



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

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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* and *Aedes albopictus*, both of which recently have been detected and become established in some California counties. Although locally acquired human infection with dengue, chikungunya, or Zika has not been detected in California to date, this is an ongoing low risk 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.

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

Introduction

The discoveries of *Aedes albopictus*, also known as the “Asian tiger mosquito,” in 2011 in Los Angeles County, and *Ae. aegypti*, also known as the “yellow fever mosquito,” in 2013 in urban areas of Fresno, Madera, and San Mateo counties demonstrated that California is vulnerable to colonization by these highly invasive mosquitoes. Both species are vectors of exotic arthropod-borne viruses (arboviruses) that include dengue, chikungunya, Zika, and yellow fever. Dengue is a viral disease with 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. Infected returned travelers or visitors to these areas presumably imported dengue virus and served as the source for these outbreaks. Chikungunya is another viral disease with fever and severe joint pain, and outbreaks had been identified 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 Caribbean islands, and the disease has since spread rapidly to other countries in South and Central America and continues to spread globally. Zika is another arboviral disease with fever, rash, and joint pain, and, before 2015, outbreaks have occurred in areas of Africa, Southeast Asia, and the Pacific Islands. In May 2015, it was detected for the first time in Brazil and has since spread rapidly in Latin America and the Caribbean. Zika is known as a relatively mild disease, but there is a growing association between Zika and microcephaly (abnormally small head and brain) in newborns and Guillain-Barré syndrome, an autoimmune neurological disease, in others. Zika virus can be sexually transmitted and pregnant women who get infected with Zika virus may pass the virus to the developing fetus. Imported human cases of dengue, chikungunya, and Zika have been detected in California. At present, none of the aforementioned arboviruses carried and transmitted by *Ae. aegypti* and *Ae. albopictus* are known to be circulating among humans or mosquitoes in California, but established mosquito populations increase the potential for local transmission to occur.

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 control a challenge. Detection and control are further complicated by eggs that resist desiccation and can remain viable for months on the dry surfaces of containers.

It is not always possible to determine the origins of *Ae. aegypti* and *Ae. albopictus* introductions into California, but transport of dormant eggs via imported tires and plants has been associated with introductions of these mosquito species in the past. In addition, 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 local public health agencies, 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 Vector-Borne Disease Section (VBDS) of any mosquitoes tentatively identified as *Ae. aegypti* or *Ae. albopictus*; send specimens to confirm identification.
 - 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 call-in 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 surveillance tools. See Appendix A.
- 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.

Post-Detection of Aedes aegypti/albopictus

- When *Aedes aegypti* or *Ae. albopictus* mosquito identification is confirmed, immediately notify the local health department 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, the disease risks they present while reassuring that the risks are low if no locally acquired human infection has been confirmed, and a request to the public to contact the local vector control agency of daytime-biting mosquitoes. See media release template in Appendix B.
- Discuss with CDPH VBDS 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 the residential or commercial premises 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 the property.
- Initiate chemical control of immature and adult mosquitoes using EPA-registered products. Define areas of control based on surveillance data, including presence, relative abundance, and distribution of *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 Davis Arbovirus Research and Training (DART) Lab at UC Davis for arboviral testing. See Appendix F.
- If notified by the local health department of any case of imported dengue, chikungunya, or Zika infection who might have been viremic while being in an *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 in the identified area(s).
 - Send pools of female mosquitoes (≤ 50 mosquitoes per pool) to DART for arboviral testing.

*Detection of *Aedes aegypti/albopictus* positive for dengue, chikungunya, or Zika virus before local human infection 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 local human case has yet been confirmed.

- 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 diagnosed imported human cases within *Aedes*-infested areas, including all applicable steps in *Post-Detection of Aedes aegypti/albopictus*.

Locally Acquired Human Infection(s) Identified

- Work collaboratively with the local health department and CDPH to issue a joint media release. See media release template in Appendix B.
- In coordination with the local health department, enhance mosquito surveillance and control in the vicinity of the case-patient(s) residence (maintaining patient confidentiality), particularly in a 150-yard radius of the residence, and 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.
- Subsequent to documented local human transmission, when notified by the local health department of any case of dengue, chikungunya, or Zika within the area of *Aedes* mosquito infestation, enhance mosquito surveillance and control in the neighborhoods of these suspected case-patients (maintaining patient confidentiality) and in other locations where exposure to invasive *Aedes* mosquitoes may have occurred.
- 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.

