

# CALIFORNIA MOSQUITO-BORNE VIRUS SURVEILLANCE & RESPONSE PLAN

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## 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). Surveillance and interagency response guidelines have been published previously by the California Department of Health Services (Walsh 1987) and the Mosquito and Vector Control Association of California (Reisen 1995). The detection of West Nile (WN) virus 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 WN. From New York, WN virus spread rapidly westward and by 2003 had been detected in 46 of the United States, including southern California. In addition to WN virus, California is vulnerable to introduction of other highly virulent mosquito-borne viruses, such as Japanese encephalitis, dengue, yellow fever, Rift Valley fever, 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 Health Services (DHS), the Mosquito and Vector Control Association of California (MVCAC), and the University of California at Davis (UCD) and Berkeley (UCB).

## Background

Mosquito-borne viruses belong to a group of viruses commonly referred to as arboviruses (for **arthropod-borne**). Although 12 mosquito-borne viruses are known to occur in California, only WN virus, western equine encephalomyelitis virus (WEE) and St. Louis encephalitis virus (SLE) are significant causes of human disease. WN is anticipated to have a serious impact upon the health of humans, horses, wild birds, and zoo collections as it spreads and becomes established statewide. Consequently, the California Arbovirus Surveillance Program emphasizes forecasting and monitoring the temporal and spatial activity of WEE, SLE, and WN. These viruses are maintained in nature 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 the western encephalitis mosquito, *Culex tarsalis*, and birds such as house finches and 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* and *quinquefasciatus*, play an important role in WN, and possibly SLE, transmission cycles in urban and suburban areas. *Ochlerotatus melanimon*, a floodwater mosquito, plays a role in a secondary transmission cycle of WEE involving rabbits. Additional mosquitoes such as *Aedes vexans* and *Culex erythrothorax* could be important bridge (i.e. bird to mammal) vectors in WN transmission.

Mosquito control is the only practical method of protecting people and animals. There are no known specific treatments or cures for diseases caused by these viruses. Vaccines are not available for public use. Infection by WEE virus tends to be most serious in very young children, whereas infection caused by SLE and WN viruses affects elderly people most seriously. WEE and WN can be an important disease in horses and emus, and WN kills a wide variety of endemic and imported birds. There are WEE and WN vaccines available to protect horses.

Mosquito-borne disease prevention strategies must be based on a well-planned, area-wide integrated pest management (IPM) based program. 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 regarding curtailing outdoor activities during peak mosquito biting times, using insect repellents, and wearing long-sleeved clothing will help reduce exposure to mosquitoes. Clinical surveillance is enhanced through education of the medical community to recognize the symptoms of WEE, SLE, and WN 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 of climatic factors, estimating immature and adult mosquito abundance, and assessing virus activity by testing mosquitoes, sentinel chickens and wild birds (including dead birds for WN), 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.

### *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 disease 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 on the effectiveness of 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 the recently published Guidelines for Integrated Mosquito Surveillance (Meyer et al. 2003) and are summarized in Appendix A.

### *Mosquito Infections*

Early detection of virus activity may be accomplished by testing adult mosquitoes for virus infection. Because *Culex tarsalis* is the primary amplifying vector of WEE and SLE and most likely WN, surveillance efforts emphasize the testing of this species. Other species that should be tested include the *Culex pipiens* complex and *Ochlerotatus melanimon*. Female mosquitoes are trapped, usually using carbon dioxide-baited or gravid traps, and pooled into groups of 50 females each for testing at the UC Davis Center for Vector-borne Diseases (CVEC). Procedures for processing mosquitoes for virus infection are detailed in Appendix B. The current surveillance system is designed to detect WN and other vector-borne viruses, in addition to 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 mosquito-borne viruses that would not otherwise be expected to be present in the state. Sampling mosquito species other than *Cx. tarsalis* may be necessary to detect the introduction of viruses that do not have a primary avian-*Culex* transmission cycle.

