Integrated Mosquito and Vector Management Program Plan

APPENDIX

D

CALIFORNIA MOSQUITO-BORNE VIRUS SURVEILLANCE AND RESPONSE PLAN

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CALIFORNIA MOSQUITO-BORNE VIRUS SURVEILLANCE AND RESPONSE PLAN

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Objectives

The California Mosquito-borne Virus Surveillance and Response Plan was developed to meet several objectives. Specifically, the Plan:

- Provides guidelines and information on the surveillance and control of mosquito-borne viruses in California, including West Nile, St. Louis encephalitis, and western equine encephalomyelitis viruses;
- Incorporates surveillance data into risk assessment models;
- Prompts surveillance and control activities associated with virus transmission risk level;
- Provides local and state agencies with a decision support system; and
- Outlines the roles and responsibilities of local and state agencies involved with mosquito-borne virus surveillance and response.

This document provides statewide guidelines, but can be modified to meet local or regional conditions.

Introduction

California has a comprehensive mosquito-borne disease surveillance program that has monitored mosquito abundance and mosquito-borne virus activity since 1969 (Reeves et al. 1990), and is an integral part of integrated mosquito management programs conducted by local mosquito and vector control agencies. Surveillance and interagency response guidelines have been published previously by the California Department of Public Health (Walsh 1987) and the Mosquito and Vector Control Association of California (Reisen 1995). The detection of West Nile virus (WNV) in New York, a virus not recognized in the Western Hemisphere prior to 1999, prompted the review and enhancement of existing guidelines to ensure that surveillance, prevention, and control activities were appropriate for WNV. From New York, WNV spread rapidly westward and by 2004 had been detected in all 48 of the continental United States. In addition to WNV, California is vulnerable to introduction of other highly virulent mosquito-borne viruses of public and veterinary health concern, such as Japanese encephalitis, dengue, Zika, chikungunya, yellow fever, Rift Valley fever, and Venezuelan equine encephalitis viruses. If an existing or introduced virus is detected, it is critical that local and state agencies are prepared to respond in a concerted effort to protect people and animals from infection and disease. The current document describes an enhanced surveillance and response program for mosquito-borne viruses in the State of California. Its contents represent the collective effort of the California Department of Public Health (CDPH), the Mosquito and Vector Control Association of California (MVCAC), and the University of California at Davis (UCD).

Background

Mosquito-borne viruses belong to a group of viruses commonly referred to as arboviruses (for **ar**thropod-**bo**rne). Although 15 mosquito-borne viruses are known to occur in California, only WNV, western equine encephalomyelitis virus (WEEV), and St. Louis encephalitis virus (SLEV) have caused significant human disease. WNV continues to seriously impact the health of humans, horses, and wild birds throughout the state. Since 2003, there have been 6,030 WNV human cases with 248 deaths and 1,255 horse cases. Consequently, the California Arbovirus Surveillance Program emphasizes monitoring and providing early detection of temporal and spatial activity of WNV, WEEV, and SLEV. These viruses are maintained in wild bird-mosquito

cycles that do not depend upon infections of humans or domestic animals to persist. Surveillance and control activities focus on this maintenance cycle, which involves primarily *Culex* mosquitoes, such as the western encephalitis mosquito, *Culex tarsalis*, and birds such as house finches and house sparrows.

Immature stages (called larvae and pupae) of *Cx. tarsalis* can be found throughout California in a wide variety of aquatic sources, ranging from clean to highly polluted waters. Most such water is associated with irrigation of agricultural crops or urban wastewater. Other mosquito species, such as *Cx. pipiens, Cx. quinquefasciatus*, and *Cx. stigmatosoma*, play an important role in the transmission cycles of WNV, and potentially SLEV, in urban and suburban areas. Additional mosquitoes such as *Aedes vexans* and *Cx. erythrothorax* also could be important bridge (i.e., bird to mammal) vectors in transmission. Lastly, *Ae. albopictus* and *Ae. aegypti* mosquitoes, important vectors of dengue, Zika, and chikungunya viruses in other parts of the world, have been detected in several locations in California in recent years and may serve as bridge vectors of WNV.

Mosquito control is the only practical method of protecting the human population from infection. There are no specific treatments or cures for diseases caused by these viruses, and vaccines are not licensed for human use. Illness caused by WEEV tends to be most serious in very young children, whereas WNV and SLEV are more likely to cause severe disease in the elderly. WNV also kills a wide variety of native and non-native birds. Vaccines for WEEV and WNV are available to protect horses, which are vulnerable to severe neurological disease caused by these viruses. Mosquito-borne disease prevention strategies must be based on a well-planned integrated pest management (IPM) program that uses near-real-time surveillance to detect problem areas, focus control, and evaluate operational efficacy. The primary components of an IPM program include education, surveillance, and mosquito control.

Education

Residents, farmers, and wetland managers can play an important role in reducing the number of adult mosquitoes by eliminating standing water that may support the development of immature mosquitoes. For instance, residents can help by properly disposing of discarded tires, cans, or buckets; emptying plastic or unused swimming pools; and unclogging blocked rain gutters around homes or businesses. Farmers and ranchers can be instructed to use irrigation practices that do not allow water to stand for extended periods, and wetland managers or duck club owners can work with mosquito control agencies to determine optimal flooding schedules. Educating the general public to curtail outdoor activities during peak mosquito biting times, use insect repellents, and wear long-sleeved clothing will help reduce exposure to mosquitoes. Clinical surveillance is enhanced through education of the medical and veterinary communities to recognize the symptoms of WEEV, SLEV, and WNV, and to request appropriate laboratory tests. Public health officials need to be alerted if a mosquito-borne viral disease case is detected, especially if the public health risk is high.

Surveillance

Surveillance includes monitoring, visualization, and analysis of data on climatic factors, immature and adult mosquito abundance, and virus activity measured by testing mosquitoes, sentinel chickens, dead birds, horses, and humans for evidence of infection. For zoonotic viruses

such as WNV, surveillance of the mosquitoes and vertebrate hosts (e.g., birds) that transmit the virus is particularly important for early warning of human disease risk. Surveillance must focus not only on mosquito-borne viruses known to exist in California, but be sufficiently broad to detect newly introduced viruses. This is especially important since the recent detection of the globally important arboviral vectors, *Ae. aegypti* and *Ae. albopictus,* in California.

Climate Variation

California's Mediterranean climate provides ideal opportunities for forecasting mosquito abundance and arbovirus activity because most precipitation falls during winter, as rain at lower elevations or as snow at higher elevations. Spring and summer temperatures then influence the rate of snow melt and runoff, mosquito population growth, the frequency of blood feeding, the rate of virus development in the mosquito, and therefore the intensity of virus transmission. In general, WEEV outbreaks have occurred in the Central Valley when wet winters are followed by warm summers, whereas SLEV and WNV outbreaks have been linked to warm, dry conditions that lead to large populations of urban *Culex*. Although climate variation may forecast conditions conducive for virus amplification, a critical sequence of events is required for amplification to reach outbreak levels.

Mosquito Abundance

Mosquito abundance can be estimated through collection of immature or adult mosquitoes. The immature stages (larvae and pupae) can be collected from water sources where mosquitoes lay their eggs. A long-handled ladle ("dipper") is used to collect water samples and estimate the number of immature mosquitoes per "dip." In most local mosquito control agencies, technicians search for new sources and inspect known habitats for mosquitoes on a 7 to 14-day cycle. These data are used to direct control operations. Maintaining careful records of immature mosquito occurrence and abundance, developmental stages treated, source sizes, and control effectiveness can be useful for estimating the expected size of future adult populations.

Adult mosquito abundance is a key factor contributing to the risk of virus transmission. Monitoring the abundance of adult mosquito populations provides important information on the size of the vector population as it responds to changing climatic factors and to control efforts. Four adult mosquito sampling methods are currently used for *Culex* in California: New Jersey light traps, carbon dioxide-baited traps, gravid female traps, and resting adult mosquito collections. The advantages and disadvantages of these sampling methods, and guidelines for the design, operation, and processing of the traps have been discussed in Guidelines for Integrated Mosquito Surveillance (Meyer et al. 2003) and are summarized in Appendix A.

Mosquito Infections

Virus activity can be monitored by testing adult mosquitoes for virus infection. Because *Cx. tarsalis* is the primary rural vector of WNV, SLEV, and WEEV, and *Cx. quinquefasciatus* and *Cx. pipiens* are important urban vectors of WNV and SLEV, surveillance efforts emphasize the testing of these species. Another species that should be tested is *Cx. stigmatosoma*, which is a highly competent but less widely distributed vector of WNV and SLEV that feeds on birds and is probably important in enzootic transmission where abundant. Female mosquitoes are trapped, usually using carbon dioxide-baited or gravid traps, identified to species, and counted into

groups (pools) of ≤ 50 females each for testing at the Davis Arbovirus Research and Training (DART) laboratory at UC Davis or by local agencies that pass annual proficiency tests. Procedures for submitting and processing mosquitoes for virus testing are detailed in Appendix B. The current surveillance system is designed to detect and measure levels of infection with WNV, SLEV, and WEEV. Mosquito testing typically begins early in the season and, with adequate trapping and testing effort, provides early warning of virus activity. Testing adult mosquitoes for infection is also one of the best methods to detect newly introduced or emerging mosquito-borne viruses. Testing non-*Culex* mosquito species may be necessary to detect the introduction of viruses that do not have a primary *Culex*-bird transmission cycle, notably dengue, Zika, or chikungunya viruses transmitted between humans by *Ae. aegypti* and *Ae. albopictus*.

Avian Infections

Detection of arboviral transmission within bird populations can be accomplished by 1) using caged chickens as sentinels and bleeding them routinely to detect development of viral antibodies (seroconversions), 2) testing dead birds reported by the public for WNV, and 3) collecting and bleeding wild birds to detect prevalence of viral antibodies (seroprevalence).

In California, flocks of 6-10 chickens, previously unexposed to arboviruses, are placed in locations where mosquito abundance is known to be high or where there is a history of virus activity. Each chicken is bled every two weeks by pricking the comb and collecting blood on a filter paper strip. The blood is tested at the CDPH Vector-Borne Disease Section for antibodies to SLEV, WEEV, and WNV. Some agencies conduct their own testing, but send positive samples to CDPH for confirmation and official reporting. Because SLEV cross-reacts with WNV in antibody testing, SLEV or WNV positive chickens are confirmed and the infecting virus is identified by western blot or cross-neutralization tests. Frequent testing of strategically placed flocks of sentinel chickens provides an effective method to monitor encephalitis virus transmission in an area, particularly as a surrogate for human risk because information on human cases often arrives too late for mosquito control decisions. Because chickens are continuously available to host-seeking mosquitoes, they are not subject to the night-to-night variation associated with mosquito trapping, and their stationary location provides a specific spatial indication of transmission when seroconversions occur. Sentinel housing, bleeding instructions, and testing protocols are provided in Appendix C.

Unlike WEEV and SLEV, WNV frequently causes death in North American birds, especially those in the family Corvidae (e.g., crows, ravens, magpies, and jays). Dead bird surveillance was initiated by CDPH in 2000 to provide early detection of WNV. Dead bird surveillance has been shown to be one of the earliest and most cost-effective indicators of WNV activity where susceptible bird species are abundant and local agencies promote this program. Dead birds are reported by the public to CDPH's dead bird hotline (1-877-WNV-BIRD) or via the California West Nile virus website (http://westnile.ca.gov). Dead birds that meet criteria for species and condition are collected by local agencies for WNV testing. Agencies collect an oral sample by swabbing the oropharyngeal cavity of the bird and pressing the swab onto an RNA preservation card, which safely preserves nucleic acids. The cards are mailed to DART for WNV RNA testing by real-time RT-PCR. Local agencies may also test dead birds in-house using RT-PCR or RAMP® tests provided they have passed annual proficiency panels. The communication and testing algorithm for the dead bird surveillance program is detailed in Appendix D.

Virus activity in wild bird populations can be monitored by bleeding young (hatching year) birds to detect initial virus infection or by bleeding a cross-section of birds in an area and comparing seroprevalence among age strata to determine if there is evidence for recent changes in prevalence of the virus. Elevated seroprevalence levels ("herd immunity") among key species during spring may limit virus transmission and dampen amplification. New infections also can be detected by bleeding banded birds in a capture-recapture scheme. In contrast to the convenience of using sentinel chickens, the repeated collection and bleeding of wild birds requires specialized permits and is labor intensive, technically difficult, and too expensive for most local mosquito control agencies to perform routinely. In addition, the actual place where a wild bird became infected is rarely known, because birds may travel over relatively long distances, and usually are collected during daytime foraging flights and not at nighttime roosting sites where they are bitten by mosquitoes.

Equine Infections

Currently, equine disease due to WEEV and WNV is no longer a sensitive indicator of epizootic activity (unusually high incidence of infections in animals other than humans) in California because of the widespread vaccination or natural immunization of equids (horses, donkeys, and mules). Nevertheless, confirmed cases in horses can indicate that WEEV or WNV has amplified to levels where tangential transmission has occurred and risk to humans is elevated in that region of the state. Numerous infectious and non-infectious causes, including other mosquito-borne viruses, can contribute to encephalitis and neurologic signs in horses. Testing of equine specimens for these possible etiologies is available through the California Animal Health and Food Safety Laboratory (CAHFS). Complete information on specimen collection and submission is available on the California Department of Food and Agriculture (CDFA) website at: http://www.cdfa.ca.gov/ahfss/Animal_Health/WNV_Lab_Submission.html. See Appendix E.

Human Infections

Local mosquito control agencies need information from the rapid detection and reporting of confirmed human cases to plan and implement emergency control activities to prevent additional infections. However, human cases of arboviral infection are an insensitive surveillance indicator of virus activity because most persons who become infected develop no or mild symptoms. For those individuals who do become ill, it may take up to two weeks for symptoms to appear, followed by additional time until the case is recognized and reported. A total of 6,030 cases of WNV have been reported in California from 2003 to 2016. Three human SLEV disease cases were detected in 2016; these were the first reported SLEV cases in California since 1997. No human WEEV cases have been reported in California in recent years.

To enhance human WNV testing and surveillance efforts throughout the state, a regional public health laboratory network was established in 2002. The laboratory network consists of the state Viral and Rickettsial Disease Laboratory (VRDL) as well as 9 county public health laboratories that are able to conduct WNV testing. Providers are encouraged to submit specimens from suspect WNV cases to their local public health laboratories. Specimens from patients with encephalitis may also be submitted directly to Neurologic Surveillance and Testing, which is based in the VRDL and offers diagnostic testing for many agents known to cause encephalitis, including WNV and other arboviruses. In addition, VRDL collaborates with reference

laboratories such as the regional laboratories of Kaiser Permanente to confirm additional suspect WNV cases.