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 not 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 *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 to describe the discovery of invasive mosquitoes, the disease risks they present while reassuring that risks are low if no local human infection has been confirmed, and a request to the public to contact the local vector control agency regarding any daytime-biting mosquitoes. See media release template in 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 not 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 in a 150-yard 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 F) and remind healthcare providers to report suspect cases. Dengue, yellow fever, and now chikungunya virus infection 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 F.
 - 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 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 local human case has yet been confirmed.
- 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.
- 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 in a 150-yard radius, to minimize the potential for arbovirus transmission.

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 in Appendix B.
- Conduct epidemiologic investigation and enhanced surveillance where the case-patient has spent the most times in the 2 weeks before onset of illness, e.g., home, neighborhood, and work place.
- Work with local vector control agency to enhance mosquito surveillance and control in the vicinity of each case-patient's residence (maintaining patient confidentiality), neighborhood, and 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 if 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 in a 150-yard 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 surveillance techniques, control options, and allocation of limited resources.
 - Human symptoms and diagnosis.
 - Human arbovirus case testing and evaluation.
 - Response to outbreak of human disease.
- 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 to Public Health” (see Appendix F).
- 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 not normally used by local agencies in California 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 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 small 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. Use of spreadsheets should be avoided because they permit inconsistent data entry and fragmentation of data across worksheets. All data on invasive *Aedes* collection efforts, including traps or door-to-door surveys that did not find mosquitoes, should be reported in the CalSurv Gateway (<https://gateway.calsurv.org>). To avoid redundant entry, agencies with in-house data systems may exchange data automatically with the CalSurv Gateway database using web services (details at <http://trac.calsurv.org/gateway>). For questions or suggestions, contact Dr. Chris Barker at UC Davis (cmbarker@ucdavis.edu).

The potential routes of invasive mosquito introduction into a given area need to be considered and a portion of the early detection activities focused on these areas. Past records suggest that commercial importers of certain goods (e.g., live plants, used tires) provide “high risk” invasion pathways; however, individual residents and visitors to urban areas can also be responsible for introducing 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 (Appendix A) 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 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 imported dengue, chikungunya, or Zika infection could have been exposed to *Aedes* mosquitoes 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(s) (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 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), 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 re-focus 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. 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 into many adult harborages 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 *Aedes* 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 be immediately notified to enhance case finding.

Locally Acquired 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. 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 imported 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 humans or mosquitoes in California and the risk of the disease being introduced into the established *Aedes* mosquito populations from infected visitors and returning travelers is low. However, a single viremic person with dengue, chikungunya, or Zika who is subsequently bitten by a female *Ae. aegypti* or *Ae. albopictus* could conceivably 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:

1. An infected individual would need to return to a locality in California where there are *Ae. aegypti* and/or *Ae. albopictus* mosquitoes while still viremic. This 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 viruses 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 temperature and humidity dependent.
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 contact 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 *Aedes* mosquito detection.

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 (CaREDIE) system maintained by CDPH. All 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, and now chikungunya virus infection 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 F contains resources for more information on dengue, chikungunya, Zika, and *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 during the incubation period, 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 mosquitoes are present, but previously undetected.

A public relations response plan that includes a media release should be prepared that can be implemented if invasive 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 mosquito-borne 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) information for the public asking that they report daytime-biting mosquitoes, minimize habitat suitable for invasive 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 invasive *Ae. aegypti* and *Ae. albopictus* mosquitoes are established in urban environments, visitors and returned travelers infected with dengue, chikungunya, or Zika virus may infect *Aedes* mosquitoes if they are bitten while viremic. The previously established public relations response plan regarding the discovery of invasive mosquitoes should be initiated. Local health departments should ensure that the local medical community is educated about the exotic arboviral disease risks associated with invasive mosquitoes, 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 within 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 confidentiality should be maintained.

