CALIFORNIA MOSQUITO-BORNE VIRUS SURVEILLANCE & RESPONSE PLAN

Arnold Schwarzenegger, Governor

California Department of Public Health
Mosquito & Vector Control Association of California
University of California

For further information contact:
Vector-Borne Disease Section
California Department of Public Health
(916) 552-9730
http://westnile.ca.gov

April 2010

CALIFORNIA MOSQUITO-BORNE VIRUS SURVEILLANCE AND RESPONSE PLAN

TABLE OF CONTENTS

Objectives 3

Introduction 3

Background 3

Education 4

Surveillance 4

- ***Climate Variation*** 5
- ***Mosquito Abundance*** 5
- ***Mosquito Infections*** 5
- ***Avian Infections*** 6
- ***Tree Squirrel Infections*** 7
- ***Equine Infections*** 7
- ***Human Infections*** 7

Mosquito Control 8

Response Levels 9

Characterization of Conditions and Responses 16

Key Agency Responsibilities 18

References 21

Appendices

Appendix A: Guidelines for Adult Mosquito Surveillance 22

Appendix B: Procedures for Processing Mosquitoes for Arbovirus Detection 28

Appendix C: Procedures for Maintaining and Bleeding Sentinel Chickens .. 30

Appendix D: Procedures for Testing Dead Birds and Squirrels 34

Appendix E: Procedures for Testing Equines and Ratites 40

Appendix F: Protocol for Submission of Specimens from Humans 45

Appendix G: West Nile Virus Surveillance Case Definition 46

Appendix H: Compounds Approved for Mosquito Control in California 49

Appendix I: Adult Mosquito Control in Urban Areas 53

Appendix J: Websites Related to Arbovirus Surveillance in California 56

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 formerly known as the California Department of Health Services (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 states in 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, yellow fever, Rift Valley fever, chikungunya and Venezuelan 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 12 mosquito-borne viruses are known to occur in California, only WNV, western equine encephalomyelitis virus (WEE) and St. Louis encephalitis virus (SLE) are significant causes of human disease. WNV is having a serious impact upon the health of humans, horses, and wild birds throughout the state. Since 2004, there have been 2874 WNV human cases with 95 deaths and 1132 horse cases. Consequently, the California Arbovirus Surveillance Program emphasizes forecasting and monitoring the temporal and spatial activity of

WNV, WEE, and SLE. 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 *Culex 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 *Culex pipiens, Culex quinquefasciatus*, and *Culex stigmatosoma*, play an important role in WNV, and possibly SLE, transmission cycles in urban and suburban areas. Historically, *Aedes melanimon*, a floodwater mosquito, played a role in a secondary transmission cycle of WEE involving rabbits. Additional mosquitoes such as *Aedes vexans* and *Culex erythrothorax* also could be important bridge (i.e. bird to mammal) vectors in transmission.

Mosquito control is the only practical method of protecting the human population from infection. There are no known specific treatments or cures for diseases caused by these viruses and vaccines are not available for public use. Infection by WEE virus tends to be most serious in very young children, whereas infections caused by WN and SLE viruses affect the elderly most seriously. WNV also kills a wide variety of native and non-native birds. There are WEE and WNV vaccines available to protect horses since both viruses can cause severe disease in horses. Mosquito-borne disease prevention strategies must be based on a well-planned integrated pest management (IPM) program that uses 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 duck club owners 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 duck club owners can work with mosquito control agencies to determine optimum 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 WEE, SLE, and WNV and to request appropriate laboratory tests. Public health officials need to be alerted if a mosquito-borne viral disease is detected, especially if the public health risk is high.

Surveillance

Surveillance includes the monitoring, visualization, and analysis of data on climatic factors, immature and adult mosquito abundance, and virus activity measured by testing mosquitoes, sentinel chickens, wild birds (including dead birds for WNV), horses, and humans for evidence

of infection. Surveillance must focus not only on mosquito-borne viruses known to exist in California, but be sufficiently broad to also detect newly introduced viruses.

Climate Variation

The California Mediterranean climate provides ideal opportunities for forecasting mosquito abundance and arbovirus activity, because most precipitation falls as rain at lower elevations or as snow at higher elevations during winter. Spring and summer temperatures then determine the rate of snow pack melt and runoff, mosquito population growth, the frequency of blood feeding, the rate of virus development in the mosquito, and therefore the frequency of virus transmission. In general, WEE virus outbreaks have occurred in the Central Valley when wet winters are followed by warm summers, whereas SLE and WN virus outbreaks seemed 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 epidemiological 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 the number of immature mosquitoes per "dip" estimated. 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, developmental stages treated, source size, and control effectiveness can provide an early warning to forecast the size of the adult population.

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 larval control efforts. Four adult mosquito sampling methods are currently used in California: New Jersey light traps, carbon dioxide-baited traps, gravid (egg-laying) 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

Early virus activity may be detected by testing adult mosquitoes for virus infection. Because *Culex tarsalis* is the primary rural vector of WNV, SLE, and WEE, and *Culex quinquefasciatus* and *Culex pipiens* are important urban vectors of WNV and SLE, surveillance efforts emphasize the testing of these species. Another species that should be tested is *Culex stigmatosoma*, which is a highly competent but less widely distributed vector of WNV and SLE that feeds on birds and is probably important in enzootic transmission where it is found in high abundance. 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 Center for Vectorborne Diseases (CVEC) at UC Davis. Procedures for submitting and processing mosquitoes for detecting virus infection are detailed in Appendix B. The current surveillance system is designed

to detect and measure levels of infection with WNV, SLE, and WEE. Although generally less sensitive than sentinel chickens, mosquito infections may be detected earlier in the season than chicken seroconversions and therefore provide an early warning of virus activity. Testing adult mosquitoes for infection is one of the best methods to detect newly introduced or emerging mosquito-borne viruses. Testing mosquito species other than *Culex* may be necessary to detect the introduction of viruses that do not have a primary avian-*Culex* transmission cycle.

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 viral antibodies (seroconversions), 2) collecting and bleeding wild birds to detect viral antibodies (seroprevalence), and 3) testing dead birds reported by the public for WNV.

In California, flocks of ten chickens 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 SLE, WEE, and WNV. Some agencies conduct their own testing, but send positive samples to CDPH for confirmation and official reporting. Because SLE cross-reacts with WNV in antibody testing, SLE 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 the most sensitive and cost-effective method to monitor encephalitis virus transmission in an area. Because chickens are continuously available to host-seeking mosquitoes, they are usually exposed to more mosquitoes than can be collected by trapping, especially when adult mosquito abundance or viral infection rates are low. Sentinel housing, bleeding instructions, and testing protocols are provided in Appendix C.

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 the prevalence of the virus in the region has changed. 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 generally is too labor intensive, technically difficult, and 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 daylight foraging flights and not at nighttime roosting sites where they are bitten by mosquitoes.

Unlike WEE and SLE, WNV frequently causes death in North American birds, especially those in the family Corvidae (e.g. crows, ravens, magpies, 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 indicators of WNV activity in a new area. Birds that meet certain criteria are necropsied at the California Animal Health and Food Safety Laboratory and kidney snips tested for WNV RNA by RT-PCR at CVEC or oral swabs of American crows tested by rapid antigen tests by local agencies. Dead birds are reported to CDPH's dead bird hotline (1-877-

WNV-BIRD) or via the website, http://westnile.ca.gov. The communication and testing algorithm for the dead bird surveillance program is detailed in Appendix D.

Since 2005, CDPH has used the Dynamic Continuous-Area Space-Time (DYCAST) model to identify areas of increased WNV activity in space and time based on the occurrence of dead bird reports. This model was developed in cooperation with the Center for Advanced Research of Spatial Information at Hunter College, City University of New York. DYCAST generates daily risk maps for the entire state of California, available on the Surveillance Gateway website, to help local agencies focus WNV surveillance, control, and public education efforts. A real-time alert system was also introduced in 2006 to provide high WNV activity counties with custom reports about WNV transmission levels. In a recent survey, local agencies reported that they used DYCAST to assist in decision-making processes for mosquito larviciding and adulticiding. In 2010, the DYCAST procedure will again be run statewide and daily maps will be made available online through the CALSURV Gateway (http://gateway.calsurv.org) from March through August.

Tree Squirrel Infections

In 2004, tree squirrels were included as a WNV surveillance tool, based upon evidence that they were susceptible to WNV and could provide information on localized WNV transmission (Padgett et al. 2007). In conjunction with dead birds, tree squirrels were reported to the California WNV hotline, necropsied at the California Animal Health and Food Safety Laboratory and kidney tissue was tested by RT-PCR at CVEC. Tree squirrels will continue to be tested for WNV in 2010 and are included in the submission protocol in Appendix D.

Equine Infections

Currently, equine disease due to WEE and WNV is no longer a sensitive indicator of epizootic (unusually high incidence of infections in animals other than humans) activity in California because of the widespread intentional or natural vaccination of equines (horses, donkeys, and mules). If confirmed cases do occur, it is a strong indication that WEE or WNV has amplified to levels where tangential transmission has occurred in that region of the State and human cases are imminent. Veterinarians are contacted annually by CDPH and the California Department of Agriculture (CDFA) to advocate equine vaccination and to describe diagnostic services that are available in the event of a suspected case of WEE or WNV encephalitis. Other mosquito-borne viruses may also cause encephalitis in horses, and testing of equine specimens for these other viruses is available (see Appendix E).

Human Infections

Local mosquito control agencies rely on 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 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. No human cases of SLE or WEE have been reported in California in recent years. However, a total of 2,877 cases of WNV have been reported in California from 2003-2009.

