 2013. An improved autocidal gravid ovitrap for the control and surveillance of *Aedes aegypti*. Parasites & Vectors 6: 225. http://www.parasitesandvectors.com/content/pdf/1756-3305-6-225.pdf

Metzger, M.E., M.H. Yoshimizu, K.A. Padgett, R. Hu, and V.L. Kramer. 2017. Detection and establishment of *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) mosquitoes in California, 2011-2015. J. Med. Entomol. 54(3): 533-543.

Unlu, I., A. Farajollahi, D. Strickman, D.M. Fonseca. 2013. Crouching tiger, hidden trouble: urban sources of *Aedes albopictus* (Diptera: Culicidae) refractory to source-reduction. PLoS ONE 8(10): e77999. Doi:10.1371/journal.pone.0077999 http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0077999

Peer-Reviewed Documents for Public Health Lessons

Porse C.C., et al. Travel-Associated Zika Cases and Threat of Local Transmission during Global Outbreak, California, USA. Emerg. Infect. Dis. 2018 Sep;24(9): 1626-1632. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6106427/

Locally acquired Dengue--Key West, Florida, 2009-2010. Centers for Disease Control and Prevention (CDC). MMWR Morb Mortal Wkly Rep. 2010 May 21; 59(19): 577-81. http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5919a1.htm

Effler P., et al. Dengue fever, Hawaii, 2001-2002. Emerg. Infect. Dis. 2005 May; 11(5): 742-9. http://wwwnc.cdc.gov/eid/article/11/5/pdfs/04-1063.pdf

Adalja A.A., et al. Lessons learned during dengue outbreaks in the United States, 2001-2011. Emerg. Infect. Dis. 2012 Apr; 18(4): 608-14. http://wwwnc.cdc.gov/eid/article/18/4/11-0968_article.htm

Surveillance and Control Manuals

American Mosquito Control Association. Best Practices for Integrated Mosquito Management: A Focused Update. January 2017.

https://cdn.ymaws.com/www.mosquito.org/resource/resmgr/docs/Resource_Center/Training_ Certification/12.21_amca_guidelines_final_.pdf

European Centre for Disease Prevention and Control. Guidelines for the surveillance of invasive mosquitoes in Europe. Stockholm: ECDC; 2012.

https://ecdc.europa.eu/sites/portal/files/media/en/publications/Publications/TER-Mosquito-surveillance-guidelines.pdf

Florida Department of Health. Surveillance and Control of selected Mosquito-Borne Disease in Florida: 2014 Guidebook http://www.floridahealth.gov/diseases-and-conditions/mosquitoborne-diseases/_documents/arboguide-2014.pdf World Health Organization: Dengue Guidelines for Diagnosis, Treatment, Prevention, and Control, 2009. https://apps.who.int/iris/handle/10665/44188

General Resources

CDPH Vector-Borne Disease Section. Includes links to dengue, chikungunya, and Zika webpages. https://www.cdph.ca.gov/Programs/CID/DCDC/Pages/VBDS.aspx

CDPH Aedes aegypti and Aedes albopictus mosquitoes https://www.cdph.ca.gov/Programs/CID/DCDC/Pages/Aedes-aegypti-and-Aedes-albopictusmosquitoes.aspx

CDPH "Information for Clinicians: *Aedes aegypti* and *Aedes albopictus* Mosquitoes in California and Reporting Patients with Suspected Dengue or Chikungunya to Public Health" https://www.cdph.ca.gov/Programs/CID/DCDC/CDPH%20Document%20Library/DengueorChikI nformationForCliniciansinCA.pdf CDPH "Traps and collection methods for *Aedes aegypti* and *Aedes albopictus* surveillance and control"

https://www.cdph.ca.gov/Programs/CID/DCDC/CDPH%20Document%20Library/CDPHAedesTrapSummaries.pdf

CDC Chikungunya Information (https://www.cdc.gov/chikungunya/)

CDC Dengue Information (https://www.cdc.gov/dengue/)

CDC Zika Information (https://www.cdc.gov/zika/)

Mosquito Submittal and Testing

Pools of captured adult female *Aedes aegypti* and *Aedes albopictus* (1 - 50 mosquitoes per pool; separated by species) should be shipped on dry ice to the following address:

Davis Arbovirus Research and Training (DART) Laboratory ATTN: Ying Fang University of California One Shields Avenue Vet Med: Pathology, Microbiology, & Immunology Building: Vet Med 3A, Room: 3336 Davis, CA 95616

Unless specified, each pool will be tested for dengue (all serotypes), chikungunya, Zika, and West Nile viruses by RTq-PCR. Pools can be tested for other *Aedes*-borne viruses such as yellow fever on request.