Detection of dengue, chikungunya, or Zika virus in Aedes mosquitoes before locally acquired infection 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 threat potential for exotic arboviral infection while acknowledging that no locally acquired infection has yet been confirmed. The public should be advised to use mosquito bite prevention measures, and the medical community encouraged to consider these diseases in patients with compatible illness 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 look for additional potential cases in the area where the locally acquired case was exposed to the infected mosquito, an epidemiologic investigation and enhanced surveillance should be implemented to cover the areas where the case-patient has spent the most times in the 2 weeks before onset of illness, e.g., home, neighborhood, and work place. The local vector control agency 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 the viremic period. Patients should be advised to take all steps to prevent mosquito bites to reduce the risk of spread to local mosquito populations. 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 *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	
<p>Advantages</p> <ul style="list-style-type: none"> • Inexpensive • Easy to deploy, inspect, and refresh • Inspection intervals can be up to 7 days 	<p>Disadvantages</p> <ul style="list-style-type: none"> • Requires that eggs be reared in the laboratory to confirm species identification • Ovitrap 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

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 tap 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 i.e., a city block with one ovitrap per property has a greater 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 indirect evidence of the presence of at least one adult female.

CDC-AGO (Autocidal Gravid Ovitrap)	
<p>Advantages</p> <ul style="list-style-type: none"> • Relatively 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. 	<p>Disadvantages</p> <ul style="list-style-type: none"> • 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 testing for viruses

Several variants of “lethal ovitraps” 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 Autocidal Gravid Ovitrap or “AGO Trap” is a patent-pending design developed by the CDC that was successfully field-tested in California in 2013. The 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 part-way 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

- Similar to standard ovitraps, detection success may be directly dependent on the number of AGO traps deployed.

Biogens (BG) Sentinel Adult Trap	
<p>Advantages</p> <ul style="list-style-type: none"> • Target-specific trap developed for capture of adult <i>Ae. aegypti</i> and <i>Ae. 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. 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 mosquitoes can be tested for arboviruses if traps are serviced frequently (e.g., every 1-2 nights) 	<p>Disadvantages</p> <ul style="list-style-type: none"> • Expensive • Selection of suitable deployment areas safe from theft, vandalism, and 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

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 similar to standard CO₂ and gravid traps currently used in California. The design is versatile in that commercially available lures can be incorporated into the body of the trap which may improve attractiveness.

Limitations

- Similar to ovitraps, detection success may be directly dependent on the number of BG Traps deployed

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* if 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 or 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, ornamental fountains, tin cans, 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]

California Department of Public Health:

<http://www.cdph.ca.gov/HealthInfo/discond/Pages/Aedes-albopictus-and-Aedes-egypti-Mosquitoes.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]

California Department of Public Health:

<http://www.cdph.ca.gov/HealthInfo/discond/Pages/Aedes-albopictus-and-Aedes-aegypti-Mosquitoes.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, myalgia, arthralgia), 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) $\geq 1,000$ 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 tissue, blood, cerebrospinal fluid (CSF), or other body fluid by 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 serum samples collected >2 weeks apart, AND confirmed by a neutralization test (e.g., plaque reduction neutralization test) with a >4 -fold 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.

Exposure

- 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 or vaccination 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. PRNT can often resolve cross-reactive serum antibodies in this situation and identify the infecting virus. However, high-titered cross-reactive antibody levels produced from multiple previous flavivirus infections cannot be resolved by PRNT. 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 testing (IgG and IgM) for exposure to dengue virus is available through commercial laboratories (e.g., Focus/Quest and ARUP), as well as from the California Department of Public

Health, Viral and Rickettsial Disease Laboratory (VRDL). The VRDL has serologic and molecular assays for dengue. 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 distinguish between dengue and other endemic flaviviruses (i.e., West Nile virus, St. Louis encephalitis virus).
- Real-time RT-PCR for acute serum specimens. This test will discriminate among the four dengue serotypes. Blood for PCR should be collected within 8 days of symptom onset.