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 26 county public health laboratories that are able to conduct WNV testing. Providers are encouraged to submit specimens for suspect WNV cases to their local public health laboratories. Specimens for patients with encephalitis may also be submitted directly to the California Encephalitis Project, 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 ascertain 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 cases of WNV infection or positive test results to their local health department. Positive WNV or other arbovirus test results are investigated by local health department officials to determine whether a patient meets the clinical and laboratory criteria for a WNV diagnosis. If so, the local health department collects demographic and clinical information on the patient using a standardized West Nile virus infection case report, 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 definition for arboviral disease, including WNV infection.

Mosquito Control

Problems detected by surveillance are mitigated through larval and adult control. Mosquito control is the only practical method of protecting people from mosquito-borne diseases. Mosquito control in California is conducted by over 70 local agencies, including mosquito and vector control districts, environmental health departments, and county health departments. Compounds currently approved for larval and adult mosquito control in California are listed in Appendix H. 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, causing discomfort, 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 times 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) and *Bacillus sphaericus*, 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 their impact on nontarget organisms and the environment.

Adult Control

When larval control is not possible or has been used to the fullest extent possible, 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, such as malathion and naled, pyrethroids, such as resmethrin, sumithrin, and permethrin, and pyrethrins such as 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.

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 agencies to plan and modulate control activities. Independent models are presented for WEE, SLE and WNV to accommodate the different ecological dynamics of these viruses (Barker et al. 2003). SLE and WN viruses are closely related, require similar environmental conditions and employ 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 (snowpack, rainfall, temperature, season)
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 (WEE 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.

For surveillance factor 2 (vector abundance), abundance is scaled as an anomaly and compared to the area average over 5 years for the same preceding two week period. The area typically encompasses the boundaries of a local mosquito and vector control district. The mosquito virus infection rate should be calculated using the most current data (prior two week period) and expresses as minimum infection rate (MIR) per 1,000 female mosquitoes tested. Calculations can also use maximum likelihood estimate (Biggerstaff 2003), which accounts for varying numbers of specimens in pools. For WNV and SLE, 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. WEE activity typically is greatest during El Niño conditions of wet winters, excessive run-off and flooding, cool springs, and increased *Culex tarsalis* abundance. Historically, WEE virus spillover into a secondary *Aedes*-rabbit cycle was common in the Central Valley, but has not been detected for the past 25 years. In contrast, SLE and perhaps WNV activity appears to be greatest during La Niña conditions of drought and hot summer temperatures and both SLE and WNV transmission risk increases when temperatures are above normal. Abundance and infection of the *Culex pipiens* complex are included in both SLE and WNV estimates of risk 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, humans), 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. WEE virus transmitted by *Culex tarsalis* typically amplifies first in rural areas and then spreads towards small and then larger communities. A risk score was included to account for where virus activity was detected. WNV and SLE virus may be amplified concurrently or sequentially in rural and urban cycles. The rural cycle is similar to WEE virus and is transmitted primarily by *Cx. tarsalis*, whereas the urban cycle is transmitted primarily by members of the *Culex 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. Climatic factors provide the earliest indication of the potential for increased mosquito abundance and virus transmission and constitute the only risk factor actually measured from the start of the calendar year through mid-spring when enzootic surveillance commences in most areas. Climate is used prospectively to forecast risk during the coming

season. Other epidemiological 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, 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; 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 decreased 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 has been enabled by tools included in the Surveillance 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. **Adult *Culex tarsalis* and *Cx. pipiens* complex relative abundance*** 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 *Culex tarsalis* and *Cx. pipiens* complex mosquitoes*** Tested in pools of 50. Test results expressed as minimum infection rate per 1,000 female 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.

SLE 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. **Adult *Culex tarsalis* and *Cx. pipiens* complex relative abundance*** 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 *Culex tarsalis* and *Cx. pipiens* complex mosquitoes*** Tested in pools of 50. Test results expressed as minimum infection rate per 1,000 female 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.

WEE 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**. Adult *Culex tarsalis* abundance** 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. Test results expressed as minimum infection rate per 1,000 female 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 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 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 seroconversions and dead bird infections may need special treatment to accommodate bleeding schedules and zip code closures, respectively. For sentinel seroconversions, use the sentinel seroconversions 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 be contiguous and immediately precede the time period being evaluated.

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

- Assessment reports will be generated and delivered to the primary contacts of each agency by email every Monday.
- The time frame of each assessment report will be for the prior two-week period ending on the previous Saturday.
- Only those agencies with active Gateway accounts and active surveillance programs will receive the reports.
- All calculations are done at the agency level, thus the specific region is the area within the agency's boundary and the broad region includes the area within 150 miles (~241 km) of the agency's boundary.
- 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 species, *Cx. tarsalis* and the *Cx. pipiens* complex.
- For sentinel seroconversions, flavivirus positives are treated as WNV positives. If SLE is found, this will be adjusted accordingly.

Characterization of Conditions and Responses

Level 1: Normal Season

Risk rating: 1.0 to 2.5

CONDITIONS
• Average or below average snowpack and rainfall; below or average seasonal temperatures (<65F) • *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 • 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
• Snowpack and rainfall and/or temperature above average (66-79F) • Adult *Culex* mosquito abundance greater than 5-year average (150% to 300% above normal) • One or more virus infections detected in *Culex* mosquitoes (MIR / 1000 is <5) • 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 • WEE virus 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
• Snowpack, rainfall, and water release rates from flood control dams and/or temperature well above average (>79F) • Adult vector population extremely high (>300%) • Virus infections detected in multiple pools of *Culex tarsalis* or *Cx. pipiens* mosquitoes (MIR / 1000 > 5.0) • 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 • 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 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://www.westnile.ca.gov/resources.php

Key Agency Responsibilities

Local Mosquito and Vector Control Agencies

- Gather, collate, and interpret regional climate and weather data.
- Monitor abundance of immature and adult mosquitoes.
- Collect and submit mosquito pools to CVEC for virus detection.
- Maintain sentinel chicken flocks, obtain blood samples, and send samples to VBDS.
- Pick-up and ship dead birds for necropsy and WNV testing, or test oral swabs from American crows locally via rapid antigen screening assays.
- Update CDPH weekly of all birds that are independently reported and/or tested by VecTest, RAMP or immunohistochemistry.
- Update the surveillance gateway weekly with mosquito pool results that are independently tested by RAMP or PCR.
- Conduct routine control of immature mosquitoes.
- 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 disperse payment for 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: www.westnile.ca.gov
- Coordinate submission of specimens for virus testing.
- Provide supplies for processing mosquito pool and sentinel chicken diagnostic specimens
- Test sentinel chicken sera for viral antibodies.
- Test human specimens for virus.
- Distribute a weekly bulletin summarizing surveillance test results.
- Send weekly surveillance results to the UC Davis interactive website.
- Provide statewide, daily DYCAST human risk maps, available through the California Vectorborne Disease Surveillance Gateway (http://gateway.calsurv.org).
- Provide analysis of DYCAST risk data and notification to local agencies when appropriate.
- Immediately notify local vector control agency and public health officials when evidence of viral 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 active surveillance for human cases.
- Provide oversight to local jurisdictions without defined vector-borne disease control program.
- Maintain inventory of antigens and antisera 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 diseases, and mosquito ecology and control.
- Test mosquito pools and dead birds for endemic and introduced viruses.
- Provide a proficiency panel of tests for identification of viruses from human, equine, bird, or arthropod vectors to local agencies to ensure quality control.
- Maintain an interactive website (http://gateway.calsurv.org) for dissemination of mosquito-borne virus information and data.
- 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 WEE and WNV and testing facilities available at UCD Center for Vectorborne Disease Research.
- Provide outreach to general public and livestock and poultry producers on the monitoring and reporting of equine and ratite encephalitides.
- Facilitate equine and ratite sample submission from the field.
- Conduct epidemiological investigations of equine cases.

California Animal Health and Food Safety Laboratory

- Identify species of dead birds submitted for WNV testing.
- Conduct necropsies and testing on dead birds.
- Submit bird tissues to CVEC for testing.
- Test equine specimens for WNV.

Local Health Departments and Public Health Laboratories

- Test human specimens for WNV.
- 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 ship carcasses to testing laboratories when needed.
- Test American crows via rapid assay or RT-PCR as resources allow.
- Participate in emergency response.
- Conduct epidemiological investigations of cases of human disease.
- Report WNV cases to CDPH.
- Conduct public education.

California Emergency Management Agency

- Coordinate the local, regional, or statewide emergency response under 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.

References

Barker, C. M., W. K. Reisen, and V. L. Kramer. 2003. California State Mosquito-borne Virus Surveillance and Response Plan: A retrospective evaluation using conditional simulations. Am. J. Trop. Med. Hyg. 68: 508-518.

Eldridge, B.F. 1987. Strategies for surveillance, prevention, and control of arbovirus diseases in western North America. Am. J. Trop. Med. Hyg. 37:77S-86S.

Eldridge, B.F. 2000. The epidemiology of arthropod-borne diseases. pp. 165-185 in B. F. Eldridge and J. Edman, Eds. Medical entomology: a textbook of public health and veterinary problems caused by arthropods. Kluwer Academic Publications. Dordrecht, the Netherlands.

Eldridge, B.F. 2000. Surveillance for arthropod-borne diseases. pp. 515-538 in B. F. Eldridge and J. Edman, Eds. Medical entomology: a textbook on public health and veterinary problems caused by arthropods. Kluwer Academic Publications. Dordrecht, Netherlands.

Hui, L.T., S.R. Husted, W.K. Reisen, C.M. Myers, M.S. Ascher, V.L. Kramer. 1999. Summary of reported St. Louis encephalitis and western equine encephalomyelitis virus activity in California from 1969-1997. Proc.Calif. Mosq. Vector Control Assoc. 67: 61-72.

Meyer, R. P., W. K. Reisen and Vector and Vector-borne Disease Committee. 2003. Integrated mosquito surveillance guidelines. Mosq. Vector. Contr. Assoc. Calif.