### *Avian Infections*

Detection of transmission of arboviruses in 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, or 3) necropsy of dead birds and testing for WN virus. 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 DHS' Viral and Rickettsial Disease Laboratory for antibodies to SLE, WEE, and WN. Some agencies conduct their own testing, but send positive samples to DHS for confirmation and official reporting. Because SLE cross-reacts with WN in antibody testing, serum drawn from SLE or WN positive chickens are confirmed at CVEC by cross neutralization tests. Frequent testing of strategically placed flocks of sentinel chickens provides the most sensitive and cost-effective method to monitor encephalitis virus activity 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 is 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 after hatching year birds to determine if the prevalence of the virus in the region has changed. New infection can be detected in recaptured banded birds. 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 local mosquito control agencies to perform routinely. In addition, the actual place where a wild bird became infected is rarely known, because birds usually are collected during daylight foraging flights and not at nocturnal roosting sites where they are most frequently bitten by mosquitoes.

Unlike the endemic encephalitides, WN virus 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 DHS in 2000 to provide early detection of WN virus. Dead bird surveillance has been shown to be one of the earliest indicators of WN virus activity in a new

area. In collaboration with many local, state, and federal agencies, birds that meet certain criteria are tested for WN virus. In 2003, a total of 8,677 dead birds were reported to DHS' dead bird hotline (1-877-WNV-BIRD). Of the 1,768 birds that were tested, 96 tested positive for WN virus. The communication and testing algorithm for the dead bird surveillance program is detailed in Appendix D.

### *Equine Infections*

Currently, equine disease due to WEE is not a sensitive indicator of epizootic (the occurrence of infections in animals other than humans) activity in California because of the widespread vaccination of equines (horses, donkeys, and mules) against WEE virus. A similar scenario may unfold for WN virus as horse owners begin vaccinating to protect their horses. If confirmed cases do occur, it is a strong indication that WEE or WN is active in that region of the State. Veterinarians are contacted annually by DHS and the California Department of Agriculture (CDFA) to ensure that equines are vaccinated and to describe diagnostic services that are available in the event of a suspected case of WEE or WN encephalitis. Other mosquito-borne viruses may also cause encephalitis in horses; testing of equine specimens for 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 human infections cause no, or only mild, symptoms. The focus of human WN, SLE and WEE surveillance is on severe cases, typically encephalitis in any age group or aseptic meningitis in adults. Since transmission may occur from blood or transplanted organs, blood banks and organ transplantation programs have begun screening procedures. In an attempt to stimulate detection of human SLE, WEE, and WN cases in California, communication with key hospitals and local health officials has been enhanced. Specimens from suspect cases entered in DHS' California Encephalitis Project are tested for 15 core agents--including SLE, WEE, and WN. For patients with extensive mosquito exposure in which SLE, WEE, and WN are negative, other arboviruses are added to the core panel. Many local health departments as well as private laboratories now have the capability to conduct screening testing for WN. Positive specimens can be submitted to the VRDL for confirmation. Physicians are required to report viral encephalitis and viral meningitis cases to their local health department. Laboratories are required to report cases of arboviral encephalitis (Title 17 Sections 2500 and 2505). Cases that are confirmed to be due to WN, SLE or WEE will be investigated by local or state health officials to determine if the infection was acquired locally, imported from a region outside the patient's residence, or acquired by a non-mosquito route of exposure such as blood transfusion, organ donation or previously unidentified exposure sources. Appendix F contains the protocol for submission of laboratory specimens for human disease and Appendix G provides the surveillance case definition for confirmed WN virus infection in humans.

## **Mosquito Control**

Mosquito control is the only practical method of protecting people and animals 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.

### *Larval Control*

Control of mosquito larvae and pupae prevents mosquitoes from becoming biting female adults capable of transmitting disease, causing discomfort, and ultimately producing another generation of mosquitoes. Larval control focuses target-specific agents in definable aquatic breeding sites. 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. Environmental management may include water management, such as increasing the water disposal rate through evaporation, percolation, recirculation, or drainage. Controlled irrigation or the careful timing of wetland flooding for waterfowl can reduce mosquito production. Environmental management also may entail 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 algal growth.