In accordance with Title 17 of the California Code of Regulations (Sections 2500 and 2505), physicians and laboratories are required to report positive test results for WNV, SLEV, and WEEV to their local health department. Positive arbovirus test results are investigated by local health department officials to determine whether a patient meets the clinical and laboratory criteria for diagnosis of arboviral disease. If so, the local health department collects demographic and clinical information on the patient using a standardized case report form, and forwards the report to the state health department. The local health department also determines whether the infection was acquired locally, imported from a region outside the patient's residence, or acquired by a non-mosquito route of transmission such as blood transfusion or organ transplantation. Appendix F contains the protocol for submission of specimens to the regional public health laboratory network for WNV testing. Appendix G provides the national surveillance case definitions for WNV, SLEV, and WEEV infections. For information on *Aedes*-transmitted diseases, such as Zika, dengue, and chikungunya, please refer to "Guidance for Surveillance of and Response to Invasive Aedes Mosquitoes and Dengue, Chikungunya, and Zika in California."

Mosquito Control

Problems detected by surveillance are mitigated through larval and adult mosquito control. Mosquito control is the only public health method of protecting people from mosquito-borne diseases. Mosquito control in California is conducted by approximately 80 local agencies, including mosquito and vector control districts, county environmental and health departments, and county agriculture departments. Agencies applying pesticides directly to a water of the United States, or where deposition may enter a water of the United States, must obtain a National Pollutant Discharge Elimination System (NPDES) permit for Biological and Residual Pesticide Discharges to Waters of the United States from Vector Control Applications (Vector Control Permit). Agencies must comply with provisions of the permit.
http://www.swrcb.ca.gov/water_issues/programs/npdes/aquatic.shtml

Compounds currently approved for larval and adult mosquito control in California are listed in Appendix H. Please refer to the Vector Control Permit, Attachments E and F, for a list of vector control pesticides that may be applied to waters of the United States, unless the receiving water has an existing impairment from a pesticide with the same active ingredient. Please review the California State Water Resources Control Board listing of impaired water bodies (303d list) prior to applying any pesticide.
http://www.swrcb.ca.gov/rwqcb4/water_issues/programs/303d_list.shtml

Additional considerations regarding adult mosquito control in urban areas are described in Appendix I.

Larval Control

Mosquito larval and pupal control methods are target-specific and prevent the emergence of adult female mosquitoes which are capable of transmitting pathogens and becoming biting nuisances, and ultimately producing another generation of mosquitoes. For these reasons, most

mosquito control agencies in California target the immature stages rather than the adult stage of the mosquito. Larval mosquito control has three key components: environmental management, biological control, and chemical control.

Environmental management decreases habitat availability or suitability for immature mosquitoes, and may include water management, such as increasing the water disposal rate through evaporation, percolation, recirculation, or drainage. Laser-leveling of fields minimizes pooling at low spots, allows even distribution of irrigation water, and precludes standing water for long periods. Controlled irrigation or the careful timing of wetland flooding for waterfowl can reduce mosquito production or limit emergence to cooler seasons of the year when virus activity is unlikely. Environmental management may include vegetation management because emergent vegetation provides food and refuge for mosquito larvae. Management strategies include the periodic removal or thinning of vegetation, restricting growth of vegetation, and controlling algae.

Biological control uses natural predators, parasites, or pathogens to reduce immature mosquito numbers. Mosquitofish, *Gambusia affinis*, are the most widely used biological control agent in California. These fish are released annually in a variety of habitats, such as rice fields, small ponds, and canals.

There are several mosquito control products that are highly specific and thus have minimal impact on non-target organisms. These include microbial control agents, such as *Bacillus thuringiensis israelensis* (Bti), *Bacillus sphaericus*, and spinosad; and insect growth regulators, such as methoprene, that prevent immature mosquitoes from developing into adults. Surface films are very effective against both larvae and pupae, but also may suffocate other surface-breathing aquatic insects. Organophosphate pesticides are used infrequently because of widespread resistance within mosquito populations and their impact on non-target organisms and the environment.

Adult Control

When larval control is not possible or more immediate control measures are needed, adult mosquito control may be required to suppress populations of infected mosquitoes and interrupt epidemic virus transmission. Adult mosquito control products may be applied using ground-based equipment, fixed wing airplanes, or helicopters. Products applied in ultralow volume (ULV) formulations and dosages include organophosphates (e.g., malathion and naled), pyrethroids (e.g., resmethrin, sumithrin, and permethrin), and pyrethrins (e.g., Pyrenone crop spray). Factors to consider when selecting an adulticide include: 1) efficacy against the target species or life cycle stage, 2) resistance status, 3) pesticide label requirements, 4) availability of pesticide and application equipment, 5) environmental conditions, 6) cost, and 7) toxicity to nontarget species, including humans.

For more information about mosquito control please see “Best Management Practices for Mosquito Control in California.” http://westnile.ca.gov/resources.php

Response Levels

The California Mosquito-borne Virus Surveillance and Response Plan was developed to provide a semi-quantitative measure of virus transmission risk to humans that could be used by local mosquito control agencies to plan and modulate control activities. Independent models are presented for WEEV, SLEV, and WNV to accommodate the different ecological dynamics of these viruses (Barker et al. 2003). SLEV and WNV are closely related, require similar environmental conditions, and are transmitted by the same *Culex* vectors. Seven surveillance factors are measured and analyzed to determine the level of risk for human involvement and thereby gauge the appropriate response level:

1. Environmental or climatic conditions (e.g., snowpack, rainfall, and temperature)
2. Adult *Culex* vector abundance
3. Virus infection rate in *Culex* mosquito vectors
4. Sentinel chicken seroconversions
5. Fatal infections in birds (WNV only)
6. Infections in humans
7. Proximity of detected virus activity to urban or suburban regions (WEEV only)

Each factor is scored on an ordinal scale from 1 (lowest risk) to 5 (highest risk). The mean score calculated from these factors corresponds to a response level as follows: normal season (1.0 to 2.5), emergency planning (2.6 to 4.0), and epidemic (4.1 to 5.0). Table 1 provides a worksheet to assist in determining the appropriate rating for each of the risk factors for each of the three viruses. Appendix J shows sources of data useful in the calculation of risk in Table 1. Surveillance data can be managed and risk level calculated in time and space using the CalSurv Gateway.

Risk calculations should be applied within a defined area, typically encompassing a local mosquito and vector control district. Use of smaller spatial units (e.g., city boundaries) is ideal due to spatial variation in virus activity and the need to define potential target areas for mosquito control at finer spatial scales. Due to spatial variation in the distributions of humans and the dominant vector species, *Cx. tarsalis* and the *Cx. pipiens* complex, separate calculation of risk for urban and rural areas is encouraged where applicable.

For surveillance factor 2 (vector abundance), abundance is expressed as a percentage of normal by comparing the current level for an area to the average over the previous 5 years for the same area and two-week period. The mosquito virus infection rate should be calculated using the most recent data (prior two-week period) and expressed as the minimum infection rate (MIR) per 1,000 female mosquitoes tested. Alternatively, when infection rates are high, they may be calculated using maximum likelihood estimates (US Centers for Disease Control and Prevention 2011), which account for varying numbers of specimens in pools and the possibility that more than one mosquito could be infected in each positive pool. For WNV and SLEV, risk may be estimated separately for *Cx. tarsalis* and the *Cx. pipiens* complex, respectively, because these species generally have different habitat requirements and therefore spatial distributions (e.g., rural vs. urban).

Each of the three viruses differs in its response to ecological conditions. WEEV activity typically is greatest during El Niño conditions of wet winters, above-normal run-off and flooding, cool springs, and increased *Cx. tarsalis* abundance. Historically, WEEV spillover into a secondary

Aedes-rabbit cycle was common in the Central Valley, but this has not been detected for more than 25 years. In contrast, SLEV and perhaps WNV activity appears to be greatest during La Niña conditions of drought and hot summer temperatures, because both SLEV and WNV transmission risk increases when temperatures are above-normal. Abundance and infection of the *Cx. pipiens* complex are included in both SLEV and WNV risk estimates because these mosquito species are important vectors, particularly in suburban/urban environments. The occurrence of dead bird infections is included as a risk factor in the WNV calculations. For surveillance factors 4–6 (chickens, birds, and humans), the specific region is defined as the area within the agency's boundary and the broad region includes the area within 150 miles (~241 km) of the agency's boundary.

Proximity of virus activity to human population centers is considered an important risk factor for all three viruses of public health concern. In the risk assessment model in Table 1 this was accommodated in two different ways. WEEV transmitted by *Cx. tarsalis* typically amplifies first in rural areas and may eventually spread into small and then larger communities. A risk score was included to account for where virus activity was detected. WNV and SLEV may be amplified concurrently or sequentially in rural and urban cycles. The rural cycle is similar to WEEV and is transmitted primarily by *Cx. tarsalis*, whereas the urban cycle is transmitted primarily by members of the *Cx. pipiens* complex. If the spatial distributions of key *Culex* species differ within an area (e.g., rural vs. urban), it may be advantageous to assess risk separately by species for abundance and infection rates in *Cx. tarsalis* and the *Cx. pipiens* complex. This would result in two estimates of overall risk for the areas dominated by each species.

Each of these surveillance factors can differ in impact and significance according to time of year and geographic region. Climate is used prospectively to forecast risk during the coming season. Climatic factors provide the earliest indication of the potential for increased mosquito abundance and virus transmission and constitute the only risk factor measured in many areas from the start of the calendar year through mid-spring when enzootic surveillance commences. Other factors that may inform control efforts as the season progresses are typically, in chronological order: mosquito abundance, infections in non-humans (e.g., dead birds for WNV, mosquitoes, and sentinel chickens), and infections in humans. Enzootic indicators measure virus amplification within the *Culex*-bird cycle and provide nowcasts of risk, whereas human infections document tangential transmission and are the outcome measure of forecasts and nowcasts. Response to the calculated risk level should consider the time of year (e.g., epidemic conditions in October would warrant a less aggressive response compared to epidemic conditions in July because cooler weather in late fall will contribute to declining risk of arbovirus transmission).

The ratings listed in Table 1 are benchmarks only and may be modified as appropriate to the conditions in each specific region or biome of the state. Calculation and mapping of risk have been enabled by tools for local agency use included in the CalSurv Gateway. Roles and responsibilities of key agencies involved in carrying out the surveillance and response plan are outlined in "Key Agency Responsibilities."

Table 1. Mosquito-borne Virus Risk Assessment.

WNV Surveillance Factor	Assessment Value	Benchmark	Assigned Value	
1. **Environmental Conditions** High-risk environmental conditions include above-normal temperatures with or without above-normal rainfall, runoff, or snowpack. Weather data link: http://ipm.ucdavis.edu	1	Avg daily temperature during prior 2 weeks ≤ 56°F		
	2	Avg daily temperature during prior 2 weeks 57–65°F		
	3	Avg daily temperature during prior 2 weeks 66–72°F		
	4	Avg daily temperature during prior 2 weeks 73–79°F		
	5	Avg daily temperature during prior 2 weeks > 79°F		
			Cx tars	*Cx pip*
2. **Relative abundance of adult female *Culex tarsalis* and *Cx. pipiens* complex mosquitoes*** Determined by trapping adults, enumerating them by species, and comparing numbers to those previously documented for an area for the prior 2-week period.	1	Vector abundance well below average (≤ 50%)		
	2	Vector abundance below average (51–90%)		
	3	Vector abundance average (91–150%)		
	4	Vector abundance above average (151–300%)		
	5	Vector abundance well above average (> 300%)		
3. **Virus infection rate in *Cx. tarsalis* and *Cx. pipiens* complex mosquitoes*** Tested in pools of ≤ 50 females. Test results expressed as minimum infection rate per 1,000 mosquitoes tested (MIR) for the prior 2-week period.	1	MIR = 0		
	2	MIR = 0.1–1.0		
	3	MIR = 1.1–2.0		
	4	MIR = 2.1–5.0		
	5	MIR > 5.0		
4. **Sentinel chicken seroconversion** Number of chickens in a flock that develop antibodies to WNV during the prior 2-week period. If more than one flock is present in a region, number of flocks with seropositive chickens is an additional consideration. Typically 10 chickens per flock.	1	No seroconversions in broad region		
	2	One or more seroconversions in broad region		
	3	One or two seroconversions in a single flock in specific region		
	4	More than two seroconversions in a single flock or two flocks with one or two seroconversions in specific region		
	5	More than two seroconversions per flock in multiple flocks in specific region		
5. **Dead bird infection** Number of birds that have tested positive (recent infections only) for WNV during the prior 3-month period. This longer time period reduces the impact of zip code closures during periods of increased WNV transmission.	1	No positive dead birds in broad region		
	2	One or more positive dead birds in broad region		
	3	One positive dead bird in specific region		
	4	Two to five positive dead birds in specific region		
	5	More than five positive dead birds in specific region		
6. **Human cases** Do not include this factor in calculations if no cases are detected in region.	3	One or more human infections in broad region		
	4	One human infection in specific region		
	5	More than one human infection in specific region		
			Cx tars	*Cx pip*
Response Level / Average Rating: Normal Season (1.0 to 2.5) Emergency Planning (2.6 to 4.0) Epidemic (4.1 to 5.0)		**TOTAL**		
		AVERAGE		

*Calculation of separate risk values for *Cx. tarsalis* and the *Cx. pipiens* complex may be useful if their spatial distributions (e.g., rural vs. urban) differ within the assessment area.