Padgett, K.A, W.K. Reisen, N. Kahl-Purcell, Y. Fang, B. Cahoon-Young, R. Carney, N. Anderson, L. Zucca, L. Woods, S. Husted, and V.L. Kramer. 2007. West Nile virus infection in tree squirrels (Rodentia: Sciuridae) in California, 2004-2005. Am. J. Trop. Med. Hyg. 76: 810-813.

Reeves, W. C., M. M. Milby and W. K. Reisen. 1990. Development of a statewide arbovirus surveillance program and models of vector populations and virus transmission. pp.: 431-458. *In:* W. C. Reeves, (ed.) Epidemiology and control of mosquito-borne arboviruses in California, 1983-1987 Sacramento, Calif. Calif. Mosq. Vector Control Assoc., Inc.

Reeves, W.C. 1990. Epidemiology and control of mosquito-borne arboviruses in California, 1943-1987. California Mosquito Vector Control Association, Sacramento.

Reeves, W.C. 2000. The threat of exotic arbovirus introductions into California. Proc. Calif. Mosq. Vector Control Assoc. 68: 9-10.

Reisen, W.K. 1995. Guidelines for surveillance and control of arbovirus encephalitis in California. pp. 1-34 in: Interagency guidelines for the surveillance and control of selected vector-borne pathogens in California. California Mosquito Vector Control Association, Inc., Sacramento.

Reisen, W.K., R.P. Meyer, S.B. Presser, and J.L. Hardy. 1993. Effect of temperature on the transmission of western equine encephalomyelitis and St. Louis encephalitis viruses by *Culex tarsalis* (Diptera: Culicidae). J. Med. Entomol. 30: 151-160.

Theophilides, C. N., S. C. Ahearn, S. Grady and M. Merlino. 2003. Identifying West Nile virus risk areas: the Dynamic Continuous-Area Space-Time System. American Journal of Epidemiology 157:843-854.

Theophilides, C. N., S. C. Ahearn, E. S. Binkowski, W. S. Paul and K. Gibbs. 2006. First evidence of West Nile virus amplification and relationship to human infections. International Journal of Geographic Information Science 20:1:103-115.

Walsh, J.D. 1987. California's mosquito-borne encephalitis virus surveillance and control program. California Department of Health Services, Sacramento.

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. This section summarizes information from Integrated Mosquito Surveillance Program Guidelines for California that recently has 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, 2) CDC or EVS style CO_2-baited trap, 3) gravid trap, and 4) adult resting collections. Collection location sites should be geocoded and registered using the Surveillance 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 metabolic 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 alive; suitable for virus detection • Without light, collects mostly mosquitoes thus reducing sorting time • Battery operated, portable	• Collects >50% nullipars (females that have never blood fed or laid eggs) • Must be set and picked-up daily • Dry ice cost high; availability can be a problem • Does not collect males or bloodfed or gravid females
Gravid Trap	
Pros	**Cons**
• Collects females that have bloodfed and digested the blood meal; may have higher infection rate than CO_2 trap • Specimens alive; suitable for virus detection • Extremely sensitive for *Cx.quinquefasciatus* in urban habitat • Bait inexpensive • Battery operated, portable	• Collects only foul-water *Culex* [mostly *pipiens* complex] • Bait has objectionable odor • Must be set and picked-up daily

Resting Catches	
Pros	**Cons**
• All metabolic states collected • Minimal equipment needed • Specimens alive; suitable for virus detection • Blooded 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 distribution of 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 phototaxic mosquitoes. Place the traps at six-foot height. This can be done by using a metal standard, 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 diminish 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.

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 for four to seven nights before the collection is retrieved (Loomis and Hanks 1959). The trap should be thoroughly cleaned 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 an enamel pan before being identified and counted at 10x magnification under a dissecting microscope. Counting aliquots or subsamples of all specimen samples 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. Traps should be hung from a 6-foot tall standard (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. *Culex tarsalis* primarily bloodfeed on birds and hunt along vegetative borders and tree canopies where birds roost and nest. *Culex 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 up-wind side of the source for *Culex tarsalis* and *Anopheles freeborni* collections. *Aedes melanimon* and *Aedes nigromaculis* are mammal feeders and typically seek hosts over open fields.

When used to supplement sentinel chickens 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 should 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 *Culex 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 (*Culex tarsalis*, *Culex pipiens, Culex quinquefasciatus, Culex stigmatosoma, Aedes melanimon*, and *Aedes 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 (i.e., *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 -80C or on dry ice for later virus testing.

Reiter/Cummings gravid traps

Trap design and components

The Reiter/Cummings gravid traps consist 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 three to four days 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 *Culex pipiens* complex. The trap is 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

Culex 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 *Culex pipiens* complex, collections may be retrieved every third day. The females are killed, identified and counted before being discarded. Autogenous females may also 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 AFS (Arbovirus Field Station) 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’ x 4’ x 6’ (Meyer 1985). Smaller 1’ x 1’ x 1’ 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 predominant wind direction.

Processing

Mosquitoes should be anesthetized with triethylamine, identified under a dissecting microscope, sorted by sex and female metabolic 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 metabolic 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/). Import from local or proprietary data systems is available. For comparisons of abundance over time, space, or collection methods, refer to Biddlingmeyer (1969).

Data usage

Mosquito collections from some or all of the four 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.

References

Barr, A.R., T.A. Smith, M.M. Boreham, and K.E. White. 1963. Evaluation of some factors affecting the efficiency of light traps in collecting mosquitoes. J. Econ. Entomol. 56:123-127.

Bidlingmeyer, W.L. 1969. The use of logarithms in analyzing trap collections. Mosq. News 29:635-640.

Cummings, R.F. 1992. The design and use of a modified Reiter gravid mosquito trap for mosquito-borne encephalitis surveillance in Los Angeles County, California. Proceedings and Papers CMVCA 60:170-176.

Loomis, E.C. and S.G. Hanks. 1959. Light trap indices of mosquito abundance: a comparison of operation for four and seven nights a week. Mosq. News 19:168-171.

Komar, N., S. Langevin, S. Hinten, N. Nemeth, E. Edwards, D. Hettler, B. Davis, R. Bowen, and M. Bunning. 2003. Experimental infection of North American birds with the New York 1999 strain of West Nile virus. Emerg. Infect. Dis. 9: 311-322.

Meyer, R.P., W.K. Reisen, B.R. Hill, and V.M. Martinez. 1983. The "AFS sweeper", a battery powered backpack mechanical aspirator for collecting adult mosquitoes. Mosq. News 43:346-350.

Meyer, R.P. 1985. The "walk-in" type red box for sampling adult mosquitoes. Proc. New Jersey Mosq. Control Assoc. 72:104-105.

Meyer, R.P. 1996. Mosquito surveillance and sampling methods *in* The Biology and Control of Mosquitoes in California (S. Durso, Ed.). Calif. Mosq. and Vector Control Assoc., Inc. Sacramento

Meyer, R. P., W. K. Reisen and Vector and Vector-borne Disease Committee. 2003. Integrated mosquito surveillance guidelines. Mosq. Vector. Contr. Assoc. Calif.

Meyer, R. P., W.K.Reisen and Vector and Vector-borne Disease Committee. 2003. Integrated mosquito surveillance guidelines. Sacramento, California: MVCAC.

Mulhern, T.D. 1953. Better results with mosquito light traps through standardizing mechanical performance. Mosq. News 13:130-133.

Pfuntner, A.P. 1979. A modified CO_2-baited miniature surveillance trap. Bull. Soc. Vector Ecol. 4:31-35.

Reeves, W. C., M. M. Milby and W. K. Reisen. 1990. Development of a statewide arbovirus surveillance program and models of vector populations and virus transmission. pp.: 431-458. *In:* W. C. Reeves, (ed.) Epidemiology and control of mosquito-borne arboviruses in California, 1983-1987 Sacramento, Calif. Calif. Mosq. Vector Control Assoc., Inc.

Reisen, W. K., B. F. Eldridge, T. W. Scott, A. Gutierrez, R. Takahashi, K. Lorenzen, J. DeBenedictis, K. Boyce, and R. Swartzell. 2002**.** Comparison of dry ice-baited CDC and NJ light traps for measuring mosquito abundance. J. Am. Mosq. Control Assoc. 18: 158-163.

Reisen, W. K., H. D. Lothrop, R. E. Chiles, M. B. Madon, C. Cossen, L. Woods, S. Husted, V. L. Kramer, and J. D. Edman. 2004. West Nile Virus in California. Emerg. Infect. Dis.8: 1369-1378.

Reisen, W. K., R. P. Meyer, R. F. Cummings, and O. Delgado. 2000. Effects of trap design and CO2 presentation on the measurement of adult mosquito abundance using CDC style miniature light traps. J. Am. Mosq. Control Assoc. 16: 13-18.

Reiter, P. 1987. A revised version of the CDC gravid mosquito trap. J. Am. Mosq. Control Assoc. 3:325-327.

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. 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 such as 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. Lots of 50 females per pool of each vector species from each collection site are then 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 starting with #1 each year after the site code; e.g., KERN-1-10; where 10 refer to year 2010. 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 "MOSQUITO POOLS SUBMITTED FORM MBVS-3" AND CAN ONLY BE TESTED FROM REGISTERED SITES. Surveillance sites should be registered online at: **http://gateway.calsurv.org/.** Faxed registration forms (MBVS-1) will be accepted from agencies without adequate internet access.

   List the site code for each pool that consists of a designated four-letter agency code followed by four digits identifying the site, i.e., KERN0001. Keep the pool numbers in sequence for the whole year regardless of the number of site codes: e.g., pool #1 may be from KERN0001, and pool #2 may be from KERN0004.