Acute samples that test positive 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

<http://www.cdph.ca.gov/programs/vrdl/Pages/CurrentVRDLSpecimenSubmittalforms.aspx>

Detailed instructions on sample submission can be found in the VRDL “Guidelines for Laboratory Services” available at

<http://www.cdph.ca.gov/programs/vrdl/Pages/default.aspx>

VRDL Contact information

Main Telephone Number (510) 307-8585

Fax Number (510) 307-8599

Mailing Address (for US Postal Service):

California Department of Public Health

Viral and Rickettsial Disease Laboratory

850 Marina Bay Parkway

Richmond, CA 94804

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.

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 $>39^{\circ}\text{C}$ [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, tissue, blood, or other body fluid, by polymerase chain reaction (PCR) test (≤ 5 days after illness onset), immunofluorescence or immunohistochemistry OR
- Demonstration of a ≥ 4 -fold rise in reciprocal immunoglobulin G (IgG) antibody titer or hemagglutination inhibition titer to chikungunya virus antigens in paired acute and convalescent serum samples, OR
- Demonstration of a > 4 -fold rise in PRNT (plaque reduction neutralization test) end point titer (as expressed by the reciprocal of the last serum dilution showing a 90% reduction in plaque counts compared to the virus infected control) between chikungunya virus and other arboviruses tested in a convalescent serum sample.

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 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 ≥ 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 testing (IgG and IgM) for exposure to chikungunya virus is available through commercial laboratories (e.g., Focus/Quest), as well as from the California Department of Public Health, Viral and Rickettsial Disease Laboratory (VRDL). The VRDL has serologic and molecular assays for chikungunya. Testing may include:

- EIA or IFA for IgM and IgG antibodies. This test is available as a validated clinical diagnostic test. 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 distinguish between chikungunya and other endemic alphaviruses (i.e., western equine encephalitis virus).
- Real-time RT-PCR for acute serum specimens. This test is validated for clinical diagnostic use. Blood for PCR should be collected within 8 days of symptom onset.

Acute samples that test positive 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

<http://www.cdph.ca.gov/programs/vrdl/Pages/CurrentVRDLSpecimenSubmittalforms.aspx>

Detailed instructions on sample submission can be found in the VRDL “Guidelines for Laboratory Services” available at

<http://www.cdph.ca.gov/programs/vrdl/Pages/default.aspx>

VRDL Contact information

Main Telephone Number (510) 307-8585

Fax Number (510) 307-8599

Mailing Address (for US Postal Service):
California Department of Public Health
Viral and Rickettsial Disease Laboratory
850 Marina Bay Parkway
Richmond, CA 94804

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 acute illness. 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, 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.

Flavivirus of undetermined species:

- Evidence of recent infection with a flavivirus where the ZIKV IgM is negative and the neutralizing antibody test results on a single specimen are insufficient to determine the identity of the infecting virus.

Zika Reporting

All infections, regardless of status (i.e., flavivirus of undetermined species, suspect, 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. All non-participating jurisdictions should report all flavivirus of undetermined species, suspect, probable, or confirmed Zika infections as a line list weekly to CDPH, followed 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

The California Department of Public Health (CDPH) has been actively working on laboratory testing for Zika and other exotic mosquito-borne diseases such as dengue and chikungunya over the last two years building upon experience with West Nile virus. Due to the ongoing Zika outbreak in the Americas, CDPH accelerated the pace of diagnostic test development and validation for this disease.

CDPH is currently using the FDA-authorized EUA Triplex Real-time RT-PCR Assay, the real-time reverse transcription polymerase chain reaction (rRT-PCR) test for clinical diagnostic use, a test that detects viral genetic material in patient blood, CSF, amniotic fluid, and urine and is used to diagnose Zika virus disease in persons who have had symptom onset within the fourteen days prior to specimen collection or in pregnant women who are positive or equivocal for Zika virus-specific IgM.