SLEV Surveillance Factor	Assessment Value	Benchmark	Assigned Value	
1. **Environmental Conditions** High-risk environmental conditions include above-normal temperatures with or without above-normal rainfall, runoff, or snowpack. Weather data link: http://ipm.ucdavis.edu	1	Avg daily temperature during prior 2 weeks ≤ 56°F		
	2	Avg daily temperature during prior 2 weeks 57–65°F		
	3	Avg daily temperature during prior 2 weeks 66–72°F		
	4	Avg daily temperature during prior 2 weeks 73–79°F		
	5	Avg daily temperature during prior 2 weeks > 79°F		
			Cx tars	*Cx pip*
2. **Relative abundance of adult female *Culex tarsalis* and *Cx. pipiens* complex mosquitoes*** Determined by trapping adults, enumerating them by species, and comparing numbers to those previously documented for an area for the prior 2-week period.	1	Vector abundance well below average (≤ 50%)		
	2	Vector abundance below average (51–90%)		
	3	Vector abundance average (91–150%)		
	4	Vector abundance above average (151–300%)		
	5	Vector abundance well above average (> 300%)		
3. **Virus infection rate in *Cx. tarsalis* and *Cx. pipiens* complex mosquitoes*** Tested in pools of ≤ 50 females . Test results expressed as minimum infection rate per 1,000 mosquitoes tested (MIR) for the prior 2-week collection period.	1	MIR = 0		
	2	MIR = 0.1–1.0		
	3	MIR = 1.1–2.0		
	4	MIR = 2.1–5.0		
	5	MIR > 5.0		
4. **Sentinel chicken seroconversion** Number of chickens in a flock that develop antibodies to SLEV during the prior 2-week period. If more than one flock is present in a region, number of flocks with seropositive chickens is an additional consideration. Typically 10 chickens per flock.	1	No seroconversions in broad region		
	2	One or more seroconversions in broad region		
	3	One or two seroconversions in a single flock in specific region		
	4	More than two seroconversions in a single flock or two flocks with one or two seroconversions in specific region		
	5	More than two seroconversions per flock in multiple flocks in specific region		
5. **Human cases** Do not include this factor in calculations if no cases are detected in region.	3	One or more human cases in broad region		
	4	One human case in specific region		
	5	More than one human case in specific region		
			Cx tars	*Cx pip*
Response Level / Average Rating: Normal Season (1.0 to 2.5) Emergency Planning (2.6 to 4.0) Epidemic (4.1 to 5.0)		**TOTAL**		
		AVERAGE		

*Calculation of separate risk values for *Cx. tarsalis* and the *Cx. pipiens* complex may be useful if their spatial distributions (e.g., rural vs. urban) differ within the assessment area.

WEEV Surveillance Factor	Assessment Value	Benchmark	Assigned Value
1. **Environmental Conditions** High-risk environmental conditions include above normal rainfall, snow pack, and runoff during the early season followed by a strong warming trend. Weather data link: http://ipm.ucdavis.edu	1	Cumulative rainfall and runoff well below average	
	2	Cumulative rainfall and runoff below average	
	3	Cumulative rainfall and runoff average	
	4	Cumulative rainfall and runoff above average	
	5	Cumulative rainfall and runoff well above average	
2. **Relative abundance of adult female *Culex tarsalis* mosquitoes** Determined by trapping adults, enumerating them by species, and comparing numbers to averages previously documented for an area for the prior 2-week period.	1	*Cx. tarsalis* abundance well below average (≤ 50%)	
	2	*Cx. tarsalis* abundance below average (51–90%)	
	3	*Cx. tarsalis* abundance average (91–150%)	
	4	*Cx. tarsalis* abundance above average (151–300%)	
	5	*Cx. tarsalis* abundance well above average (> 300%)	
3. **Virus infection rate in *Cx. tarsalis* mosquitoes** Tested in pools of ≤ 50 females. Test results expressed as minimum infection rate per 1,000 mosquitoes tested (MIR) for the prior 2-week collection period.	1	*Cx. tarsalis* MIR = 0	
	2	*Cx. tarsalis* MIR = 0.1–1.0	
	3	*Cx. tarsalis* MIR = 1.1–2.0	
	4	*Cx. tarsalis* MIR = 2.1–5.0	
	5	*Cx. tarsalis* MIR > 5.0	
4. **Sentinel chicken seroconversion** Number of chickens in a flock that develop antibodies to WEEV during the prior 2-week period. If more than one flock is present in a region, number of flocks with seropositive chickens is an additional consideration. Typically 6-10 chickens per flock.	1	No seroconversions in broad region	
	2	One or more seroconversions in broad region	
	3	One or two seroconversions in a single flock in specific region	
	4	More than two seroconversions in a single flock or two flocks with one or two seroconversions in specific region	
	5	More than two seroconversions per flock in multiple flocks in specific region	
5. **Proximity to urban or suburban regions** (score only if virus activity is detected) Risk of outbreak is highest in urban areas because of high likelihood of contact between humans and vectors.	1	Virus detected in rural area	
	3	Virus detected in small town or suburban area	
	5	Virus detected in urban area	
6. **Human cases** Do not include this factor in calculations if no cases found in region or in agency.	3	One or more human cases in broad region	
	4	One human case in specific region	
	5	More than one human case in specific region	
Response Level / Average Rating: Normal Season (1.0 to 2.5) Emergency Planning (2.6 to 4.0) Epidemic (4.1 to 5.0)		**TOTAL**	
		AVERAGE	

General suggestions for applying the risk assessment model locally

- Use a consistent time period for environmental conditions, adult mosquito abundance, mosquito infection rates, and human cases. If you use a period that differs from the prior two-week period defined in the risk assessment, such as the prior month, use the same period for all other relevant measures. Note that sentinel chicken seroconversions may need special treatment to accommodate bleeding schedules and dead bird data need to accommodate zip code closures. For sentinel seroconversions, use data from the most recent collection.
- If you have multiple trap types in your surveillance program, determine the vector abundance anomaly for each trap type and species and use the most sensitive trap type's value in the risk assessment.
- When determining the vector abundance anomaly, there should be at least two and preferably five years of prior data to provide a comparative baseline for the particular trap type. Ideally, the prior years should use the same or very similar trap locations and be contiguous and immediately precede the time period being evaluated.

Risk assessment as implemented by the CalSurv Gateway (http://gateway.calsurv.org)

- Statewide maps at a resolution of 1 km^2 are generated and delivered to the primary contacts of each agency by email every Monday.
- Only those agencies with active Gateway accounts and defined primary contacts will receive the weekly maps.
- Mapped risk surfaces are generated for all areas of California that have one or more surveillance inputs within 8 km. The risk for each pixel in the map image is based on a spatially weighted summary of all available surveillance data within 8 km. Pixels > 8 km from the nearest surveillance do not have assigned risk values.
- Due to privacy concerns and delays in detection and reporting, human cases are not part of the Gateway's risk assessment.
- All of the general suggestions from the prior section are used in the Gateway's implementation.
- Risk estimates based on mosquito abundance and infection rates will be calculated separately for the key mosquito taxa, *Culex tarsalis* and the *Cx. pipiens* complex.
- The risk assessment model is implemented also as an online calculator for use by local vector control agencies that allows user definition of locations, date ranges, and other criteria.

Characterization of Conditions and Responses for State and Local agencies

Level 1: Normal Season

Risk rating: 1.0 to 2.5

CONDITIONS
• Cool to moderate seasonal temperatures (< 65°F) • *Culex* mosquito abundance at or below five year average (key indicator = adults of vector species) • No virus infection detected in mosquitoes • No seroconversions in sentinel chickens • No recently infected WNV-positive dead birds • No human cases
RESPONSE
• Conduct routine public education (eliminate standing water around homes, use personal protection measures) • Conduct routine mosquito and virus surveillance activities • Comply with National Pollutant Discharge Eliminations System (NPDES) permit if applying pesticides to waters of the United States • Conduct routine mosquito control with emphasis on larval control • Inventory pesticides and equipment • Evaluate pesticide resistance in vector species • Ensure adequate emergency funding • Release routine press notices • Send routine notifications to physicians and veterinarians • Establish and maintain routine communication with local office of emergency services personnel; obtain Standardized Emergency Management System (SEMS) training

Level 2: Emergency Planning

Risk rating: 2.6 to 4.0

CONDITIONS
• Temperature above average (66–79°F) • Adult *Culex* mosquito abundance greater than 5-year average (150% to 300% above normal) • One or more virus infections detected in *Culex* mosquitoes (MIR < 5 per 1,000 tested) • One or more seroconversions in single flock or one to two seroconversions in multiple flocks in specific region • One to five recently infected WNV-positive dead birds in specific region • One human case in broad or specific region • WEEV detected in small towns or suburban area
RESPONSE
• Review epidemic response plan • Enhance public education (include messages on the signs and symptoms of encephalitis; seek medical care if needed; inform public about pesticide applications if appropriate) • Enhance information to public health providers • Conduct epidemiological investigations of cases of equine or human disease • Increase surveillance and control of mosquito larvae • Increase adult mosquito surveillance • Increase number of mosquito pools tested for virus • Conduct or increase localized chemical control of adult mosquitoes as appropriate • Contact commercial applicators in anticipation of large scale adulticiding • Review candidate pesticides for availability and susceptibility of vector mosquito species • Ensure notification of key agencies of presence of viral activity, including the local office of emergency services

Level 3: Epidemic Conditions

Risk rating: 4.1 to 5.0

CONDITIONS
• Temperature well above average (> 79°F) • Adult vector population extremely high (> 300% above normal) • Virus infections detected in multiple pools of *Culex tarsalis* or *Cx. pipiens* mosquitoes (MIR > 5 per 1,000 tested) • More than two seroconversions per flock in multiple flocks in specific region • More than five recently infected WNV-positive dead birds and multiple reports of dead birds in specific region • More than one human case in specific region • WEE virus detection in urban or suburban areas
RESPONSE
• Conduct full-scale media campaign • Alert physicians and veterinarians to expect cases • Conduct active human case detection • Conduct epidemiological investigations of cases of equine or human disease • Continue enhanced larval surveillance and control of immature mosquitoes • Broaden geographic coverage of adult mosquito surveillance • Accelerate adult mosquito control as appropriate by ground and/or air • Coordinate the response with the local Office of Emergency Services or if activated, the Emergency Operation Center (EOC) • Initiate mosquito surveillance and control in geographic regions without an organized vector control program • Determine whether declaration of a local emergency should be considered by the County Board of Supervisors (or Local Health Officer) • Determine whether declaration of a "State of Emergency" should be considered by the Governor at the request of designated county or city officials • Ensure state funds and resources are available to assist local agencies at their request • Determine whether to activate a Standardized Emergency Management System (SEMS) plan at the local or state level • Continue mosquito education and control programs until mosquito abundance and enzootic virus activity is substantially reduced and no additional human cases are detected

For more detailed information on responding to a mosquito-borne disease outbreak, please refer to:

Operational Plan for Emergency Response to Mosquito-Borne Disease Outbreaks, California Department of Public Health (supplement to California Mosquito-Borne Virus Surveillance and Response Plan). http://westnile.ca.gov/resources.php

Key Agency Responsibilities

Local Mosquito and Vector Control Agencies

- Acquire and interpret local climate and weather data.
- Monitor abundance of immature and adult mosquitoes.
- Collect and submit mosquito pools for virus detection at DART or local laboratories.
- Maintain sentinel chicken flocks, obtain blood samples, and send samples to VBDS.
- Pick-up and sample dead birds by oral swabs using RNA preservation cards for WNV testing, or test oral swabs from suitable bird species locally via RT-PCR or RAMP® screening assays.
- Update the CalSurv Gateway weekly to record all birds that are independently reported and/or tested by RAMP® or RT-PCR.
- Update the CalSurv Gateway weekly to report mosquito pool results that are independently tested by RAMP® or RT-PCR.
- Conduct routine control of immature mosquitoes.
- Comply with NPDES permit if applying pesticides to waters of the United States.
- Conduct control of adult mosquitoes when needed.
- Educate public on mosquito avoidance and reduction of mosquito breeding sites.
- Coordinate with local Office of Emergency Services personnel.
- Communicate regularly with neighboring agencies.

Mosquito and Vector Control Association of California

- Coordinate purchase of sentinel chickens.
- Receive, track, and disburse payment for mosquito surveillance expenses.
- Coordinate surveillance and response activities among member agencies.
- Serve as spokesperson for member agencies.
- Establish liaisons with press and government officials.

California Department of Public Health

- Collate adult mosquito abundance data submitted by local agencies; provide summary of data to local agencies.
- Maintain a WNV information and dead bird reporting hotline, 1-877-WNV-BIRD, and a WNV website: http://westnile.ca.gov.
- Coordinate submission of specimens for virus testing.
- Provide supplies for sentinel chicken diagnostic specimens.
- Test sentinel chicken sera for viral antibodies.
- Test human specimens for virus.
- Distribute a weekly bulletin summarizing surveillance test results.
- Report weekly surveillance results to the CDC ArboNET surveillance system.
- Immediately notify local vector control agency and public health officials when evidence of virus activity is found.
- Conduct epidemiological investigations of cases of human disease.
- Coordinate and participate in a regional emergency response in conjunction with California Emergency Management Agency.
- Conduct surveillance for human cases.

- Provide oversight to local jurisdictions without defined vector-borne disease control program.
- Maintain inventory of antigens, antisera, and RNA assays to detect exotic viruses.
- Provide confirmation of tests done by local agencies.

University of California at Davis

- Conduct research on arbovirus surveillance, transmission of mosquito-borne pathogens, and mosquito ecology and control.
- Test mosquito pools and dead bird samples on RNA preservation cards for endemic and introduced viruses.
- Provide a proficiency panel to local agencies annually to evaluate local tests used for identification of viruses from birds or arthropod vectors to ensure quality control.
- Maintain an interactive website (http://gateway.calsurv.org) for management and dissemination of data on mosquito-borne virus surveillance and control.
- Maintain inventory of antigens, antisera, and viruses to detect the introduction of exotic viruses.
- Provide confirmation of tests done by local or state agencies.

California Department of Food and Agriculture

- Notify veterinarians and veterinary diagnostic laboratories about WEEV and WNV testing available at CAHFS.
- Provide outreach to general public and livestock managers of the need to monitor and report equine and ratite encephalitides.
- Facilitate equine and ratite sample submission from veterinarians.
- Conduct investigations of confirmed WNV and WEEV equine cases.

California Animal Health and Food Safety Laboratory

- Test equine and other animal specimens for evidence of WNV or other arbovirus infection.

Local Health Departments and Public Health Laboratories

- Test human specimens for WNV or other arboviruses.
- Refer human specimens to CDPH for further testing.
- Notify local medical community, including hospitals and laboratories, if evidence of viral activity is present.
- Collect dead birds and send oral swab samples on RNA preservation cards to testing laboratory as resources allow.
- Test American crows or other suitable bird species via RAMP® or RT-PCR as resources allow.
- Participate in emergency response.
- Conduct epidemiological investigations of cases of human disease.
- Report WNV and other arbovirus cases to CDPH.
- Conduct public education.

California Emergency Management Agency

- Coordinate the local, regional, or statewide emergency response to epidemic conditions in conjunction with CDPH via the Standardized Emergency Management System (SEMS).
- Serve as liaison with the Federal Emergency Management Agency (FEMA) in the event that a federal disaster has been declared.

Federal Centers for Disease Control and Prevention

- Provide consultation to state and local agencies in California if epidemic conditions exist.
- Provide national surveillance data to state health departments.
- Provide diagnostic consultation.