4. Freeze pools immediately at -70°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 CVEC for testing by real time multiplex RT-PCR. Pools received by noon on Wednesday will be tested and reported by Friday or sooner using the Gateway website and automated email notification, in addition to the routine reporting within the weekly Arbovirus Surveillance Bulletin. Each pool is screened for WNV, SLE, and WEE viruses by a multiplex assay, with positives confirmed by a singleplex RT-PCR. Pools from selected areas also are screened for additional viruses using Vero cell culture with isolates identified following sequencing.

Care must be taken not to allow pools to defrost during storage or shipment, because each freeze-thaw cycle may result in a 10-fold decrease in viral titer, and all virus will be lost if the specimens sit at room temperature for extended periods. Address shipment to: Center for Vectorborne Diseases, University of California, Old Davis Road, Davis CA 95616.

5. Local agencies that conduct their own testing by 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 CDPH 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. Ten sentinel chickens can be housed in a 3Wx6Lx3H ft 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 easier enter the coop and the coops be placed in locations with a history of arbovirus transmission and/or high mosquito abundance. The site of 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 bi-weekly 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 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 zip lock 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. VERY IMPORTANT: CHICKEN SERA MUST BE ACCOMPANIED BY SENTINEL CHICKEN BLOOD

FORM (MBVS- 2) OUTSIDE THE ZIP-LOCK BAG. Do not staple the form to the bag. Samples from each bleeding date then can be placed into a mailing envelope and sent to:

Department of Public Health, Richmond Campus
Specimen Receiving Unit Room B106 **(ATTN: ARBO)**
850 Marina Bay Parkway
Richmond, CA 94804

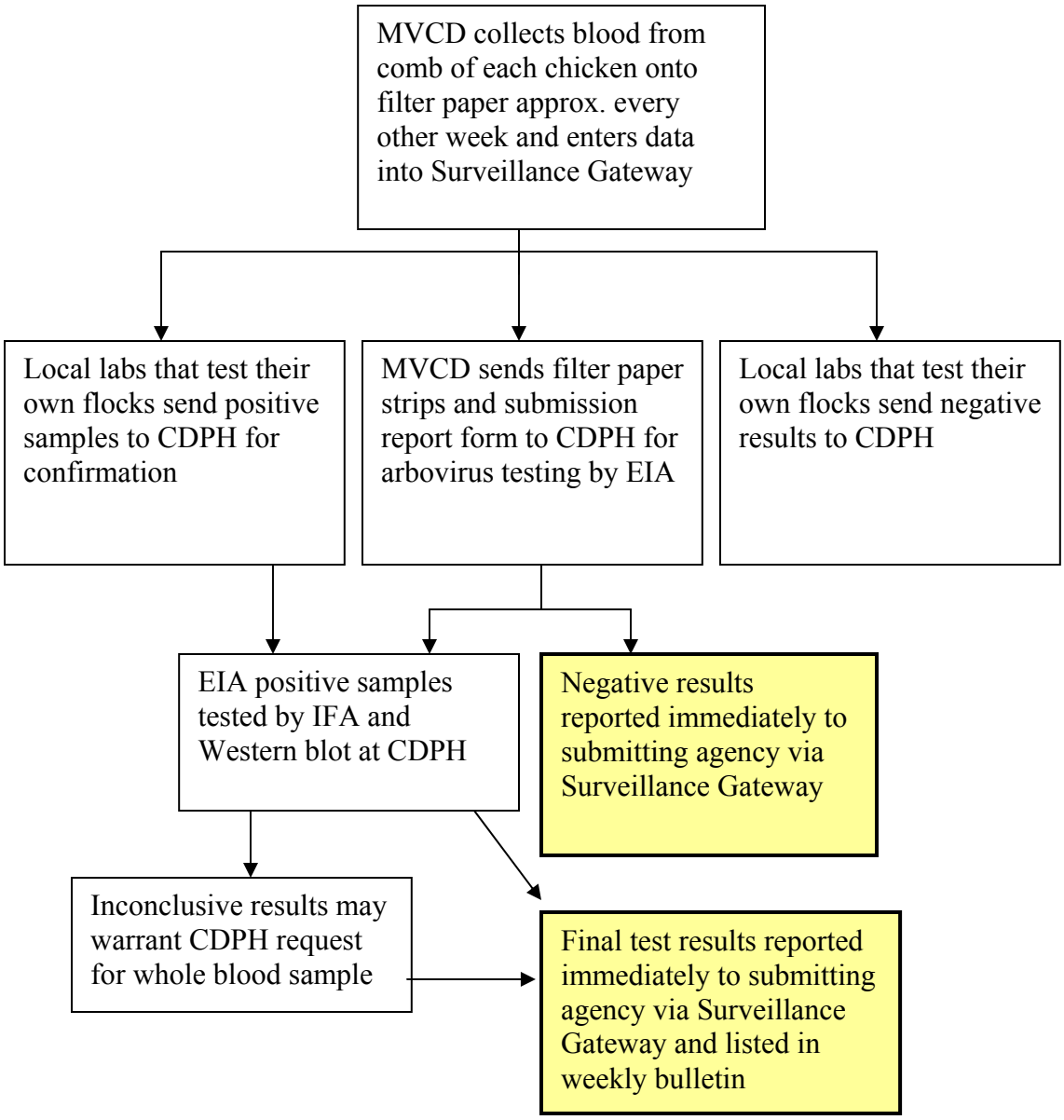
Specimens should be mailed to arrive no later than noon on Tuesdays for testing to begin that week.

6. In the laboratory, a single punch is removed from the blooded end of the paper and placed into one well of a 96-well plate with 150 µl of diluent. Specimens are allowed to soak for 2 hours on a rotator and the eluate is tested for WEE, SLE, and WNV IgG antibody using ELISA. Positive specimens are tested further with an indirect fluorescent antibody test and confirmed with a Western blot. Inconclusive SLE or WNV positives are confirmed and identified by cross-neutralization tests. Test results are made available online at: http://gateway.calsurv.org/.

Reference

Reisen, W.K. 1995. Guidelines for Surveillance and Control of Arboviral Encephalitis in California, In: Interagency Guidelines for the Surveillance and Control of Selected Vector-borne Pathogens in California, Mosquito and Vector Control Association of California, Sacramento.

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



Key:

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

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 mosquito pool testing by UC Davis Center for Vectorborne Diseases (CVEC) through the Mosquito and Vector Control Association (MVCAC). Sentinel chicken testing should be ordered through the California Department of Public Health (CDPH). Agencies will be billed in advance for the number of samples to be tested.

Agencies are responsible for registering 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 form "SENTINEL CHICKEN BLOOD – 2010" (MBVS-2) 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

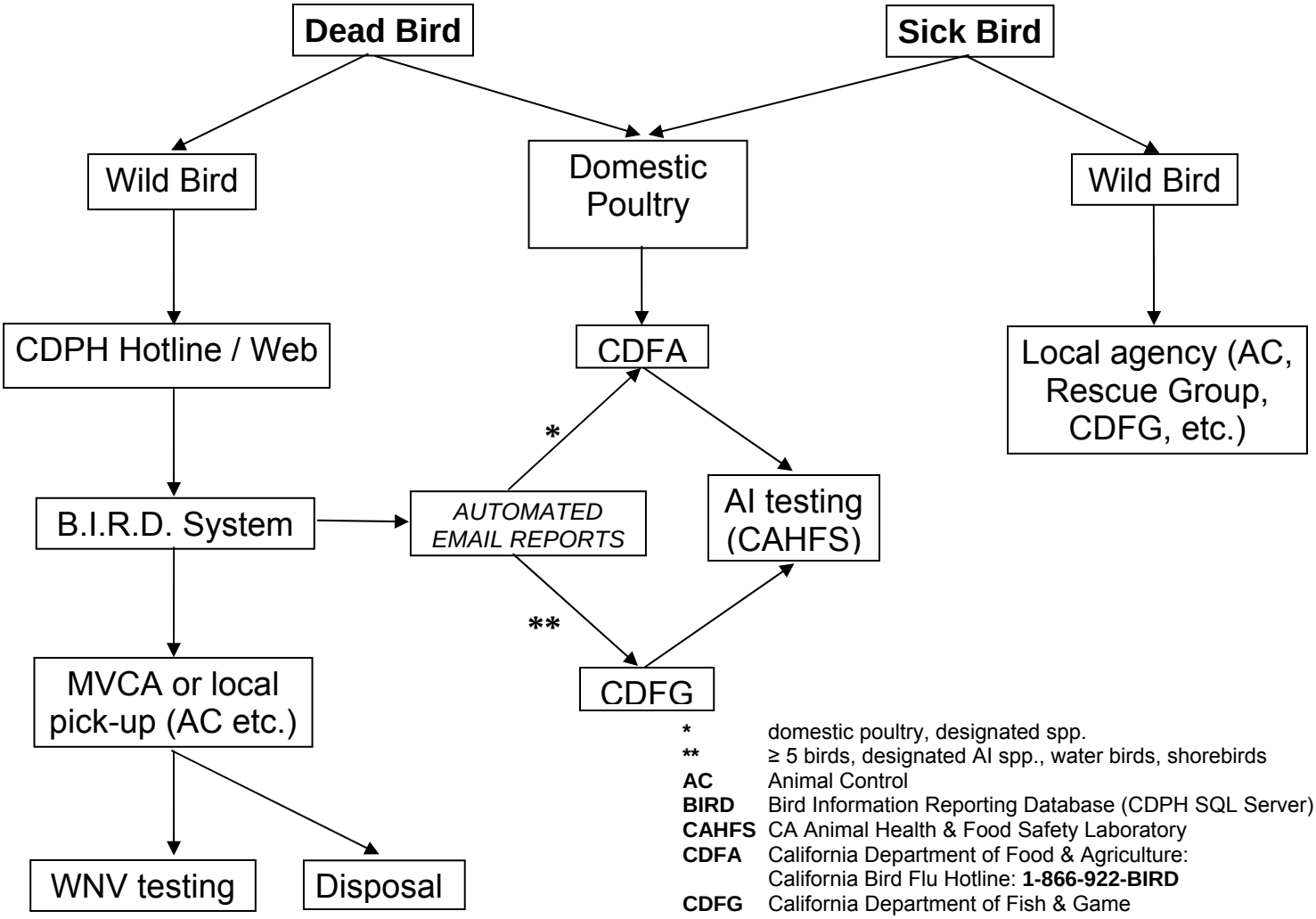
Registration of new sites used for collection of mosquitoes for virus testing may be accomplished by accessing the California Vectorborne Disease Surveillance Gateway http://gateway.calsurv.org/. Beginning in 2010, the CalSurv Gateway has enhanced spatial capabilities that allow users the option of directly 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 (MBVS-3, revised 01/12/06), including your agency code, your site code for the site and geographic coordinates.