CDPH also performs serological testing by IFA and the FDA-authorized CDC MAC ELISA to detect Zika virus-specific IgM antibodies in patient blood that is indicative of a recent infection. For symptomatic individuals with indicated travel history, serum samples should be collected three or more days after illness onset for serological testing. For asymptomatic pregnant women who have lived in an area with Zika virus transmission or who have traveled to areas with ongoing Zika virus transmission, the blood sample should be collected between two and 12 weeks after return from travel for IgM testing. The test is part of initial screening of Zika virus. For specimens that test positive using this test, CDPH conducts a confirmatory test for neutralizing antibodies that may distinguish Zika virus from other viruses.

To submit samples to VRDL for Zika testing, you must complete the VRDL “General Purpose Specimen Submittal Form” available at <http://tinyurl.com/VRDLLab300>.

Detailed instructions on sample submission and the VRDL “Zika Laboratory Testing Guidance” are available on the VRDL Zika website, <https://www.cdph.ca.gov/programs/vrdl/Pages/zikainfo.aspx>.

When completing this form, the following information is required for testing to be performed:

- Your local public health department contact information
- Date of symptom onset and sample collection date
- Travel history – list countries visited and date of departure from risk area
- List of clinical symptoms
- Pregnancy status and estimated due date, as applicable (testing will be expedited for all pregnant women)

For asymptomatic pregnant women:

- For Disease Onset date, enter “N/A”
- Under Other clinical findings, state “Asymptomatic”

- Pregnancy status and estimated due date, as applicable (testing will be expedited for all pregnant women)

Please email electronic copies of all forms to VRDL.submittal@cdph.ca.gov.

VRDL Contact information

Main Telephone Number (510) 307-8585

Fax Number (510) 307-8599

Mailing Address (for US Postal Service):
California Department of Public Health
Viral and Rickettsial Disease Laboratory
850 Marina Bay Parkway
Richmond, CA 94804

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

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.
<http://docserver.ingentaconnect.com/deliver/connect/esa/00222585/v50n3/s1.pdf?expires=1391713101&id=0000&titleid=10266&checksum=959A687384154C796A1DA096E9419C61>

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>

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

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 AA, 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-](http://wwwnc.cdc.gov/eid/article/18/4/11-0968_article.htm)

[0968_article.htm](http://wwwnc.cdc.gov/eid/article/18/4/11-0968_article.htm)

Surveillance and Control Manuals

World Health Organization,: Dengue Guidelines for Diagnosis, Treatment, Prevention, and Control, 2009. <http://www.who.int/rpc/guidelines/9789241547871/en/>

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

<http://www.ecdc.europa.eu/en/publications/publications/ter-mosquito-surveillance-guidelines.pdf>

Florida Department of Health. Surveillance and Control of selected Mosquito-Borne Disease in Florida: 2013 Guidebook

[http://www.floridahealth.gov/diseases-and-conditions/disease-reporting-and-management/disease-reporting-and-surveillance/ documents/mosquito-guide-2013.pdf](http://www.floridahealth.gov/diseases-and-conditions/disease-reporting-and-management/disease-reporting-and-surveillance/documents/mosquito-guide-2013.pdf)

General Resources

CDPH Vector-Borne Disease Section. Includes links to dengue, chikungunya, and Zika webpages. <http://www.cdph.ca.gov/programs/vbds/Pages/default.aspx>

CDPH *Aedes aegypti* and *Aedes albopictus* mosquitoes

<https://www.cdph.ca.gov/HEALTHINFO/DISCOND/Pages/Aedes-albopictus-and-Aedes-aegypti-Mosquitoes.aspx>

CDPH “Information for Clinicians: *Aedes aegypti* and *Aedes albopictus* Mosquitoes in California and Reporting Patients with Suspected Dengue or Chikungunya to Public Health”

<http://www.cdph.ca.gov/HealthInfo/discond/Documents/DengueorCHIKInformationForClinicianinCA.pdf>

CDC Chikungunya Information: <http://www.cdc.gov/chikungunya/>

CDC Dengue Information: <http://www.cdc.gov/dengue/>

CDC Zika Information: <http://www.cdc.gov/zika/>

Mosquito Submittal and Testing

Pools of captured female *Aedes* mosquitoes (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 qRT-PCR. Pools can be tested for other *Aedes*-borne viruses such as yellow fever on request.

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