State Water Resources Control Board

- Review NPDES permit applications and respond in a timely manner.
- Review vector control pesticides registered by the California Department of Pesticide Regulation for inclusion on the Vector Control NPDES permit.

Appendix A: Guidelines for Adult Mosquito Surveillance

The objective of Appendix A is to standardize mosquito sampling and reporting procedures to provide comparable and interpretable abundance measures among collaborating mosquito control agencies in California. Specific sampling methods for invasive *Aedes* have been summarized in the document "Guidance for Surveillance of and Response to Invasive *Aedes* Mosquitoes and Dengue, Chikungunya, and Zika in California." Appendix A summarizes information from Integrated Mosquito Surveillance Program Guidelines for California that have been adopted by the Mosquito and Vector Control Association (MVCAC) (Meyer et al. 2003). The MVCAC guidelines recommend stratifying the use of different sampling methods in rural, small town, and urban environments for each of the major biomes of California and provide a listing of target vector and nuisance mosquito species. The stratified sampling approach monitors vector populations and virus activity in rural enzootic foci, agricultural or suburban amplification sites, and densely populated urban centers to provide estimates of early, eminent, and current epidemic risk.

The four sampling methods currently used by mosquito control agencies are: 1) New Jersey (American) light trap (Mulhern 1942); 2) CO_2-baited trap, such as CDC/EVS style (Newhouse et al. 1966; Sudia and Chamberlain 1962); 3) gravid trap (Cummings 1992; Reiter 1983); and 4) adult resting collections (Loomis and Sherman 1959). Collection location sites should be geocoded and registered using the CalSurv Gateway (http://gateway.calsurv.org/). Studies comparing trap design and efficiency for surveillance purposes have been published (Reisen et al. 2000; Reisen et al. 2002). These guidelines describe: 1) a comparison of the sampling methods, 2) equipment design, 3) operation, 4) specimen processing, 5) data recording and analysis, and 6) data usage.

Advantages and Disadvantages of Mosquito Sampling Methods:

New Jersey Light Trap	
Pros	**Cons**
• All female gonotrophic states and males collected • Minimal collection effort (can be run nightly without service) • Long history of use in California	• Selective for phototactic nocturnally active mosquitoes • Ineffective in the presence of competing light sources • Sorting time excessive because of other insects in traps • Specimens dead; less useful for virus detection • Collects comparatively few specimens
CDC/EVS CO_2 Trap	
Pros	**Cons**
• Samples biting population • Collects large numbers of virus vector species • Specimens are alive and suitable for virus detection • Without light, collects mostly mosquitoes and reduces sorting time • Battery operated, portable	• Collects >50% newly emerged females that have never blood fed, implying lower probability of infection • Must be set and picked-up daily • Dry ice cost may be high and availability can be a problem • Does not collect males or bloodfed and gravid females

Gravid Trap	
Pros	**Cons**
• Primarily collects females that have bloodfed and digested a blood meal; may have higher infection rate than CO_2 trap • Specimens are alive and suitable for virus detection • Effective for *Culex quinquefasciatus and Cx. pipiens* in urban habitats • Bait is inexpensive, consisting of water and organic matter • Battery operated, portable	• Collects only foul-water *Culex* (mostly *Cx. pipiens* complex) • Bait has an objectionable odor • Must be set and picked-up daily
Resting Catches	
Pros	**Cons**
• All female reproductive stages collected (unfed, bloodfed, and gravid) • Minimal equipment needed • Specimens are collected alive and suitable for virus detection • Bloodfed and gravid specimens can be tested to improve sensitivity of virus surveillance	• Standardization is difficult due to: 1. Variable shelter size and type 2. Variable collector efficiency • Labor intensive; difficult to concurrently sample a large number of sites

New Jersey (American) Light Trap (NJLT)

Operation

At a minimum, one trap should be located in each principal municipality of a district or have a density of about one trap/township (36 sq. mi.). Correct placement of the NJLT is a critical factor in its performance as an effective surveillance mechanism for measuring the relative abundance of phototactic mosquitoes. Place the traps at six-foot height. This can be done by using a metal stand, or by hanging the traps from tree limbs or roof eaves. These distances should maximize attractancy over a 360 degree radius. The trap should be placed on the leeward side of a structure or tree line to decrease the influence of wind on trap catch.

Traps should be kept away from smoke or chemical odors that may be repellent to the mosquitoes. Traps should be away from buildings in which animals are housed and not be in the immediate vicinity of sentinel flocks to minimize attractancy competition. Traps should be placed away from street and security lights that may diminish attractancy of the trap bulb. A trap should be placed approximately 100–200 feet from each sentinel chicken flock when possible to link abundance with seroconversions.

Traps should be operated from week 14 to week 44 of the calendar year for districts north of the Tehachapi Mountains and all year long for districts south of the Tehachapi. Ideally, the traps should run consecutively for four to seven nights before the collection is retrieved (Loomis and Hanks 1959). The trap should be cleaned thoroughly at each visit with a brush to remove spider webs or any other debris that may hinder airflow through the trap. A regular cleaning schedule should be maintained during the trapping season to maintain trap efficiency.

Processing

Adult mosquitoes from the NJLT collection should be sorted from the other insects in a white pan before being identified and counted at 10x magnification under a dissecting microscope. Counting aliquots or subsamples of all specimens should be discouraged because vector species may comprise only a small fraction of the total mosquito collection.

CDC style CO_2-baited trap

Operation

Carbon dioxide-baited traps can be used for abundance monitoring or capturing mosquitoes for virus testing, and increased trapping density will result in increased certainty for estimates of mosquito abundance and infection rates (Healy et al. 2015). Traps should be hung from a 6-foot tall standard pole (approximately 4 feet above ground level) to standardize trap placement for population and virus infection rate monitoring. Knowledge of the host-seeking patterns of the target species is essential in determining CO_2-baited trap placement in the habitat to enhance catch size and therefore sampling sensitivity. *Cx. tarsalis* primarily bloodfeed on birds and seek bloodmeals along vegetative borders and tree canopies where birds roost and nest. *Cx. erythrothorax* are best collected within wetland areas near dense stands of tules and cattails. In large, open breeding sources such as rice fields, CO_2-baited traps could be hung on standards on the upwind side of the source for *Cx. tarsalis* and *Anopheles freeborni* collections. *Aedes melanimon* and *Ae. nigromaculis* are mammal feeders and typically seek hosts over open fields.

When used for arbovirus surveillance, traps should be operated at different locations to enhance geographical coverage and thus surveillance sensitivity. Labor and time constraints determine the extent of sampling. When used to monitor population abundance, traps should be operated weekly or biweekly at the same fixed stations. Temperature, wind speed, wind direction, and rainfall should be recorded because these factors affect catch size. The mini-light may be removed, because it attracts other phototactic insects that may hinder sorting and/or damage female mosquitoes in the collection container and may repel members of the *Cx. pipiens* complex. The CO_2-baited trap should not be placed in immediate proximity to the sentinel chicken flock, because it will compete with, and therefore lessen, exposure of the sentinel birds, but may be placed within a 100–200 foot radius of the sentinel flock site, but no closer than 100 feet from the flock.

Processing

Mosquitoes collected for arbovirus surveillance should be processed according to the procedures outlined in Appendix B. If possible, ten pools of a species (*Cx. tarsalis*, *Cx. pipiens, Cx. quinquefasciatus, Cx. stigmatosoma, Ae. melanimon*, and *Ae. dorsalis*) should be submitted for virus testing from a given geographical location at a given time. Only live mosquitoes should be pooled for virus testing. Dead, dried specimens should be counted and discarded. Only whole specimens should be submitted; avoid including detached body parts (which may be from other mosquito species) or other Diptera (e.g., *Culicoides*, etc.) in the pool to prevent sample contamination. Avoid freezing specimens before sorting and counting. Mosquitoes collected for population monitoring should be anesthetized in a well-ventilated area or under a chemical hood using triethylamine, identified to species under a dissecting microscope, counted, pooled, and immediately frozen at -80°C or on dry ice for later virus testing.

Reiter/Cummings gravid traps

Trap design and components

The Reiter/Cummings gravid trap consists of a rectangular trap housing (plastic tool box) with an inlet tube on the bottom and an outlet tube on the side or top. The rectangular housing is provided with legs to stabilize the trap over the attractant basin containing the hay-infusion

mixture (Cummings 1992). The oviposition attractant consists of a fermented infusion made by mixing hay, Brewer's yeast, and water. The mixture should sit at ambient temperature for a minimum of three to four days prior to use to allow fermentation and increase attractancy. New solutions should be made at least biweekly to maintain consistent attractancy.

Operation

The Reiter/Cummings gravid trap is primarily used in suburban and urban residential settings for surveillance of gravid females in the *Cx. pipiens* complex. As for CO_2-baited traps, increased trapping density will result in increased certainty for estimates of mosquito abundance and infection rates (Healy et al. 2015). Gravid traps are placed on the ground near dense vegetation that serves as resting sites for gravid females. Specimens may be retrieved on a one to three-day basis.

Processing

Cx. pipiens complex females collected with the gravid trap for arbovirus surveillance should be retrieved daily and the protocol for mosquito pool submission as outlined in Appendix B should be followed. For population monitoring of the *Cx. pipiens* complex, collections may be retrieved every third day. The females are killed, identified, and counted before being discarded. Autogenous females also may be attracted to the gravid trap.

Adult resting collections

Trap design and operation

A flashlight and mechanical aspirator can be used to collect adult mosquitoes resting in habitats such as shady alcoves, buildings, culverts, or spaces under bridges. Highest numbers usually are collected at humid sites protected from strong air currents. Adults resting in vegetation may be collected using a mechanical sweeper such as the Arbovirus Field Station (AFS) sweeper (Meyer et al. 1983). For quantification, time spent searching is recorded and abundance expressed as the number collected per person-hour.

Red boxes were developed to standardize collections spatially. Different researchers have used red boxes of varying dimensions. Largest catches are made in semi-permanent walk-in red boxes which measure 4'x4'x6' (Meyer 1985). Smaller 1'x1'x1' foot boxes typically collect fewer specimens, but are readily portable. The entrance of the walk-in red box should be left open, draped with canvas, or closed with a plywood door. The canvas or plywood door should have a 1 or 2 ft gap at the bottom to allow entry of mosquitoes, while affording some protection from the wind and decreasing the light intensity within the box. The box entrance should not face eastward into the morning sun or into the prevailing wind direction.

Processing

Mosquitoes should be anesthetized with triethylamine, identified under a dissecting microscope, sorted by sex and female gonotrophic status (i.e., empty or unfed, blood fed, or gravid), and counted. Females may be counted into ten pools of approximately 50 females per site per collection date for virus monitoring (see Appendix B). Only living females should be used for arbovirus surveillance. Data on gonotrophic status may indicate population reproductive age as well as diapause status.

Data recording and analysis

Counts from NJLTs, EVS, and gravid traps and information on pools submitted for testing or tested locally should be entered directly in electronic format through the California Vectorborne Disease Surveillance Gateway (http://gateway.calsurv.org). Data import from local or proprietary data systems is available. For comparisons of abundance over time, space, or collection methods, refer to Bidlingmayer (1969).

Data usage

Mosquito collections from some or all of the four adult sampling methods collectively can be used to:

1. Assess control efforts.
2. Monitor arbovirus vector abundance and infection rates.
3. Compare mosquito abundance from collections with the number of service requests from the public to determine the tolerance of neighborhoods to mosquito abundance.
4. Determine proximity of breeding source(s) by the number of males present in collections from the NJLTs and red boxes.
5. Determine age structure of females collected by CO_2 traps and resting adult collections; such data are critical to evaluating the vector potential of the population.

Appendix B: Procedures for Processing Mosquitoes for Arbovirus Detection

1. Collect mosquitoes alive and return them immediately to the laboratory. Collections should be kept humid during transport with moist toweling to prevent desiccation. Females should be offered 5–10 percent sucrose if held overnight or longer before processing.

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 (Kramer et al. 1990). 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, sorting to species and enumeration 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 parts (i.e., legs, wings) or other small insects (e.g., chironomids or *Culicoides*) are not inadvertently included in the pools. This is extremely important because diagnostics have transitioned from virus isolation to sensitive RT-PCR methods of viral detection. Count and discard dead and dried mosquitoes. Pools are comprised of up to 50 females of each vector species from each collection site counted into individual polystyrene vials with snap caps containing two 5mm glass beads. Recommended sampling effort is ten pools of 50 females of each species from each site per week to detect minimum infection rates (MIRs) ranging from 0 to 20 per 1,000 females tested. Vials with pools should be labeled sequentially each year with the pool number and year after the agency code (e.g., KERN-1-17, where 17 refers to year 2017). Number pools consecutively starting with 1 for each calendar year within your agency.

   Data on each pool can be entered directly in electronic format through the California Vectorborne Disease Surveillance Gateway (http://gateway.calsurv.org/). POOLS MUST BE ACCOMPANIED BY A "MOSQUITO POOL SUBMISSION FORM " AND CAN ONLY BE TESTED FROM REGISTERED SITES. Surveillance sites should be registered online at: http://gateway.calsurv.org/.

   Register the surveillance site code for each pool in the CalSurv Gateway that consists of a designated four-letter agency code followed by six digits identifying the site (e.g., KERN000001). Pool numbers do not need to follow the ordering of site codes (e.g., pool #1 may be from KERN000001, pool #2 may be from KERN000004, pool #3 may be from KERN000003, etc.).

4. Freeze pools immediately at -80°C either on dry ice in an insulated container or in an ultra-low temperature freezer. Pools should be shipped frozen on dry ice to DART for testing by real-time multiplex RT-PCR. Agencies will receive an automated email notification that results have been entered into the CalSurv Gateway as well as a summary of positive pools; additionally, positive pools will be reported weekly in the California Arbovirus Surveillance Bulletin. Each pool is screened for West Nile virus (WNV), St. Louis encephalitis virus

(SLEV), and western equine encephalomyelitis virus (WEEV) by a multiplex RT-PCR assay. Positives with Ct scores >35 are confirmed by a singleplex RT-PCR with a different set of virus species-specific primers and probes. Pools from selected areas also are screened for additional viruses using Vero cell culture with isolates identified by genetic sequencing. 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 mosquito pool shipments to the following address:

ATTN: Ying Fang
University of California
One Shields Avenue
Vet Med: PMI
Room 3336 Vet Med 3A
Davis, CA 95616

For UPS shipments only:
Ying Fang
VM://PMI 3336 Vet Med 3A
1285 Veterinary Medicine Mall
University of California, Davis
Davis, CA 95616

5. Local agencies that conduct their own testing by RT-PCR or RAMP® tests need to complete and pass a proficiency panel each year for the results to be reported by CDPH.