The geographic coordinates will be used to generate computer maps that show all registered sites and test results for each site. Also, as part of a collaborative effort, CVEC will host real-time maps in ArcGIS format at http://maps.calsurv.org. In addition to these maps, agencies can access maps using Google Earth through the California Vectorborne Disease Surveillance Gateway (http://gateway.calsurv.org) that provide enhanced functionality and detail.

Appendix D: Procedures for Testing Dead Birds and Squirrels

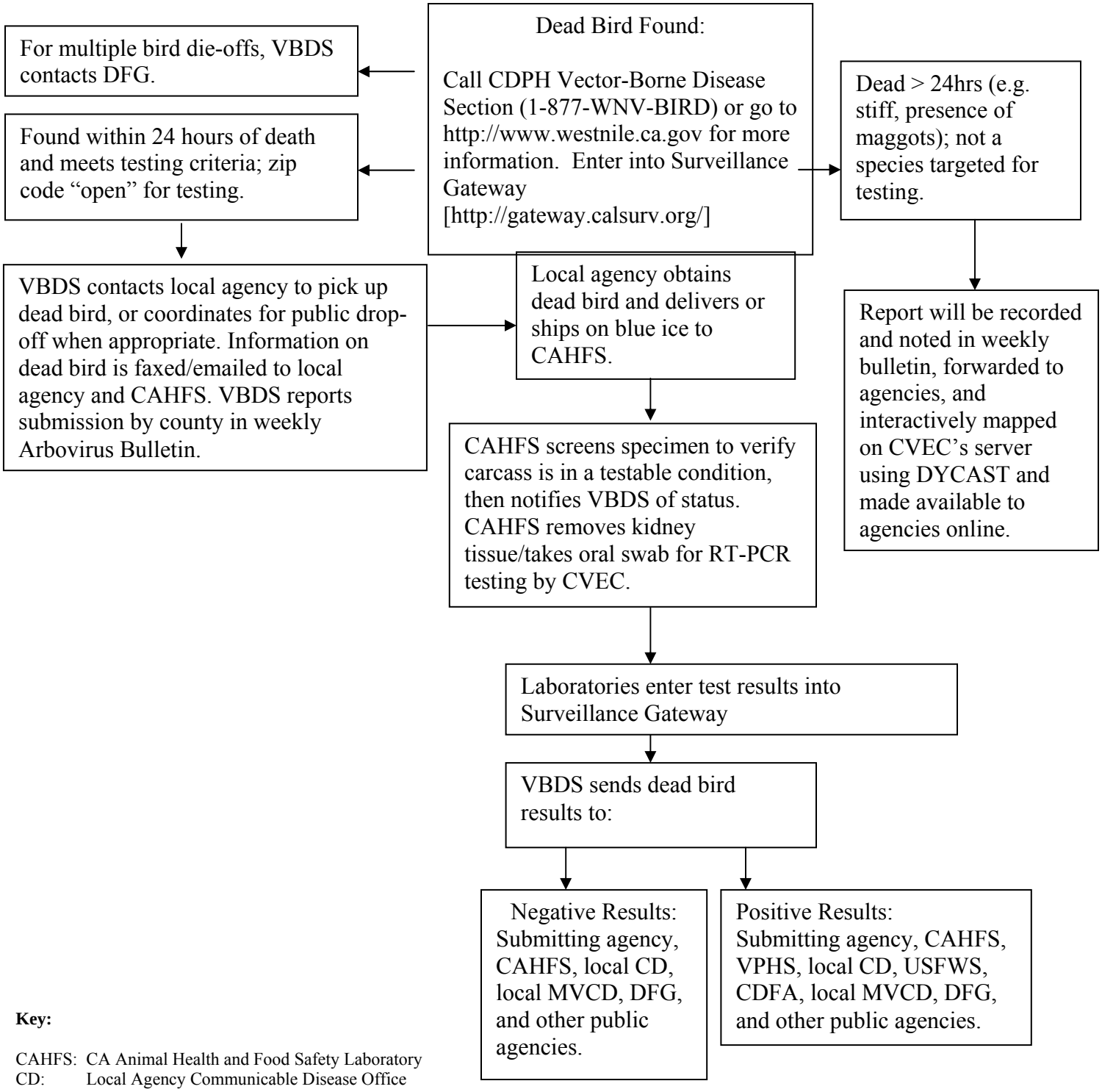
In 2000, CDHS initiated a dead bird surveillance program in collaboration with other public agencies. CDPH annually notifies about 600 agencies, organizations, and veterinarians involved with wildlife, including rehabilitation centers, about the program. The public is also notified about the program through the media and outreach materials. Dead birds and squirrels are reported to CDPH or data entered electronically through the Surveillance Gateway [http://gateway.calsurv.org/] and shipped to the California Animal Health & Food Safety (CAHFS) laboratory at UC Davis for screening and removal of kidney tissue (an oral swab is taken instead if the bird is an American Crow), which is then sent to the UC Davis Center for Vectorborne Diseases (CVEC) for WNV RNA detection via RT-PCR. Beginning in 2010, results from RT-PCR testing at CVEC will distinguish between birds that have been recently infected with WNV ("positive-recent") and those with older, chronic infections that are of limited value for surveillance ("positive-chronic") based on cycle threshold (Ct) values. 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
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**
CDFG	California Department of Fish & Game http://www.dfg.ca.gov/regions/index.html
CDPH	California Department of Public Health West Nile virus & Dead Bird hotline: **1-877-968-BIRD** website: www.westnile.ca.gov
MVCA	Mosquito & Vector Control Agency

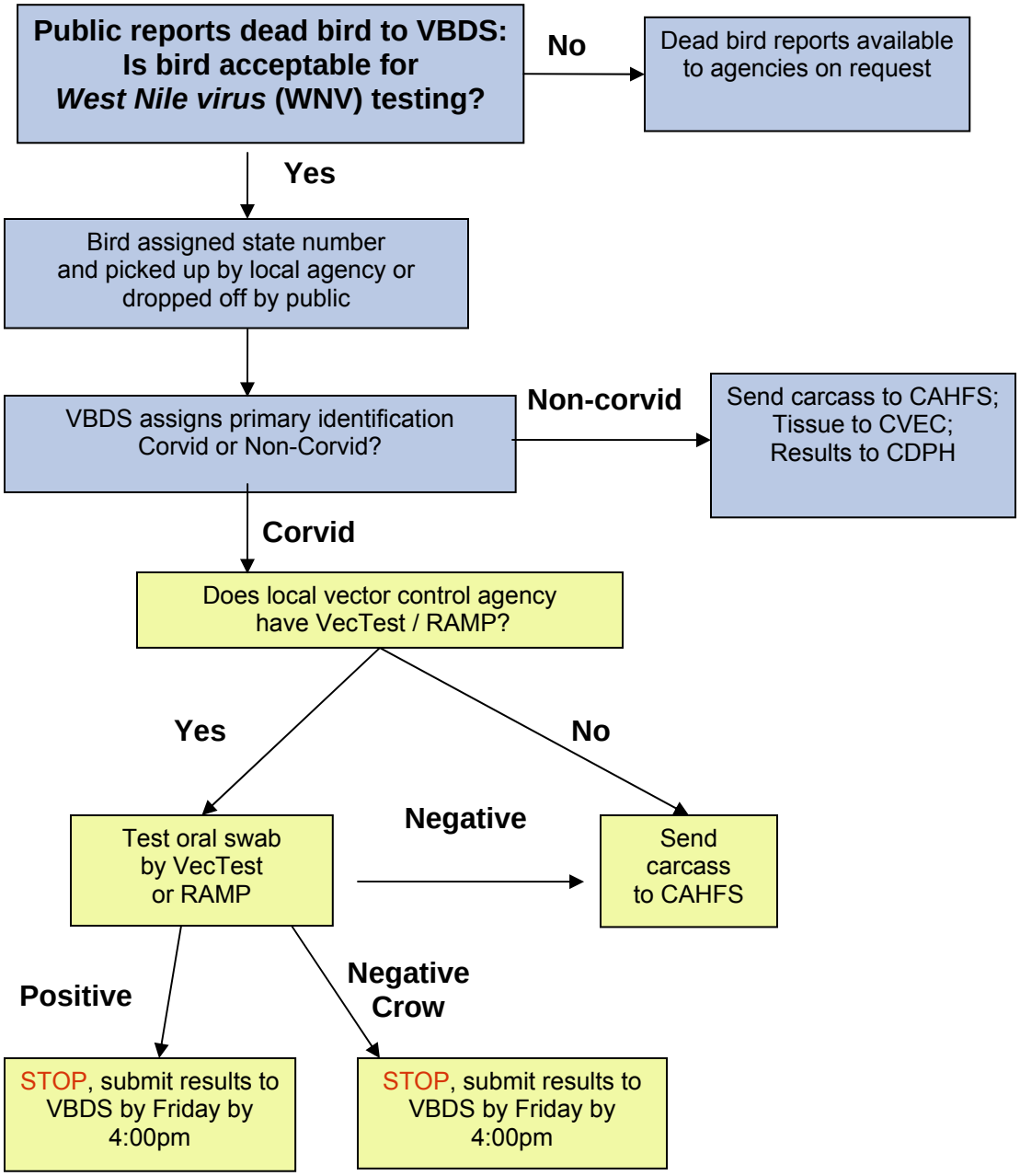
Procedures for Testing Dead Birds: RT-PCR