Appendix C: Procedures for Maintaining and Bleeding Sentinel Chickens

1. Procure hens in March or when they become available as notified by MVCAC when the chickens are 14–18 weeks of age to ensure minimal mortality during handling. Hens at this age have not yet begun to lay eggs, but they should have received all their vaccinations and been dewormed.
2. Recommended housing for chickens. Flocks of 6–10 sentinel chickens can be housed in a 3Wx6Lx3H foot coop framed with 2x2 and 2x4 inch construction lumber and screened with no smaller than 1x1 inch welded wire. It is critical that the wire mesh be large enough to allow the mosquitoes to easily enter the coop and the coops be placed in locations with a history of arbovirus transmission and/or high mosquito abundance. The site and band numbers located at each coop must be registered online at: http://gateway.calsurv.org/. Faxed registration forms (MBVS-1) will be accepted from agencies without adequate internet access. Coops should be at least two feet off the ground to reduce predator access, facilitate capture of the birds for bleeding, and allow the free passage of the feces through the wire floor to the ground. A single, hinged door should be placed in the middle of the coop, so that the entire coop is accessible during chicken capture. After construction, the lumber and roof should be protected with water seal. A self-filling watering device should be fitted to one end of the coop and a 25 lb. feeder suspended in the center for easy access. In exchange for the eggs, a local person (usually the home owner, farm manager, etc.) should check the birds (especially the watering device) and remove the eggs daily. If hung so the bottom is about four inches above the cage floor and adjusted properly, the feeder should only have to be refilled weekly (i.e., 100 lb. of feed per month per flock of ten birds). Therefore, if proper arrangements can be made and an empty 55-gallon drum provided to store extra feed, sentinel flocks need only be visited biweekly when blood samples are collected.
3. Band each bird in the web of the wing using metal hog ear tags and appropriate pliers. This band number, the date, and site registration number must accompany each blood sample sent to the laboratory for testing.
4. Bleed each hen from the distal portion of the comb using a standard lancet used for human finger "prick" blood samples. The bird can be immobilized by wedging the wings between the bleeder's forearm and thigh, thereby leaving the hand free to hold the head by grabbing the base of the comb with the thumb and forefinger. Use alcohol swabs on comb before bleeding. Blood samples are collected on half-inch wide filter paper strips, which should be labeled with the date bled and wing band number. The comb should be "pricked" with the lancet and blood allowed to flow from the "wound" to form a drop. Collect the blood by touching the opposite end of the pre-labeled filter paper strip to the wound. THE BLOOD MUST COMPLETELY SOAK THROUGH ON A ¾ INCH LONG PORTION OF THE STRIP. Place the labeled end of the strip into the slot of the holder (or "jaws" of the clothes pin) leaving the blood soaked end exposed to air dry.
5. Attach the completely dry filter paper strips to a 5x7 inch card in sequential order, from left to right by stapling the labeled end towards the top edge of the card, and leaving the blood soaked end free so that the laboratory staff can readily remove a standard punch sample. Write the county, agency code, site, and date bled onto the card and place it into a Ziplock plastic bag. Do not put more than one sample card per bag. It is important that blooded ends do not become dirty, wet, or touch each other. CHICKEN SERA MUST BE

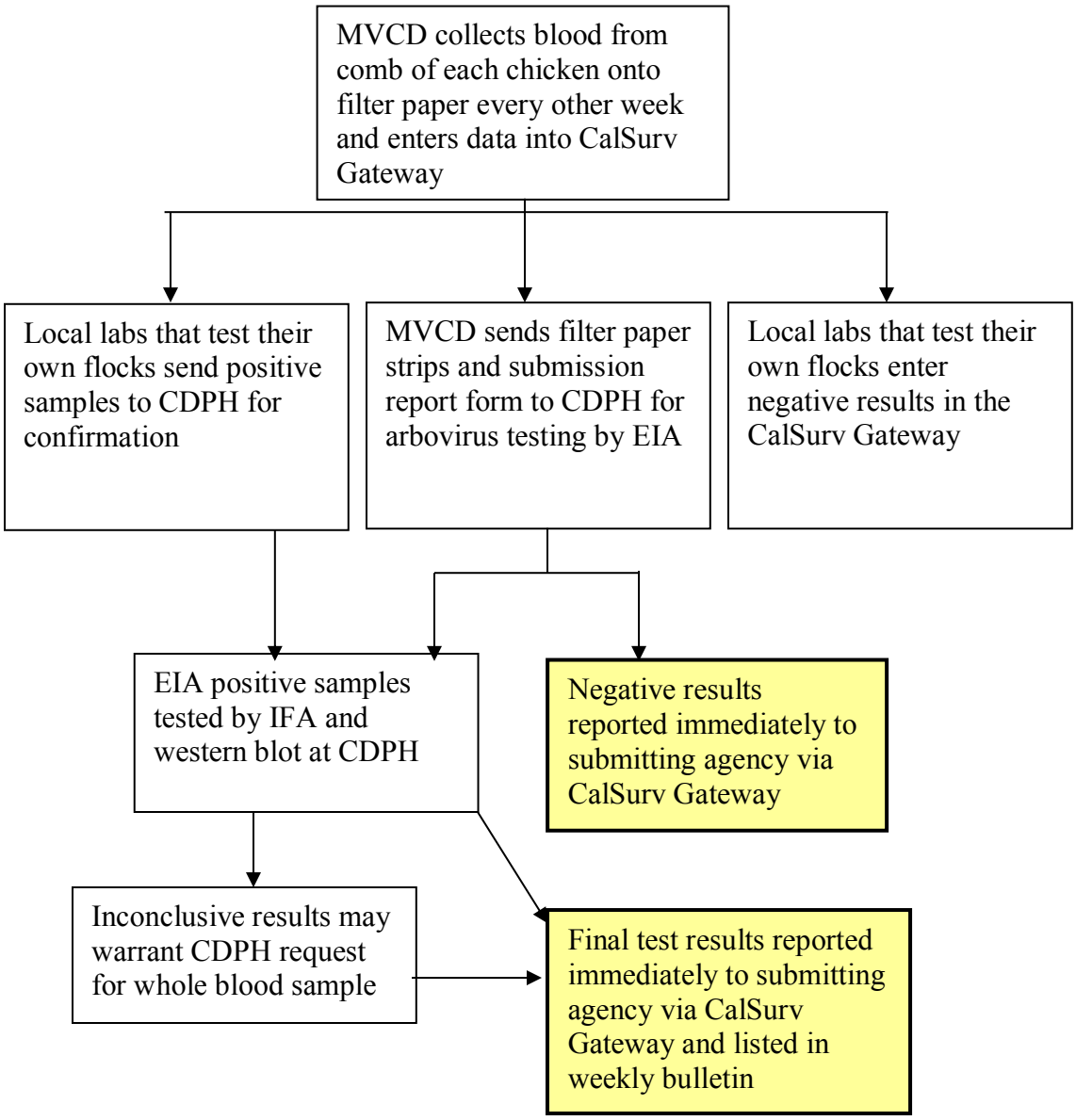
ACCOMPANIED BY A SENTINEL CHICKEN BLOOD FORM OUTSIDE THE ZIPLOCK BAG. Do not staple the form to the bag. Samples from each collection date can be placed into a mailing envelope and sent to:

ATTN: R. Payne
California Department of Public Health
Vector-Borne Disease Section, G164
850 Marina Bay Parkway
Richmond, CA 94804

Specimens will be tested within 1–3 days upon receipt by the laboratory.

6. In the laboratory, a single punch is removed from the blooded end of the paper and tested for West Nile virus (WNV), St. Louis encephalitis virus (SLEV), and western equine encephalomyelitis virus (WEEV) IgG antibodies using an ELISA (Patiris et al. 2008; Taketa-Graham et al. 2010). Positive specimens are confirmed with an indirect fluorescent antibody test and/or a Western blot. Samples yielding inconclusive SLEV or WNV results are tested further by cross-neutralization tests. Agencies will receive an automated email notification that results have been entered into the CalSurv Gateway. Additionally, positive chickens will be reported in the weekly California Arbovirus Surveillance Bulletin.

California Procedure for Testing Sentinel Chickens for the Presence of Antibodies to Flaviviruses (SLEV and WNV) and WEEV



Key:

EIA:	Enzyme immunoassay test
IFA:	Indirect fluorescent antibody test
MVCD:	Local Mosquito and Vector Control District/Health Dept.
SLEV:	St. Louis encephalitis virus
CDPH:	CDPH Vector-Borne Disease Section, Richmond
WEEV:	Western equine encephalomyelitis virus
WNV:	West Nile virus

Surveillance for Mosquito-borne Viruses
Registration of Agencies and Sites

1. Participation of agencies

Agencies interested in participating in the statewide surveillance program for mosquito-borne viruses should place orders for sentinel chicken testing through the California Department of Public Health (CDPH). Agencies will be billed in advance for the number of samples to be tested. Mosquito pool testing by the Davis Arbovirus Research and Training (DART) laboratory at UC Davis, will be billed through the Mosquito and Vector Control Association (MVCAC).

Agencies are responsible for registering and maintaining updated information for their sites online at: http://gateway.calsurv.org/.

2. Registration of sentinel flock sites and wing band numbers

Agencies must use the unique band numbers assigned to their district by CDPH each year. Prior to submitting any sentinel chicken blood samples to CDPH, each agency must ensure that each flock site and accompanying band numbers are registered online at: http://gateway.calsurv.org. CDPH will only test samples if they are accompanied by the "SENTINEL CHICKEN BLOOD – 2017" form for each flock site, which includes the registered agency code, the registered site code (assigned by local agency), the wing band numbers assigned to that site, and date bled. **Also, the form should indicate any changes made and match the sample card exactly.**

3. Registration of mosquito sampling sites

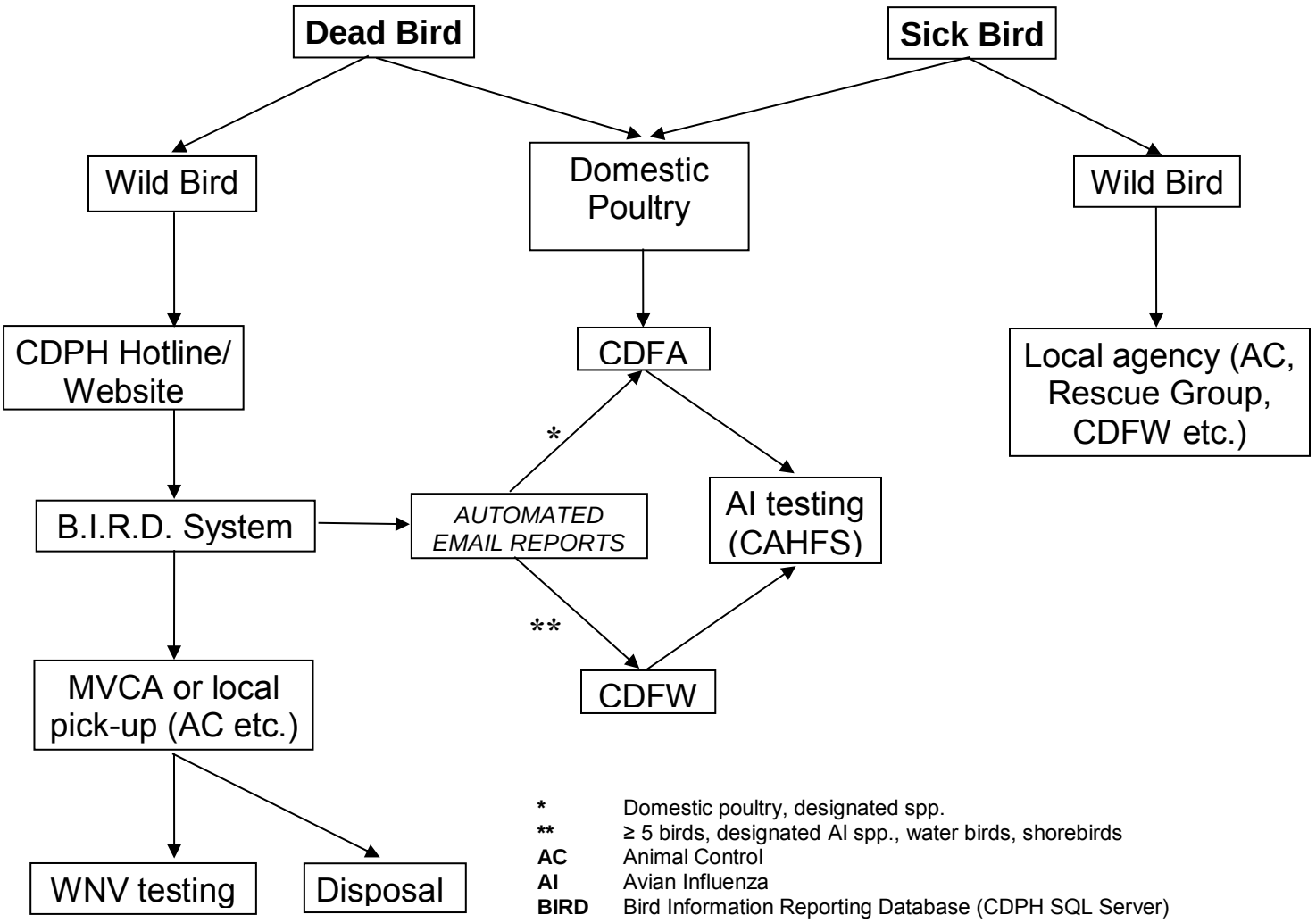
Registration of new sites used for collection of mosquitoes for virus testing may be accomplished by accessing the California Vectorborne Disease Surveillance Gateway (http://gateway.calsurv.org/). Since 2010, the CalSurv Gateway has included enhanced spatial capabilities that allow users the option of directly entering geographic coordinates for sites or interactively selecting the location using a new Google Maps-based interface. The laboratory will test the pools provided that adequate information is provided on the "MOSQUITO POOL SUBMISSION" form including your agency code, site code, and geographic coordinates.

Recording the geographic coordinates of all surveillance sites allows users to filter data spatially for analysis, and the locations are used to generate computer maps that show all registered sites and test results. As part of a collaborative effort, the DART laboratory hosts real-time maps at http://maps.calsurv.org. Local agencies can log in on the mapping website or the CalSurv Gateway (http://gateway.calsurv.org) to access more detailed maps and enhanced analysis tools.

Appendix D: Procedures for Testing Dead Birds

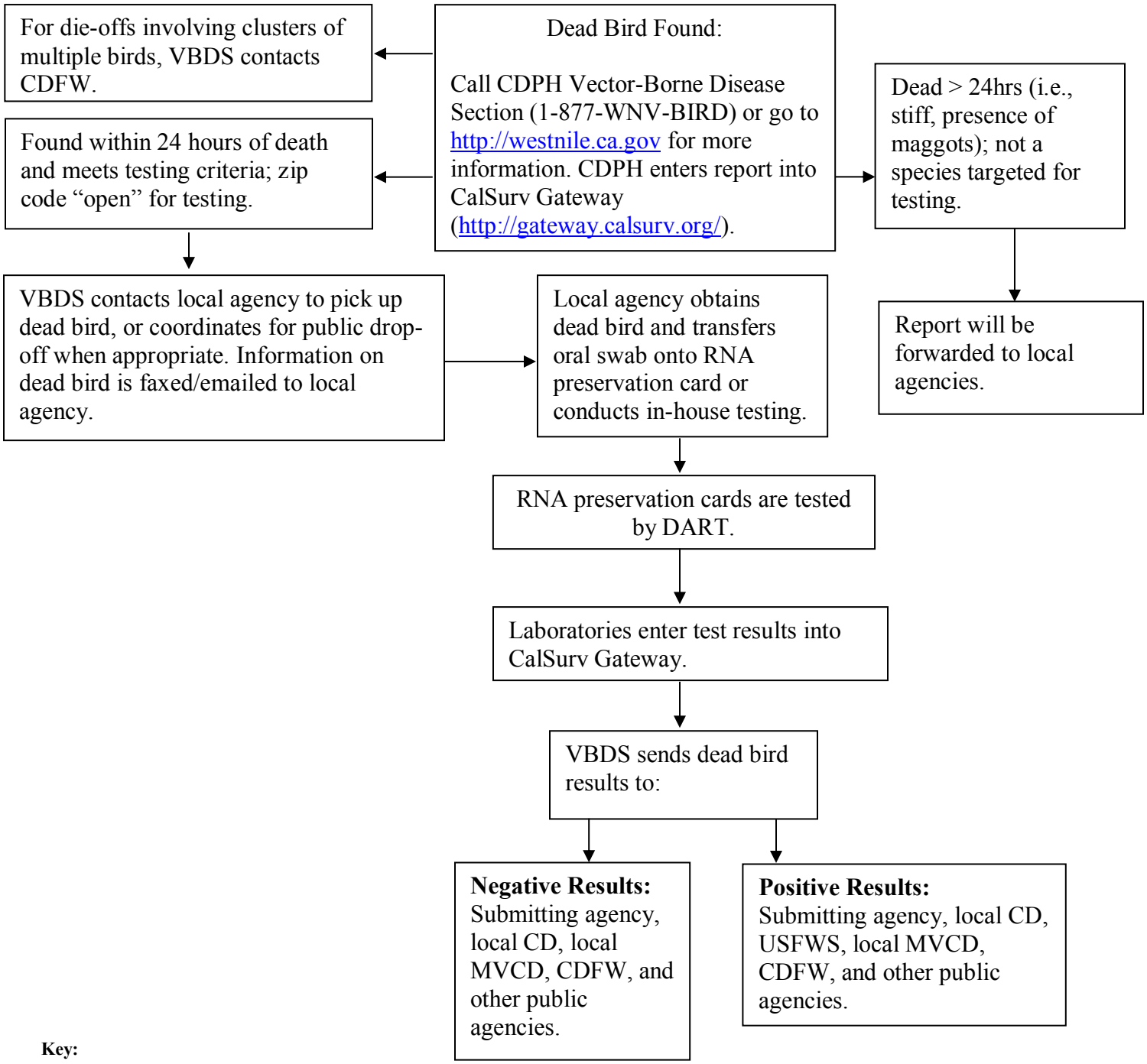
In 2000, CDPH initiated a dead bird surveillance program in collaboration with other public agencies. The public is notified about the program through the media and outreach materials, and it is important for local agencies to publicize the need to report dead birds to ensure that the system will be effective. Dead birds are reported to CDPH or data entered electronically through the CalSurv Gateway (http://gateway.calsurv.org/). An oral sample is taken from the bird, pressed on an RNA preservation card, and sent to the Davis Arbovirus Research and Training (DART) laboratory at UC Davis for West Nile virus (WNV) RNA detection via RT-PCR. Overviews of the dead bird reporting and testing algorithms are provided below.

Sick/Dead Bird Reporting Protocol for Public and Local Agencies



*	Domestic poultry, designated spp.
**	≥ 5 birds, designated AI spp., water birds, shorebirds
AC	Animal Control
AI	Avian Influenza
BIRD	Bird Information Reporting Database (CDPH SQL Server)
CAHFS	CA Animal Health & Food Safety Laboratory
CDFA	California Department of Food & Agriculture: California Bird Flu Hotline: **1-866-922-BIRD**
CDFW	California Department of Fish & Wildlife Investigations Lab: **1-916-358-2790** website: https://www.wildlife.ca.gov/
CDPH	California Department of Public Health West Nile virus & Dead Bird hotline: **1-877-WNV-BIRD** website: http://westnile.ca.gov
MVCA	Mosquito & Vector Control Agency

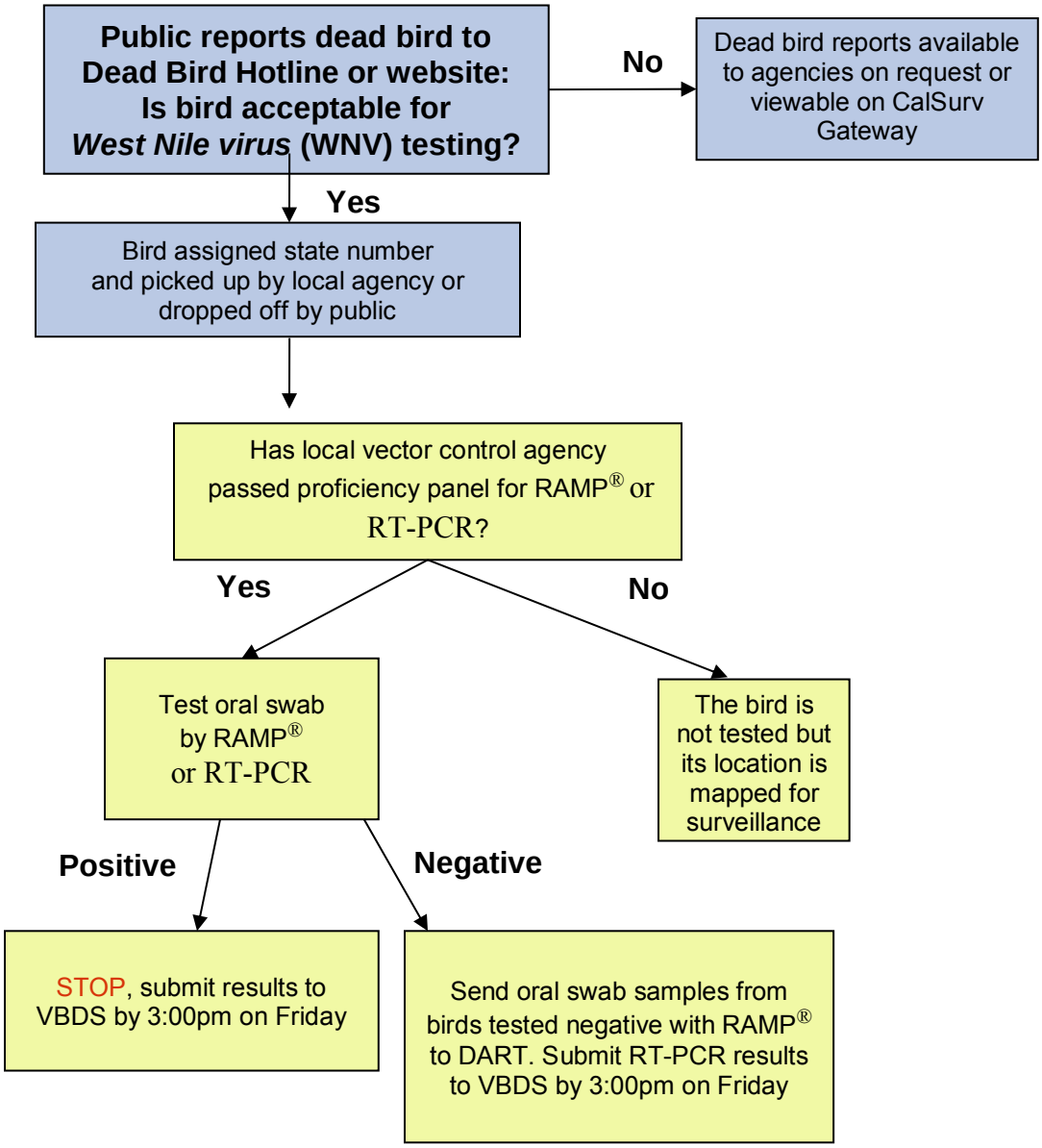
Procedures for Testing Dead Birds: RT-PCR



Key:

CD: Local Agency Communicable Disease Office
CDFW: CA Dept. of Fish and Wildlife
DART: Davis Arbovirus Research and Training laboratory
MVCD: Local Mosquito and Vector Control District
USFWS: US Fish and Wildlife Service
VBDS: CDPH Vector-Borne Disease Section

Procedures for Testing Dead Birds: Rapid Assays (e.g. RAMP®) and RT-PCR



DART = Davis Arbovirus Research and Training laboratory, UC Davis
VBDS = Vector-Borne Disease Section, California Department of Public Health

VBDS

Local Agencies

Dead Bird Reporting and Submission Instructions for Local Agencies
California West Nile Virus (WNV) Dead Bird Surveillance Program
California Department of Public Health (CDPH)
Division of Communicable Disease Control

When your agency receives a call from the public about a dead bird or your staff finds any dead bird, please immediately refer them to the **CDPH West Nile Virus and Dead Bird Hotline at 1-877-WNV-BIRD (968-2473) or the online report page at http://westnile.ca.gov. Crows, ravens, magpies, jays, and raptors are especially vulnerable to WNV, but other bird species will be accepted for testing as well (except for doves, quails, and pigeons).**

The Dead Bird Hotline will be staffed **8:00am–4:30pm, Sunday–Friday (6 days a week from mid-April to mid-October with the exception that Sunday coverage does not begin until May).** Reports can also be made on the WNV website (http://westnile.ca.gov) or after hours via voicemail prompts. CDPH will assess the suitability of the dead bird for testing and contact your agency if the carcass is approved for pickup.

Agencies may call directly **(510-412-4601)** to coordinate bird pick-ups with hotline operators. If your agency collects a dead bird for testing and it is in suitable condition, you can call this number to receive a dead bird number and submission form prior to sampling and/or testing.

Only agencies listed under the permit issued to CDPH from the California Department of Fish & Wildlife are authorized to pick up dead birds. The agencies covered include local mosquito abatement districts, environmental health departments, and other designated agencies. Dead tree squirrels and lagomorphs may also be picked up, but will no longer be tested in the program. If your agency would like to test tree squirrels and lagomorphs, the Center for Animal Health and Food Safety (CAHFS) offers a fee-based testing service (http://www.cahfs.ucdavis.edu/lab_tests/).

Members of the public may salvage dead birds found on their property or place of residence if the local agency has indicated to CDPH they will accept public salvage. **The public must first call the Dead Bird Hotline and obtain a Dead Bird Number**; a corresponding public salvage submission form will then be faxed to the appropriate agency. The public will be instructed by the hotline staff to double-bag the carcasses and drop it off at the designated agency within 24 hours, between 9 am–3 pm, Monday–Friday. **Note: only dead birds, not live, may be brought in by the public to local agencies for sampling or testing.**

***web link*: http://westnile.ca.gov/bird_descriptions_frameset.htm**

Collect fresh carcasses. Badly decomposed or scavenged carcasses are of limited diagnostic value. Signs that a bird has been dead for too long (over 24–48 hours) are the presence of maggots; an extremely lightweight carcass; missing eyes; skin discoloration; skin or feathers that rub off easily; strong odor; or a soft, mushy carcass.

If the carcass is found to be unacceptable upon pick-up (e.g., a species your agency or CDPH is not accepting or a badly decomposed specimen), please collect the carcass,

double-bag it, and dispose of it in a secure garbage can or dumpster. Please call CDPH immediately and notify us that the animal will no longer be submitted.

Once the submission is approved, your agency can collect an oral swab from the bird for an RNA preservation card (please see protocol below) and mail the card to the Davis Arbovirus Research and Training (DART) laboratory at UC Davis for WNV testing. Testing expenses will be paid by CDPH, but agencies must purchase the RNA preservation cards.

To ensure your safety when handling carcasses, please follow these instructions:

Dead Bird Oral Swab Sampling Procedure

1. Avoid direct contact with the dead bird by using disposable gloves and/or handle the carcass only with plastic bags as described below.

2. Dead birds should be handled in a Class II biosafety cabinet within a laboratory. If it is not possible to work with the bird carcass in a biosafety cabinet, work should be conducted outside while wearing an N95 respirator. One option is to collect the oral swab sample at the dead bird collection site.

3. **Refrigeration:** It is recommended to refrigerate carcasses until ready for swabbing in lieu of maintaining at room temperature. RNA preservation cards must be stored in the refrigerator.

4. Partially unwrap the disposable swab.

5. Open the bag containing the bird to expose the head. With gloved hands, pry open the beak with a metal spatula, and put swab into the mouth. Aggressively swab the mouth and oropharyngeal cavity (throat).

6. Wipe, press, and roll the contents of the swab onto the target area of the RNA preservation card (over the two perforated discs). The sample may be dry; this is normal. Make sure to label the card with the dead bird number assigned to the bird by the WNV hotline.

7. Discard the swab into the bag containing the dead bird.

8. Wipe the inside of cabinet and metal spatula used for opening the beak with a fresh solution of 10% bleach, followed by 70 to 100% ethanol or isopropyl alcohol and change gloves after each bird. Cavicide®, a product that kills viruses without corroding stainless steel, may also be used.

9. Allow cards to dry in back of cabinet or outside in the shade for 2 hours. Make sure the dead bird number corresponding to the dead bird is written at the bottom of each card. Seal RNA preservation cards back into the small individual bags in which they were shipped.

10. Place all cards into a Ziplock bag and ship in regular business or manila envelope to DART (address below). **IMPORTANT:** Include an inventory list of bird numbers corresponding to RNA preservation card samples in each shipment.