Key:

CAHFS: CA Animal Health and Food Safety Laboratory
CD: Local Agency Communicable Disease Office
CDFA: CA Dept. of Food and Agriculture
CVEC: UC Davis Center for Vectorborne Diseases
DFG: CA Dept. of Fish and Game
MVCD: Local Mosquito and Vector Control District
USFWS: US Fish and Wildlife Service
VBDS: CDHS Vector-Borne Disease Section, Richmond
VPHS: CDHS Veterinary Public Health Section, Sacramento
IHC: Immunohistochemistry

Procedures for Testing Dead Birds: Rapid Assays



CVEC = Center for Vectorborne Disease Research
VBDS = Vector-Borne Disease Section, California Department of Public Health
CAHFS = California Animal Health and Food Safety Laboratory

VBDS

Local Agencies

Dead Bird and Tree Squirrel Reporting and Submission Instructions for Local Agencies
California West Nile Virus (WNV) Dead Bird & Tree Squirrel 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 (especially recently dead crows, ravens, magpies, jays, or raptors) or dead tree squirrel, or one of your staff finds any dead bird, please immediately refer them to the **CDPH West Nile Virus and Dead Bird Hotline at 1-877-968-BIRD (2473)**.

The Dead Bird Hotline is monitored **8am - 5pm, 7 days a week.** CDPH will assess the suitability of the dead bird or tree squirrel for testing and contact your agency only if the carcass is approved for pickup. Any carcasses sent without prior notification will not be tested.

Only agencies listed under the permit issued to CDPH from the California Department of Fish & Game are authorized to pick up dead birds and tree squirrels. The agencies covered include local mosquito abatement districts, environmental health departments, and other designated agencies.

Members of the public may salvage dead birds found on their property or place of residence. **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 them off at the designated agency within 24 hours, between 9 am - 3 pm, Monday – Friday, and **only in areas where local agencies are not picking up dead birds** (e.g., closed zip codes), unless otherwise requested by the local agency. **Note: only dead birds may be brought in by the public to local agencies for shipping. We discourage public salvage of all squirrels because ground squirrels, which could be infected with plague, may be misidentified as tree squirrels.**

***web links*:** bird and tree squirrel ID chart (pdf) tree squirrel surveillance Q&A (pdf)

Once the submission is approved, your agency can ship the carcass to the California Animal Health & Food Safety laboratory at UC Davis (CAHFS Central). CAHFS Central removes specific tissues and forwards the samples to the UC Davis Center for Vectorborne Diseases (CVEC) for WNV testing. Shipping and testing expenses will be paid by CDPH. Carcasses are considered **Category B, Biological Substances.** This replaces the old designation, "Diagnostic Specimen".

To ensure the carcass arrives at CAHFS in a testable condition, to protect your safety, and to comply with shipping regulations, please follow these instructions:

- Only dead birds and tree squirrels can be picked up under our permit.

- Wear rubber or latex gloves when handling all carcasses. If gloves are not available, use a plastic bag -- turned inside out -- over your hand and invert the bag to surround the carcass. Do not touch a carcass with bare hands.

- **Collect fresh carcasses.** Badly decomposed or scavenged carcasses are of limited diagnostic value. Signs that a bird or squirrel 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 upon pick-up the carcass is found to be unacceptable (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.** California Department of Fish & Game prefers that you burn or bury the carcass, but disposing of it in a dumpster is also acceptable. **Please call CDPH immediately and notify us that the animal will no longer be submitted.**

- Place each carcass into two sealed (zip-locked) plastic bags. **Double-bagging prevents cross-contamination and leakage**. **There should always be two bags separating the carcass from shipping documents.**

- Enclose the shipping documents into a SEPARATE ZIP-LOCK BAG. The primary shipping document is a copy of the dead bird submission form which contains the dead bird number and which is located on the Surveillance Gateway [http://gateway.calsurv.org/] or faxed by CDPH. CAHFS prefers that you put this separate zip-lock bag inside the <u>outer</u> bag containing the dead bird or squirrel.

- **Pack the carcass with blue ice packs.** Please limit the number of ice packs to the number required to keep the carcass fresh, as the weight of extra ice packs add to the shipping charges. In accordance to shipping regulations, an absorbent material such as newspaper must be included in the box to prevent any leakage.

- Ship the carcass in a hard-sided plastic cooler or a styrofoam cooler placed in a cardboard box. Unprotected styrofoam containers cannot be shipped without an outer box or container, as they may break into pieces during shipment. **Contact UPS/GSO directly to arrange for carrier pickup Monday through Thursday; this guarantees arrival at CAHFS before the weekend.**

- Contact **UPS** to pick up carcasses either by web (https://wwwapps.ups.com/pickup/schedule?loc=en_US) or by phone **1-800-PICK UPS** (1-800-742-5877)**. Select "UPS Next Day Air"** and **estimate the weight of the box** (generally 10 lbs for a single large bird packed with ice). Please DO NOT UNDER-ESTIMATE the weight of a package. For billing, the **UPS account number is: <u>48R89V</u>.**

- Carcasses that need to be stored for an extended time period (over 2 days) should be put on dry ice or stored at -70ºC. If it is not possible to store carcass at -70ºC, a carcass may be stored at 0ºC (regular freezer) for a short period of time. **Refrigerating** the carcass is recommended for **overnight storage only** (this slows virus deterioration, but does not stop it).

- CDPH will provide prepared shipping boxes with appropriate labels. Any empty boxes shipped to your agency from CDPH will have its caution labels covered by a sheet of paper with "EMPTY BOX" printed on it. Please discard this sheet of paper before using the box to ship out a dead bird. If you need additional boxes, please contact VBDS at (510) 412-6251 or email arbovirus@cdph.ca.gov.

- Once West Nile virus is found in an area, agencies may test corvids via VecTest or RAMP assays. While results can be entered directly into the Surveillance Gateway, please **notify CDPH with results by 4:00pm Friday of each week to have results included in reports for the following week's State WNV updates.** Reporting forms can be found at (http://www.westnile.ca.gov/resources.php). **Note: any positive bird must be disposed of as biomedical waste (incineration).**

Dead Bird Shipping List

Please verify that your agency has the following items:

- CAHFS Address (see below)
- UPS preprinted labels
- WNV hotline number (877-968-BIRD; manned 8am - 5pm, 7 days a week)
- Crumpled newspapers or another absorbent material
- Rubber or Latex Gloves
- Packing tape
- Dead Bird Shipping Boxes
  - inner zip-lock bag
  - outer zip-lock bag
  - inner styrofoam box
  - outer cardboard box
  - blue ice packs

California Animal Health & Food Safety (CAHFS) laboratories:

CAHFS Central (530) 754-7372
ATTN: WNV
Jacquelyn Parker
University of California, Davis
West Health Science Drive
Davis, CA 95616

Appendix E: Procedures for Testing Equines and Ratites

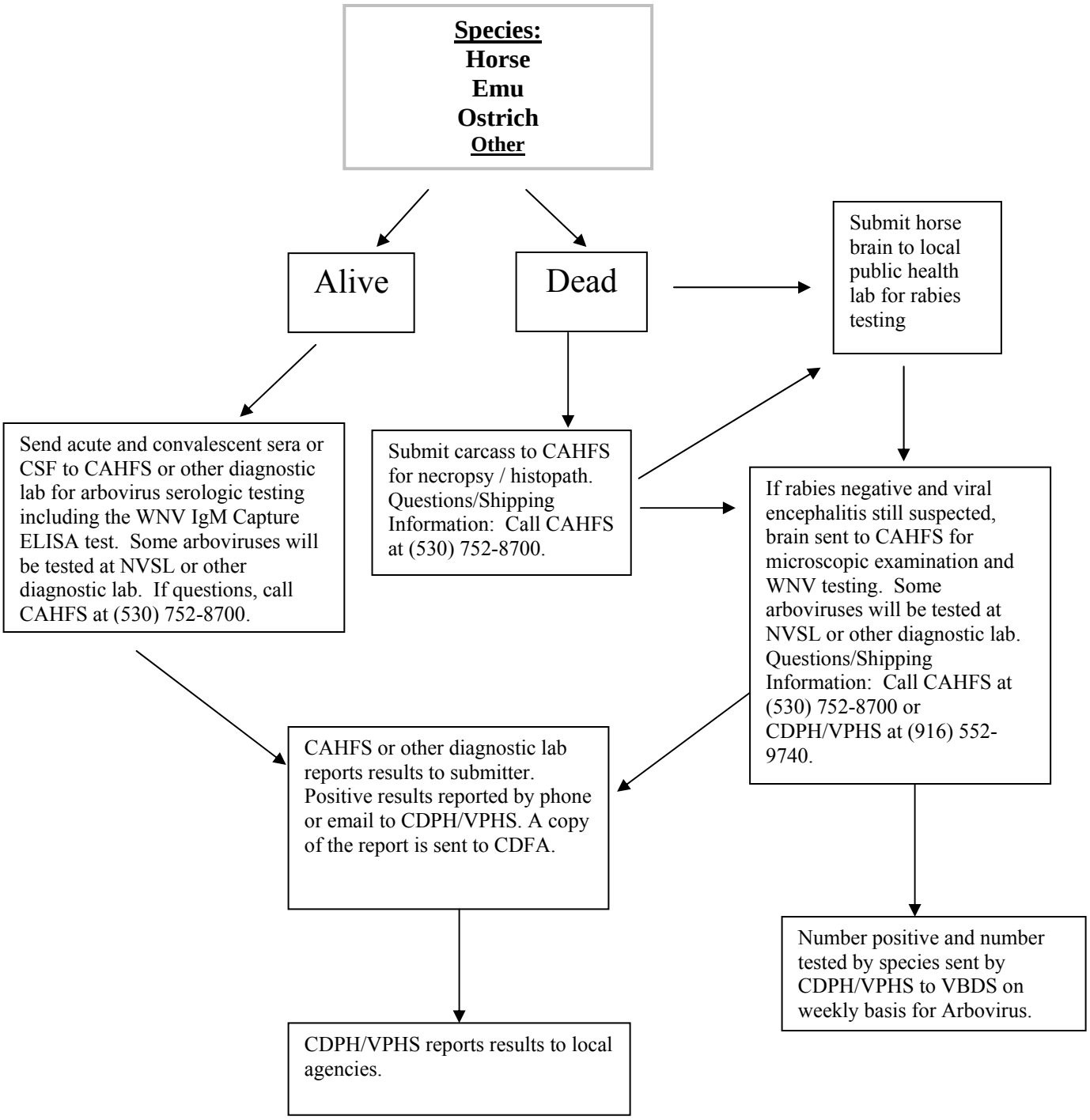
Effective June 1, 2009, the responsibility for equine and ratite West Nile Virus (WNV) surveillance was assumed by The California Department of Public Health (CDPH). The Department of Food and Agriculture (CDFA) was previously the lead agency for WNV surveillance. CDPH and CDFA developed a well-established passive surveillance program for equine and ratite encephalomyelitis. Equine encephalomyelitides are legally reportable to CDFA by veterinarians and diagnostic laboratories pursuant to Section 9101 of the Food and Agricultural Code. Venezuelan equine encephalitis is an emergency animal disease that must be reported to CDFA by telephone within 24 hours. Eastern and Western Encephalomyelitis and West Nile virus (WNV) are a classified as conditions of regulatory importance and must be reported to CDFA within 2 days.