Shipping options:

a. Seal all cards with card inventory list in another Ziplock bag and add to weekly mosquito pool shipments. The cold temperature of the mosquito boxes are fine for the cards, but cards should be protected from moisture.
b. Ship batches of cards via overnight delivery (FedEx, GSO). Ship on Monday for fastest turnaround times during the testing season.
c. Regular U.S. Postal Service mail is accepted; adding shipment tracking and requiring a signature upon receipt is highly recommended to help avoid lost packages.

11. Dead bird carcasses and used polyester swabs which are double-bagged can be discarded in the trash. If you sample birds at the place of collection, the resident may dispose of the carcass in an outdoor trash can, or you may do it for them. Agencies conducting in-house testing must dispose of any WNV-positive birds as biohazard waste (incinerate); negative birds can be discarded in the trash.

12. **Ship cards to the address below:**

ATTN: Ying Fang
University of California
One Shields Avenue
Vet Med: PMI
Room 4206 Vet Med 3A
Davis, CA 95616

For UPS shipments only:
Ying Fang
VM://PMI 4206 Vet Med 3A
1285 Veterinary Medicine Mall
University of California, Davis
Davis, CA 95616

13. Once your agency receives test results, telephone the citizens who reported dead birds which tested WNV-positive to let them know the bird had WNV and deliver risk prevention information if needed.

Materials

- Biosafety cabinet or N95 respirator masks
- Refrigerator to store RNA preservation cards and carcasses
- Disposable nitrile or latex gloves
- Lab coat
- **RNA preservation cards** (specifically, RNASound ReadyPunched™ cards). Order online at http://www.fortiusbio.com/RNA_Sampling_Card.html. Quantities of 25 ($140) or 10 ($60.20) are available. **Note:** cards expire after 12 months (order as needed).

- Individually-wrapped polyester swabs are included with the cards. If more are needed: Fisher brand cat. no. 23-400-116.
- Sandwich-size Ziplock bags
- Small metal spatula
- Permanent markers
- Envelopes for shipping (manila or business size)

For agencies conducting in-house testing by RAMP® or RT-PCR of tissues:
Once agencies pass the yearly proficiency panel, agencies may conduct in-house testing. Results can be entered directly into the CalSurv Gateway. **Note: any positive bird must be disposed of as biomedical waste (incineration).**

Appendix E: Procedures for Testing Equines

The California Department Food and Agriculture (CDFA) has primary responsibility for investigation of West Nile virus (WNV) in equids. Veterinarians and diagnostic laboratories are required to report cases of WNV and other equine encephalomyelitides to CDFA (California Food and Agriculture Code §9101; Title 9 California Code of Regulations §161.4(f))

Each spring, CDFA sends information on the California West Nile Surveillance Program to approximately 1,200 veterinarians, animal health branch personnel, and other interested parties. The mailing includes case definitions for equine WNV and instructions for collection and submission of specimens for diagnostic testing. Specimen submission is coordinated through the California Animal Health and Food Safety Laboratory System (CAHFS) and other laboratories or individual veterinarians. Equine serum and cerebrospinal fluid are tested by CAHFS using the IgM-capture ELISA. Equine neurologic tissue specimens are also sent to CAHFS for microscopic examination and, as indicated by clinical findings, forwarded to the USDA National Veterinary Services Laboratories (NVSL) for further arbovirus testing. All fatal cases of equine encephalitis should also be evaluated for rabies at the local or state public health laboratory.

Outreach is an important component of the program. Additional information on WNV for veterinarians, horse owners, and ratite owners is available from CDFA, Animal Health Branch (916) 900-5002, and at the CDFA website: http://www.cdfa.ca.gov/AHFSS/Animal_Health/WNV_Info.html. Information on submission of laboratory samples is available from CAHFS (530) 752-8700 and at CAHFS website: http://cahfs.ucdavis.edu.

Appendix F: Protocol for Submission of Laboratory Specimens for Human West Nile Virus (WNV) Testing

WNV testing within the regional public health laboratory network (i.e., the California Department of Public Health Viral and Rickettsial Disease Laboratory and participating local public health laboratories) is recommended for individuals with the following symptoms, particularly during WNV "season," which typically occurs from July through October in California:

A. Encephalitis
B. Aseptic meningitis (Note: Consider enterovirus for individuals ≤ 18 years of age)
C. Acute flaccid paralysis, atypical Guillain-Barré Syndrome, transverse myelitis, or
D. Febrile illness*
   - Illness compatible with West Nile fever and lasting ≥ 7 days
   - Must be seen by a health care provider

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*The West Nile fever syndrome can be variable and often includes headache and fever (T ≥ 38°C). Other symptoms include rash, swollen lymph nodes, eye pain, nausea, or vomiting. After initial symptoms, the patient may experience several days of fatigue and lethargy.

Required specimens:

- Acute serum: ≥ 2cc serum

If a lumbar puncture is performed and residual CSF is available:

- Cerebral spinal fluid (CSF): 1-2cc CSF

If West Nile virus is highly suspected and acute serum is negative or inconclusive, request:

- 2nd serum: ≥ 2cc serum collected 3-5 days after acute serum

Contact your local health department for instructions on where to send specimens.

Appendix G: Surveillance Case Definitions for Arbovirus Infection in Humans

Infections with West Nile virus (WNV), St. Louis encephalitis virus (SLEV), and western equine encephalomyelitis virus (WEEV) are reportable to local health departments under Title 17 of the California Code of Regulations. Local health departments should report cases to CDPH. Blood donors testing positive for WNV through blood bank screening should also be reported to CDPH, regardless of clinical presentation.

CASE DEFINITION: Arboviral Diseases, Neuroinvasive and Non-neuroinvasive (including WNV, SLEV, and WEEV)

NOTE: This definition is for public health surveillance purposes only. It is not intended for use in clinical diagnosis.

Symptomatic Cases (adapted from 2015 CSTE case definition http://wwwn.cdc.gov/nndss/conditions/arboviral-diseases-neuroinvasive-and-non-neuroinvasive/case-definition/2015/)

Clinical criteria for diagnosis

Neuroinvasive disease
- Meningitis, encephalitis, acute flaccid paralysis, or other acute signs of central or peripheral neurologic dysfunction, as documented by a physician, AND
- Absence of a more likely clinical explanation.

Non-neuroinvasive disease
- Fever or chills as reported by the patient or a health-care provider, AND
- Absence of neuroinvasive disease, AND
- Absence of a more likely clinical explanation.

Case classification

Confirmed = A case that meets the above clinical criteria and one or more of the following laboratory criteria for a confirmed case:
- Isolation of virus from, or demonstration of specific viral antigen or nucleic acid in tissue, blood, CSF, or other body fluid, OR
- Four-fold or greater change in virus-specific quantitative antibody titers in paired sera, OR
- Virus-specific immunoglobulin M (IgM) antibodies in serum with confirmatory virus-specific neutralizing antibodies in the same or a later specimen, OR
- Virus-specific IgM antibodies in CSF and a negative result for other IgM antibodies in CSF for arboviruses endemic to the region where exposure occurred.

Probable = A case that meets the above clinical criteria and the following laboratory criteria:
- Virus-specific IgM antibodies in serum but with no other testing.*

*CDPH recommends that virus-specific IgG antibody testing (e.g., EIA or IFA) also be performed. A specimen that is IgM-positive only (i.e., IgG-negative) may be a false positive, while a specimen that is both WNV IgM- and IgG-positive is more likely a true infection.

Presumptive Viremic Donors (Asymptomatic)

Asymptomatic infection with WNV, which is generally identified in blood donors, is also reportable. Blood donors who test positive for WNV may not necessarily be ill, nor will they initially have positive IgM or IgG antibody test results. Local health departments should report blood donors who meet the following criteria for being a presumptively viremic donor to CDPH:

A presumptively viremic donor (PVD) is a person with a blood donation that meets at least one of the following criteria:

a) One reactive nucleic acid amplification (NAT) test with signal-to-cutoff (S/CO) ≥ 17
b) Two reactive NATs

Additional serological testing is not required. Local health departments should follow up with the donor after two weeks of the date of donation to assess if the patient subsequently became ill. If the donor did become ill as a result of WNV infection, the disease incident should be reclassified as "West Nile virus – Non-neuroinvasive" or "West Nile virus – Neuroinvasive," depending on the individual's clinical symptoms. Similarly, organ donors testing positive for WNV should also be reported to CDPH and receive public health follow-up by the local health department.

Appendix H: Compounds Approved for Mosquito Control in California

Label rates and usage vary from year to year and geographically; consult your County Agricultural Commissioner and the California Department of Fish and Game before application. Examples of products containing specific active ingredients are provided below, but this list is not exhaustive, nor does it constitute product endorsement. For more information on pesticides and mosquito control, please refer to the Environmental Protection Agency website: http://www.epa.gov/opp00001/factsheets/westnile.htm.

Larvicides:

1. *Bacillus thuringiensis* subspecies *israelensis* (Bti: e.g. Aquabac 200G, VectoBac® 12AS, Teknar HP-D)
   Use: Approved for most permanent and temporary bodies of water.
   Limitations: Only works on actively feeding stages. Does not persist well in the water column.

2. *Bacillus sphaericus* (Bs: e.g. VectoLex® CG)
   Use: Approved for most permanent and temporary bodies of water.
   Limitations: Only works on actively feeding stages. Does not work well on all species. May persist and have residual activity in some sites.

3. Spinosad (e.g. Natular™ G30)
   Limitations: Effective against all larval stages and moderately effective against pupal stage. Toxic via ingestion and contact. Some formulations approved for use in OMRI certified organic crops.

4. IGRs (Insect Growth Regulators)
   a. (S)-Methoprene (e.g. Altosid® Pellets)
   Use: Approved for most permanent and temporary bodies of water.
   Limitations: Works best on older instars. Some populations of mosquitoes may show some resistance.
   b. Diflurobenzamide (e.g. Dimilin®25W)
   Use: Impounded tail water, sewage effluent, urban drains and catch basins.
   Limitations: Cannot be applied to wetlands, crops, or near estuaries.

5. Larviciding oils (e.g. Bonide)
   Use: Ditches, dairy lagoons, floodwater. Effective against all stages, including pupae.
   Limitations: Consult with the California Department of Fish and Game for local restrictions.

6. Monomolecular films (e.g. Agnique® MMF)
   Use: Most standing water including certain crops.
   Limitations: Does not work well in areas with unidirectional winds in excess of ten mph.

7. Temephos (e.g. Abate® 2-BG)
   Use: Non-potable water; marshes; polluted water sites

Limitations: Cannot be applied to crops for food, forage, or pasture. This material is an organophosphate compound and may not be effective on some *Cx. tarsalis* populations in the Central Valley. May require sampling and testing per General Vector Control National Pollutant Discharge Elimination System (NPDES) permit requirements if applied to waters of the United States.

Adulticides:

1. Organophosphate compounds
   Note: Many *Cx. tarsalis* populations in the Central Valley are resistant at label OP application rates.
   a. Malathion (e.g. Fyfanon® ULV)
      Use: May be applied by air or ground equipment over urban areas, some crops including rice, wetlands.
      Limitations: Paint damage to cars; toxic to fish, wildlife and bees; crop residue limitations restrict application before harvest.
   b. Naled (e.g. Dibrom® Concentrate, Trumpet® EC)
      Use: Air or ground application on fodder crops, swamps, floodwater, residential areas.
      Limitations: Similar to malathion.

2. Pyrethrins (natural pyrethrin products: e.g. Pyrenone® Crop Spray, Pyrenone® 25-5, Evergreen)
   Use: Wetlands, floodwater, residential areas, some crops.
   Limitations: Do not apply to drinking water, milking areas; may be toxic to bees, fish, and some wildlife. Some formulations with synergists have greater limitations.

3. Pyrethroids (synthetic pyrethrin products containing deltamethrin, cyfluthrin, permethrin, resmethrin, sumithrin or etofenprox: e.g. Suspend® SC, Tempo Ultra SC, Aqua-Reslin®, Scourge® Insecticide, Anvil® 10+10 ULV, Zenivex E20, and Duet – which also contains the mosquito exciter prallethrin)
   Use: All non-crop areas including wetlands and floodwater.
   Limitations: May be toxic to bees, fish, and some wildlife; avoid treating food crops, drinking water or milk production.

PESTICIDES USED FOR MOSQUITO CONTROL IN CALIFORNIA

Larvicides

Active Ingredient	Trade name	EPA Reg. No.	Mfgr.	Formulation	Application	Pesticide classification
Bacillus sphaericus, (Bs)	VectoLex CG / WSP	73049-20	Valent BioSciences	Granule Water soluble packet	Larvae	Biorational
Bacillus sphaericus, (Bs)	VectoLex WDG	73049-57	Valent BioSciences	Water dispersible granule	Larvae	Biorational
Bacillus thuringiensis var. *israelensis* (Bti)	VectoBac WDG	73049-56	Valent BioSciences	Water dispersible Granules	Larvae	Biorational
Bacillus thuringiensis var. *israelensis* (Bti)	VectoBac 12AS	73049-38	Valent BioSciences	Liquid	Larvae	Biorational
Bacillus thuringiensis var. *israelensis* (Bti)	VectoBac AS	275-52	Abbott Labs	Liquid	Larvae	Biorational
Bacillus thuringiensis var. *israelensis* (Bti)	VectoBac G	73049-10	Valent BioSciences	Granule Flake	Larvae	Biorational
Bacillus thuringiensis var. *israelensis* (Bti)	VectoBac GS	73049-10	Valent BioSciences	Granule Flake	Larvae	Biorational
Bacillus thuringiensis var. *israelensis* (Bti)	VectoBac Tech. Pdr.	73049-13	Valent BioSciences	Technical powder	Larvae	Biorational
Bacillus thuringiensis var. *israelensis* (Bti)	Aquabac 200G	62637-3	Becker Microbial	Granule	Larvae	Biorational
Bacillus thuringiensis var. *israelensis* (Bti)	Consume MP	62637-3	Spartan Chemical	Granule	Larvae	Biorational
Bacillus thuringiensis var. *israelensis* (Bti)	Aquabac XT	62637-1	Becker Microbial	Liquid	Larvae	Biorational
Bacillus thuringiensis var. *israelensis* (Bti)	Bactimos PT	73049-452	Valent BioSciences	Granular flake	Larvae	Biorational
Bacillus thuringiensis var. *israelensis* (Bti)	Teknar HP-D	73049-404	Valent BioSciences	Liquid	Larvae	Biorational
Bacillus thuringiensis var. *israelensis* (Bti)	Fourstar SBG	85685-1	Fourstar Microbials LLC	Granule	Larvae	Biorational
Bti / Bs combination	Vectomax G, CG, WSP	73049-429	Valent BioSciences	Granular and water soluble packet	Larvae	Biorational
Bti / Bs combination	Fourstar Briquettes	83362-3	Fourstar Microbials LLC	Briquette	Larvae	Biorational
Spinosad	Natular 2EC	8329-82	Clarke	Liquid concentrate	Larvae and pupae	Biorational
Spinosad	Natular G	8329-80	Clarke	Granule	Larvae and pupae	Biorational
Spinosad	Natural G30	8329-83	Clarke	Granule	Larvae and pupae	Biorational
Spinosad	Natular T30	8329-85	Clarke	Tablet	Larvae and pupae	Biorational
Spinosad	Natular XRT	8329-84	Clarke	Tablet	Larvae and pupae	Biorational
Monomolecular film	Agnique MMF	53263-28	Cognis Corp.	Liquid	Larvae and pupae	Surface film
Monomolecular film	Agnique MMF G	53263-30	Cognis Corp.	Granular	Larvae and pupae	Surface film