This appendix contains information sent to veterinarians, public health lab directors, local health officers, public health veterinarians, animal health branch personnel, and interested parties every spring to inform them about the California Equine and Ratite Arbovirus Surveillance Program. The mailing includes a case definition for equine encephalomyelitides and instructions for specimen collection and submission for both equine and ratite samples. The information is distributed to approximately 1,200 practitioners, equine organizations, and other interested parties. Specimen submission is coordinated through the California Animal Health and Food Safety Laboratory System's (CAHFS) and its 3 regional branches, and other laboratories or individual veterinarians. Equine WNV serum and cerebrospinal fluid testing is performed by CAHFS, using the ELISA test for WNV IgM. Equine neurologic tissue specimens are also sent to CAHFS for microscopic examination and as dictated by clinical findings, forwarded to the National Veterinary Services Laboratories (NVSL) for further arbovirus testing. All fatal cases of equine encephalitides are first rabies ruled out at the local public health laboratory. An algorithm outlining the protocol for specimen submission and reporting is available for participants in the program and is included in this appendix.

Outreach is an important component of the program. CDPH and CDFA have developed and distributed educational materials concerning the diagnosis and reporting of arboviruses in equines and ratites.

Additional information on WNV for veterinarians, horse owners, and ratite owners, is available from CDFA, Animal Health Branch (916) 654-1447, 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. A brochure containing facts about California WNV surveillance and general information about prevention and control is available from CDPH (916) 552-9730 and at CDPH's website: http://www.westnile.ca.gov; a special section for veterinarians and horse owners is available at: http://www.westnile.ca.gov/resources.php.

Algorithm for Submission of Specimens from Domestic Animals with Neurologic Symptoms



Key:
CAHFS: California Animal Health and Food Safety Laboratory
NVSL: National Veterinary Services Laboratory
VPHS: CDPH Veterinary Public Health Section
VBDS: CDPH Vector-Borne Disease Section
CDFA: California Department of Food and Agriculture
CDPH: California Department of Public Health

SURVEILLANCE CASE DEFINITIONS FOR WEST NILE VIRUS DISEASE IN EQUINES

NOTE: A HORSE WITH SIGNS OF ENCEPHALITIS MAY HAVE RABIES – TAKE PROPER PRECAUTIONS

CONFIRMED CLINICAL CASE:

A horse with compatible clinical signs including ataxia (stumbling, staggering, wobbly gait, or in-coordination) or at least two of the following: fever, circling, hind limb weakness, inability to stand, multiple limb paralysis, muscle fasciculation, proprioceptive deficits, blindness, lip droop/paralysis, teeth grinding, acute death.

Plus one or more of the following:

- Isolation of West Nile (WNV) virus from tissues[1]
- Detection of IgM antibody to WNV by IgM-capture ELISA in serum or CSF
- An associated 4-fold or greater change in plaque-reduction neutralization test (PRNT) antibody titer to WNV in appropriately timed[2], paired sera
- Positive polymerase chain reaction (PCR)[3] for WNV genomic sequences in tissues[1]
- Positive IHC for WNV antigen in tissue (Note: this test has low sensitivity in equids)

SUSPECT CLINICAL CASE[4]:

- Compatible clinical signs

EXPOSED EQUID:

- Detection of IgM antibody to WNV by IgM-capture ELISA in serum or CSF without any observable or noted clinical signs.

Assumptions on which case definition is based:

- Antibody in serum may be due to vaccination or a natural exposure; additional testing must be done to confirm WNV infection in a vaccinated horse.
- IgM antibody in equine serum is relatively short-lived; a positive IgM-capture ELISA means exposure to WNV or rarely a closely related flavivirus (SLE) has occurred, very likely within the last three months.

---

[1] Preferred diagnostic tissue are equine brain or spinal cord; although tissues may include blood or CSF, the only known reports of WNV isolation or positive PCR from equine blood or CSF have been related to experimentally infected animals.
[2] The first serum should be drawn as soon as possible after onset of clinical signs and the second drawn at least seven days after the first.
[3] For horses it is recommended that RT-nested polymerase chain reaction assay be used to maximize sensitivity of the test (Emerg. Infect. Dis. 2001 Jul-Aug; 7(4):739-41)
[4] An equine case classified as a suspect case should, if possible, undergo further diagnostic testing to confirm or rule out WNV as the cause of the clinical illness.

Protocol for Submission of Laboratory Specimens for Equine Neurological Disease Diagnosis and Surveillance

Complete information on specimen collection and submission is available on the CDFA website at: http://www.cdfa.ca.gov/ahfss/Animal_Health/WNV_Lab_Submission.html

1. Specimen collection and submission:

A. Blood

- Acute sample (5-10 ml) / no later than 7 days after onset
- Convalescent sample (5-10 ml) / 14-21 days after onset
  Red top tubes of whole blood or serum (no preservatives or anticoagulants) should be submitted at ambient temperature to the California Animal Health and Food Safety (CAHFS) Laboratory* in your area. Do not freeze whole blood.
- **NOTE**: For WNV, an acute sample only is required since the assay used detects IgM (and vaccine does not interfere). For the other encephalitis viruses, the acute sample should be submitted immediately, and a convalescent sample may be requested later to assist with the interpretation and differentiation of vaccine titers from active infection.

B. Brain

- The local health department and CDFA/Animal Health District Office should be contacted if rabies is suspected.
- All equine specimens submitted to local public health laboratories for rabies testing and found to be negative, should be sent to CAHFS for arbovirus testing.
- Submission of the intact head is preferable because: 1) brain is better preserved (anatomically and virus titer) when left in the skull during transport, 2) specimens will be ruined if removal is not done correctly, and 3) brain removal in field conditions may increase the risk of exposure to rabies.
- **The intact head should be chilled (refrigerated, *not* frozen) immediately after removal. Submit it to a CAHFS Laboratory* in your area as quickly as possible.** Prepare a leak-proof insulated transporting container with "cold packs" to keep the specimen at 4° C while in transit. *When it is impossible for the CAHFS Laboratory to receive the chilled intact head within 48 hours, the submission protocol should be coordinated with the laboratory.*
- Specimens will then be forwarded by CAHFS to: 1) a Public Health Laboratory to confirm or rule out rabies, and 2) The National Veterinary Services Laboratories (NVSL) for arboviral testing. *In addition, brain will be examined microscopically for changes compatible with viral encephalitis or other causes of neurologic disease.*

C. Other specimens for differential neurological diagnoses

- Protocol for submission of serum, CSF or carcasses may be coordinated through CAHFS*. Protocol for submission of these specimens may be coordinated through the CAHFS Laboratory, and may include sampling for

equine herpesvirus, EPM, or other agents associated with clinical neurological presentations.

2. **Submission forms**: Complete and include the transmittal forms supplied by CAHFS. Call 530-752-8700 or visit the CAHFS website at http://cahfs.ucdavis.edu. The submittal form for each specimen should be placed in a leak-proof plastic bag. The specimen is collected in a leak proof plastic tube or bag and then placed in to a secondary leak proof plastic bag. The submission form is then attached to the corresponding container and shipped to CAHFS.
3. **Shipment:** Check with the CAHFS Laboratory in your area for assistance with shipping regulations governing the transportation of infectious materials.

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

West Nile virus (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 West Nile virus "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

---

* 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
- Cerebral spinal fluid (CSF): 1-2cc CSF <u>if lumbar puncture is performed</u>

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 Definition for West Nile Virus Infection in Humans

West Nile virus infection (neuroinvasive disease, fever, and asymptomatic infection) is reportable to CDPH under Title 17 of the California Code of Regulations. Below is the summary statement by the Council of State and Territorial Epidemiologists (available at http://www.cste.org/ps/2004pdf/04-ID-01-final.pdf) including the case definition for West Nile neuroinvasive disease, followed by the case definitions for West Nile fever and asymptomatic West Nile virus infection.