Monomolecular film	Agnique MMF G Pak 35	53263-30	Cognis Corp.	Water soluble pack	Larvae and pupae	Surface film
Petroleum oil	Masterline Kontrol	73748-10	Univar	Liquid	Larvae and pupae	Surface film
Petroleum oil	BVA 2	70589-1	B-V Assoc.	Liquid	Larvae and pupae	Surface film
Dimilin	Dimilin 25W	400-465	Uniroyal Chemical	Wettable powder	Larvae	IGR
S-Methoprene	Altosid ALLC	2724-446	Wellmark-Zoecon	Liquid concentrate	Larvae	IGR
S-Methoprene	Altosid ALL	2724-392	Wellmark-Zoecon	Liquid concentrate	Larvae	IGR
S-methoprene	Altosid Briquets	2724-375	Wellmark-Zoecon	Briquet	Larvae	IGR
S-methoprene	Altosid Pellets / WSP	2724-448	Wellmark-Zoecon	Pellet-type granules / water soluble packet	Larvae	IGR
S-methoprene	Altosid SBG	2724-489	Wellmark-Zoecon	Granule	Larvae	IGR
S-methoprene	Altosid XR Briquets	2724-421	Wellmark-Zoecon	Briquet	Larvae	IGR
S-methoprene	Altosid XR-G	2724-451	Wellmark-Zoecon	Granule	Larvae	IGR
Temephos	Abate 2-BG	8329-71	Clarke	Granule	Larvae	OP
Temephos	5% Skeeter Abate*	8329-70	Clarke	Granule	Larvae	OP
Temephos	Abate 4E	8329-69	Clarke	Liquid	Larvae	OP

PESTICIDES USED FOR MOSQUITO CONTROL IN CALIFORNIA

Adulticides

Active Ingredient	Trade name	EPA Reg. No.	Mfgr.	Formulation	Stage	Pesticide classification
Malathion	Fyfanon® ULV	67760-34	Cheminova	Liquid	Adults	OP
Naled	Trumpet™ EC	5481-481	AMVAC	Liquid	Adults	OP
Prallethrin Sumithrin	AquaDuet Adulticide	1021-2562-8329	Clarke	Liquid	Adults	Pyrethroid
Prallethrin Sumithrin	Duet Dual Action Adulticide	1021-1795	Clarke	Liquid	Adults	Pyrethroid
Deltamethrin	Suspend® SC	432-763	Aventis	Liquid	Adults	Pyrethroid
Cyfluthrin	Tempo SC Ultra	432-1363	Bayer	Liquid	Adults	Pyrethroid
Permethrin	Aqua-Kontrol	73748-1	Univar	Liquid	Adults	Pyrethroid
Permethrin	Aqualeur 20-20	769-985	Value Garden Supply	Liquid	Adults	Pyrethroid
Permethrin	Aqua-Reslin®	432-796	Bayer	Liquid	Adults	Pyrethroid
Permethrin	Biomist® 4+4	8329-35	Clarke	Liquid	Adults	Pyrethroid
Permethrin	Biomist® 4+12 ULV	8329-34	Clarke	Liquid	Adults	Pyrethroid
Permethrin	Evoluer 4-4 ULV	769-982	Value Garden Supply	Liquid	Adults	Pyrethroid
Permethrin	Kontrol 2-2	73748-3	Univar	Liquid	Adults	Pyrethroid
Permethrin	Kontrol 4-4	73748-4	Univar	Liquid	Adults	Pyrethroid
Permethrin	Kontrol 30-30	73748-5	Univar	Liquid	Adults	Pyrethroid
Permethrin	Permanone 31-66	432-1250	Bayer	Liquid	Adults	Pyrethroid
Permethrin	Permanone® Ready-To-Use	432-1277	Bayer	Liquid	Adults	Pyrethroid

Permethrin	Perm-X UL 4-4	655-898	Prentiss	Liquid	Adults	Pyrethroid
Pyrethrins	Aquahalt	1021-1803	Clarke	Liquid	Adults	Pyrethroid
Pyrethrins	Evergreen 60-6	1021-1770	MGK	Liquid	Adults	Pyrethroid
Pyrethrins	Pyrenone® 25-5	432-1050	Bayer	Liquid	Adults	Pyrethroid
Pyrethrins	Pyrenone® Crop Spray	432-1033	Bayer	Liquid	Adults	Pyrethroid
Pyrethrins	Pyrocide® 7453	1021-1803	MGK	Liquid	Adults	Pyrethroid
Pyrethrins	Pyrocide® 7395	1021-1570	MGK	Liquid	Adults	Pyrethroid
Pyrethrins	Pyrocide® 7396	1021-1569	MGK	Liquid	Adults	Pyrethroid
Pyrethrins	Pyronyl Crop Spray	655-489	Prentiss	Liquid	Adults	Pyrethroid
Pyrethrins	Pyronyl Oil 525	655-471	Prentiss	Liquid	Adults	Pyrethroid
Pyrethrins	Pyronyl Oil 3610A	655-501	Prentiss	Liquid	Adults	Pyrethroid
Resmethrin	Scourge® Insecticide (4%)	432-716	Bayer	Liquid	Adults	Pyrethroid
Resmethrin	Scourge® Insecticide (18%)	432-667	Bayer	Liquid	Adults	Pyrethroid
Sumithrin	Anvil 2+2 ULV	1021-1687	Clarke	Liquid	Adults	Pyrethroid
Sumithrin	Anvil® 10+10 ULV	1021-1688	Clarke	Liquid	Adults	Pyrethroid
Sumithrin	AquaANVIL	1021-1807	Clarke	Liquid	Adults	Pyrethroid
Etofenprox	Zenivex E4 RTU	2724-807	Wellmark Intl.	Liquid	Adults	Pyrethroid
Etofenprox	Zenivex E20	2724-791	Wellmark, Intl.	Liquid	Adults	Pyrethroid
Lambda-cyhalothrin	Demand CS	100-1066	Syngenta	Liquid	Adults	Pryethroid

Appendix I: Adult Mosquito Control in Urban Areas

Adult mosquito control via ultralow volume (ULV) application is an integral part of an integrated mosquito management program. This response plan recommends the consideration of adult mosquito control to break local virus transmission cycles and reduce the risk of human infection. The following provides guidelines for local agencies considering ground or aerial ULV control of adult mosquitoes. Agencies should ensure they are complying with National Pollutant Discharge Elimination System (NPDES) permit requirements.

Preparatory steps for aerial application contracts

- Send out request for proposals (RFP) to commercial applicators well in advance of any potential need for actual treatment. Specify required equipment and abilities in the RFP such as: 1) application equipment capable of producing desired droplet spectrum and application rate, 2) aircraft availability time frames (remember FAA requires 2-engine aircraft for applications over urban areas), and 3) the demonstrated ability to apply the chosen product to the target area in accordance with label requirements.
- Outline the desired capabilities and equipment within the RFP such as: 1) onboard real time weather systems, and 2) advanced onboard drift optimization and guidance software.
- Determine in advance whether the vector control agency or contractor will secure and provide pesticides. If the contractor will supply the pesticide, verify their knowledge of and ability to comply with regulations regarding the transport, use, and disposal of all pesticide and containers.
- Enter into a contingency contract with the commercial applicator.
- Consider acquiring non-owned, multiple engine aircraft insurance with urban application endorsement for added protection.
- Determine product and application rate to be used, along with a contingency plan. The product choice may be subject to change depending on product availability, the determination of resistance, labeling restrictions, environmental conditions, or other unforeseen factors.

Preparatory steps for ground-based applications

- Ensure that application equipment has been properly calibrated and tested for droplet size and flow rate. The vector control agency should have enough equipment, operators, and product available to finish the desired application(s) between sunset and midnight, or within 2-3 hours pre-sunrise (or when mosquitoes are demonstrated to be most active) to maximize efficacy.
- Ensure that vehicles are equipped with safety lighting and appropriate identifying signs; use sufficient personnel.
- Contact local law enforcement and provide them with locations to be treated and approximate time frames.
- Consider using lead and trailing vehicles particularly if the area has not been treated before and personnel are available.

Implementing an aerial application contract

- Contact commercial applicator and determine availability.
- Review long-term weather forecasts. Ideally applications should be scheduled during periods of mild winds to avoid last minute cancellations.

Contractor should:

- Contact Local Flight Standards District Office (FSDO) for low flying waiver.
- Arrange for suitable airport facilities.
- Contact local air traffic control.
- Locate potential hazards prior to any application and implement a strategy to avoid those hazards during the application – often in darkness.
- Provide equipment and personnel for mixing and loading of material (if previously agreed upon in contract).
- Register with applicable County Agricultural Commissioner's office.

Vector control agency should:

- Delineate treatment block in a GIS format and send to contractor.
- Identify areas that must be avoided during an application and include detailed maps of those areas to contract applicators (e.g. open water, registered organic farms, any area excluded by product label).
- Send authorization letter to FSDO authorizing contractor to fly on the agency's behalf; contractor should provide contact information and assistance.
- Send map of application area and flight times / dates to local air traffic control; contractor should provide contact information and assistance.
- Consult with County Agricultural Commissioner's office. Commissioner's office can provide guidance on contacting registered bee keepers and help identify any registered organic farms that may need to be excluded from application.
- If vector control agency is providing material, ensure adequate quantity to complete mission and that the agency has means to transport material.

Efficacy evaluation for aerial or ground based application

- Choose appropriate method(s) for evaluating efficacy of application
  - Determine changes in adult mosquito population via routine or enhanced surveillance.
  - Conduct three day pre and post-trapping in all treatment and control areas.
  - Set out bioassay cages with wild caught and laboratory reared (susceptible) mosquitoes during application.
- Ensure adequate planning so surveillance staff is available and trained, equipment is available, and trap / bioassay cage test locations are selected prior to application.
- Ensure efficacy evaluation activities are timed appropriately with applications.
- Enlist an outside agency such as CDPH and/or university personnel to help evaluate efficacy of application as appropriate.

Actions at time of application

- Confirm application rate with contractor.
- Confirm treatment block.
- Coordinate efficacy evaluations.

Public notification

Notification of the public prior to a mosquito control pesticide application by a vector control agency signatory to a Cooperative Agreement with CDPH, or under contract for such agency is not a legal requirement in California (California Code of Regulations – Title 3: Food and Agriculture: Division 6. Pesticides and Pest Control Operations: Section 6620a). However, public notification of pending adult mosquito control is recommended as early as possible prior to the treatment event.

Basic notification steps

- Provide notification of pending application as early as possible.
- Post clearly defined treatment block map online or through appropriate media outlet.
- Post product label and material safety data sheet (MSDS) online or through appropriate media outlet.
- Post and/or have available scientific publications regarding the efficacy of aerial or ground based applications (as appropriate), including effects on non-target organisms and risk-assessments.

Public relations considerations

- Ensure staffing is adequate to handle a significant increase in phone calls.
- Ensure website capability is adequate to handle a rapid increase in visitors.
- Train personnel answering phones to address calls from citizens concerned about personal and environmental pesticide exposure.
- Ensure adequate follow-through for calls related to sporting events, concerts, weddings, and other outdoor events that may be scheduled during the application and within the treatment block.

Appendix J: Websites Related to Arbovirus Surveillance, Mosquito Control, Weather Conditions and Forecasts, and Crop Acreage and Production in California

Website	URL	Available information
California West Nile Virus Website	http://westnile.ca.gov	Up to date information on the spread of West Nile virus throughout California, personal protection measures, online dead bird reporting, bird identification charts, mosquito control information and links, clinician information, local agency information, public education materials.
California Department of Public Health	http://cdph.ca.gov	Use search box to find information on mosquitoes, mosquito-borne diseases, or other vectors and diseases.
Davis Arbovirus Research and Training Laboratory at UC Davis	http://dart.ucdavis.edu	Information on mosquito and arbovirus surveillance in California and related research.
Mosquito and Vector Control Association of California	http://www.mvcac.org	News, membership information, event calendars, and other topics of interest to California's mosquito control agencies.
California Vectorborne Disease Surveillance Maps	http://maps.calsurv.org	Maps showing locations of arbovirus activity and detections of invasive mosquitoes.
California Data Exchange Center	http://cdec.water.ca.gov	Water-related data from the California Department of Water Resources, including historical and current stream flow, snow pack, and precipitation information.
UC IPM Online	http://www.ipm.ucdavis.edu	Precipitation and temperature data for stations throughout California; also allows calculation of degree-days based on user-defined data and parameters.
National Weather Service – Climate Prediction Center	http://www.cpc.ncep.noaa.gov/products/predictions/	Short-range (daily) to long-range (seasonal) temperature and precipitation forecasts. Also provides El Niño-related forecasts.
California Agricultural Statistics Service	http://www.nass.usda.gov/Statistics_by_State/California	Crop acreage, yield, and production estimates for past years and the current year's projections. Reports for particular crops are published at specific times during the year – see the calendar on the website.
State Water Resources Control Board	http://www.swrcb.ca.gov/water_issues/programs/npdes/aquatic.shtml	National Pollutant Discharge Elimination System (NPDES) permit for vector control information.
US Environmental Protection Agency – Mosquito Control	http://www.epa.gov/pesticides/health/mosquitoes	Describes the role of mosquito control agencies and products used for mosquito control.
US Centers for Disease Control and Prevention – West Nile Virus	http://www.cdc.gov/ncidod/dvbid/westnile/index.htm	Information on the transmission of West Nile virus across the United States, viral ecology and background on WNV, and personal protection measures in various languages.

Appendix K: Reference List

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