CASE DEFINITION: Neurotropic Domestic Arboviral Diseases

Clinical description

Arboviral infections may be asymptomatic or may result in febrile illnesses of variable severity sometimes associated with central nervous system (CNS) involvement. When the CNS is affected, clinical syndromes include aseptic meningitis, myelitis and encephalitis, which are clinically indistinguishable from similar syndromes caused by other viruses. Arboviral meningitis is usually characterized by fever, headache, stiff neck, and pleocytosis in cerebrospinal fluid. Arboviral myelitis is usually characterized by fever and acute limb paresis or flaccid paralysis.
Arboviral encephalitis is usually characterized by fever, headache, and altered mental status ranging from confusion to coma with or without additional signs of brain dysfunction. Less common neurological syndromes can include cranial and peripheral neuritis/neuropathies, including Guillain-Barré syndrome.

Non-neuroinvasive syndromes caused by these usually neurotropic arboviruses can rarely include myocarditis, pancreatitis, or hepatitis. In addition, they may cause febrile illnesses (e.g.,West Nile fever [WNF]) that are non-localized, self-limited illnesses with headache, myalgias, arthralgias, and sometimes accompanied by skin rash or lymphadenopathy. Laboratory-confirmed arboviral illnesses lacking documented fever can occur, and overlap among the various clinical syndromes is common.

Clinical criteria for diagnosis

Cases of arboviral disease are classified either as neuroinvasive or non-neuroinvasive, according to the following criteria:

Neuroinvasive disease requires the presence of fever and at least one of the following, as documented by a physician and in the absence of a more likely clinical explanation:

- Acutely altered mental status (e.g., disorientation, obtundation, stupor, or coma), or
- Other acute signs of central or peripheral neurologic dysfunction (e.g., paresis or paralysis, nerve palsies, sensory deficits, abnormal reflexes, generalized convulsions, or abnormal movements)

- Pleocytosis (increased white blood cell concentration in cerebrospinal fluid [CSF]) associated with illness clinically compatible with meningitis (e.g., headache or stiff neck)

Non-neuroinvasive disease requires, at minimum, the presence of documented fever, as measured by the patient or clinician, the absence of neuroinvasive disease (above), and the absence of a more likely clinical explanation for the illness. Involvement of non-neurological organs (e.g., heart, pancreas, liver) should be documented using standard clinico-laboratory criteria.

Laboratory criteria for diagnosis

Cases of arboviral disease are also classified either as confirmed or probable, according to the following laboratory criteria:

Confirmed case:

• Fourfold or greater change in virus-specific serum antibody titer, or
• Isolation of virus from or demonstration of specific viral antigen or genomic sequences in tissue, blood, CSF, or other body fluid, or
• Virus-specific immunoglobulin M (IgM) antibodies demonstrated in CSF by antibody-capture enzyme immunoassay (EIA), or
• Virus-specific IgM antibodies demonstrated in serum by antibody-capture EIA and confirmed by demonstration of virus-specific serum immunoglobulin G (IgG) antibodies in the same or a later specimen by another serologic assay (e.g., neutralization or hemagglutination inhibition).

Probable case:

• A single or stable (less than or equal to a twofold change) but elevated titer of virus-specific serum antibodies, or
• Serum IgM antibodies detected by antibody-capture EIA but with no available results of a confirmatory test for virus-specific serum IgG antibodies in the same or a later specimen.

Case definition: A case must meet one or more of the above clinical criteria and one or more of the above laboratory criteria.

Comment

Because closely related arboviruses exhibit serologic cross-reactivity, positive results of serologic tests using antigens from a single arbovirus can be misleading. In some circumstances (e.g., in areas where two or more closely related arboviruses occur, or in imported arboviral disease cases), it may be epidemiologically important to attempt to pinpoint the infecting virus by conducting cross-neutralization tests using an appropriate battery of closely related viruses. This is essential, for example, in determining that antibodies detected against St. Louis encephalitis virus are not the result of an infection with West Nile (or dengue) virus, or vice versa, in areas where both of these viruses occur. Because dengue fever and West Nile fever can be clinically indistinguishable, the importance of a recent travel history and appropriate serologic testing cannot be overemphasized. In some persons, West Nile virus-specific serum IgM antibody can wane slowly and be detectable for more than one year following infection. Therefore, in areas where West Nile virus has circulated in the recent past, the co-existence of West Nile virus-specific IgM antibody and illness in a given case may be coincidental and

unrelated. In those areas, the testing of serially collected serum specimens assumes added importance.
The seasonality of arboviral transmission is variable and depends on the geographic location of exposure, the specific cycles of viral transmission, and local climatic conditions. Reporting should be etiology-specific (see below; the six diseases printed in bold are nationally reportable to CDC):

- **St. Louis encephalitis virus disease**
- **West Nile virus disease**
- **Powassan virus disease**
- **Eastern equine encephalitis virus disease**
- **Western equine virus disease**
- **California serogroup virus disease** (includes infections with the following viruses: La Crosse, Jamestown Canyon, snowshoe hare, trivittatus, Keystone, and California encephalitis viruses)

West Nile Fever: West Nile fever is reportable in California. The following definition is used: West Nile fever syndrome can be variable and often includes headache and fever (T≥38˚C or 100.4˚F). 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. For the purposes of surveillance, an individual is considered to be a West Nile fever case if he or she has a febrile illness compatible with West Nile fever, and laboratory confirmation (as described above).

Asymptomatic West Nile Virus Infection: Asymptomatic infection with WNV, which is generally identified in blood donors, is also reportable. WNV-positive blood donors detected by blood banks are reported directly to local health departments. 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, an updated case report form should be sent to VRDL so that the blood donor may be reclassified as a clinical case.

Note: Due to the continued risk of unintentional or intentional introduction of exotic arboviruses into the United States (e.g., Venezuelan equine encephalitis virus), or the reemergence of indigenous epidemic arboviruses (e.g., St. Louis encephalitis and western equine encephalitis viruses), physicians and local public health officials should maintain a high index of clinical suspicion for cases of potential exotic or unusual arboviral etiology, and consider early consultation with arboviral disease experts at state health departments and CDC.

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 is not an inclusive list nor constitutes product endorsement. For more information on pesticides and mosquito control, please refer to the Environmental Protection Agency (EPA) Web site:
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. 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.

4. Larviciding oils (e.g. Mosquito Larvicide GB-1111)
   Use: Ditches, dairy lagoons, floodwater. Effective against all stages, including pupae.
   Limitations: Consult with the California Department of Fish and Game for local restrictions.

5. 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.

6. 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 *Culex tarsalis* populations in the Central Valley.

Adulticides:

1. Organophosphate compounds
   Note: Many *Culex 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	73049-20	Valent BioSciences	Granule	Larvae	Biorational
Bacillus sphaericus, (Bs)	VectoLex WDG	73049-57	Valent BioSciences	Water dispersible granule	Larvae	Biorational
Bacillus sphaericus, (Bs)	VectoLex WSP	73049-20	Valent BioSciences	Water soluble packet	Larvae	Biorational
Bacillus thuringiensis var. *israelensis* (Bti)	VectoBac 12AS	73049-38	Valent BioSciences	Liquid	Larvae	Biorational
Bacillus thuringiensis var. *israelensis* (Bti)	VectoBac G	73049-10	Valent BioSciences	Granule	Larvae	Biorational
Bacillus thuringiensis var. *israelensis* (Bti)	VectoBac Tech. Powder	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)	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
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
Petroleum oil	GB-1111	8329-72	Clarke	Liquid	Larvae and pupae	Surface film
Dimilin	Dimilin 25W	400-465	Uniroyal Chemical	Wettable powder	Larvae	IGR
S-Methoprene	Altosid ALL	2724-446	Wellmark-Zoecon	Liquid concentrate	Larvae	IGR
S-methoprene	Altosid Briquets	2724-375	Wellmark-Zoecon	Briquet	Larvae	IGR
S-methoprene	Altosid Pellets	2724-448	Wellmark-Zoecon	Pellet-type granules	Larvae	IGR
S-methoprene	Altosid SBG	2724-489	Wellmark-Zoecon	Granule	Larvae	IGR
S-methoprene	Altosid XR-G	2724-451	Wellmark-Zoecon	Briquet	Larvae	IGR
Temephos	Abate 2-BG	8329-71	Clarke	Granule	Larvae	OP
Temephos	5% Skeeter Abate	8329-70	Clarke	Granule	Larvae	OP

PESTICIDES USED FOR MOSQUITO CONTROL IN CALIFORNIA

Adulticides

Active Ingredient	Trade name	EPA Reg. No.	Mfgr.	Formulation	Application	Pesticide classification
Malathion	Fyfanon® ULV	67760-34	Cheminova	Liquid	Adults	OP
Naled	Dibrom® Concentrate	5481-480	AMVAC	Liquid	Adults	OP
Naled	Trumpet™ EC	5481-481	AMVAC	Liquid	Adults	OP
Prallethrin	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-Reslin®	432-796	Bayer	Liquid	Adults	Pyrethroid
Permethrin	Biomist® 4+12 ULV	8329-34	Clarke	Liquid	Adults	Pyrethroid
Permethrin	Permanone® Ready-To-Use	432-1277	Bayer	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® 7396	1021-1569	MGK	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® 10+10 ULV	1021-1688	Clarke	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 ultra low 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.

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, 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 Commissioners 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 Commissioners 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 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.
UC Davis Center for Vectorborne Diseases	http://cvec.ucdavis.edu/	Frequently updated reports and interactive maps on arbovirus surveillance and mosquito occurrence in California.
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 Gateway	http://gateway.calsurv.org	Data management system for California's mosquito control agencies.
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/ca/	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.
US Environmental Protection Agency – Mosquito Control	http://www.epa.gov/pesticides /factsheets/skeeters.htm	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/dv bid/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.

Reference List

Biggerstaff,BJ. 2003. Pooled infection rate. http://www.cdc.gov/ncidod/dvbid/westnile/software.htm : 1-5.