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*. Insect growth regulators, such as methoprene, 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 stem an epidemic. Adult mosquito control products may be applied using ground-based equipment, fixed wing airplanes, or helicopters. These products include organophosphates, such as malathion and naled, and pyrethroids, such as resmethrin, sumithrin, and permethrin.

Factors to consider when selecting a pesticide include: 1) efficacy against the target species or life cycle stage, 2) pesticide resistance, 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 that could be used by local agencies to plan and modulate control activities. Independent models are presented for WEE, SLE, and WN to accommodate the different ecological dynamics of the three viruses (Barker et al. 2003). Six to eight surveillance factors are analyzed to determine the potential for virus transmission and thereby gauge the appropriate response level:

1. Environmental conditions (snowpack, rainfall, temperature, season)
2. Adult mosquito vector abundance
3. Virus isolations from mosquitoes
4. Sentinel chicken seroconversions
5. Fatal infections in birds
6. Infections in equids and ratites
7. Infections in humans
8. Proximity of detected virus activity to urban or suburban regions

Each factor is scored on an ordinal scale from 1 (least severe) to 5 (most severe). 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 I shows sources of data useful in the calculation of risk in Table 1. The term “average” refers to averages over non-epidemic years in a specific region, such as that within the boundaries of a local mosquito and vector control district. Averages typically are determined for the preceding five-year period (perhaps longer for environmental variables). 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. Roles and responsibilities of key agencies involved in carrying-out the surveillance and response plan are outlined in “Key Agency Responsibilities.”

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 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. Other biological factors that emerge as the season progresses are typically, in order: mosquito abundance, infections in non-humans (e.g., mosquitoes, sentinel chickens, equids), and infections in humans.

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, cool springs, increased *Cx. tarsalis* abundance, and virus spillover into *Ochlerotatus* populations. In contrast, SLE activity appears to be greatest during La Niña conditions of drought and hot summer temperatures. Because equine infections with SLE do not result in disease, equine cases are not included in the SLE risk assessment. The SLE response to climatic factors serves as a proxy for WN until further research elucidates the ecology of this virus in California. Abundance and infection of the *Culex pipiens* complex are included in both SLE and WN estimates of risk because of possible transmission by this mosquito in urban environments. The occurrence of dead bird infections is included as a risk factor in the WN calculations.



Table 1. Mosquito-borne Virus Risk Assessment

WEE Surveillance Factor	Assessment Value	Benchmark	Assigned Value
<b>1. Environmental Conditions</b>  Favorable environmental conditions include above normal rainfall, snow pack, and runoff and cool early season ambient temperature followed by a strong warming trend (El Niño season).	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	
<b>2. Adult <i>Culex tarsalis</i> and <i>Ochlerotatus melanimon</i> (bridge vector) abundance</b>  Determined by trapping adults, identifying them to species, and comparing numbers to averages previously documented for an area.	1	<i>Cx. tarsalis</i> abundance well below average (<50%)	
	2	<i>Cx. tarsalis</i> abundance below average (50-90%)	
	3	<i>Cx. tarsalis</i> abundance average (90-150%)	
	4	<i>Cx. tarsalis</i> and <i>Oc. melanimon</i> abundance above average (150-300%)	
	5	<i>Cx. tarsalis</i> and <i>Oc. melanimon</i> abundance well above average (>300%)	
<b>3. Virus isolation rate in <i>Cx. tarsalis</i> and <i>Oc. melanimon</i> mosquitoes</b>  Tested in pools of 50. Test results expressed as minimum infection rate (MIR) per 1,000 female mosquitoes tested (or per 20 pools).	1	<i>Cx. tarsalis</i> MIR / 1000 = 0	
	2	<i>Cx. tarsalis</i> MIR / 1000 = 0–1.0	
	3	<i>Cx. tarsalis</i> MIR / 1000 = 1.1-2.0	
	4	<i>Cx. tarsalis</i> MIR / 1000 = 2.1-5.0 and/or <i>Oc. melanimon</i> MIR/1000 > 0	
	5	<i>Cx. tarsalis</i> MIR / 1000 > 5.0 and <i>Oc. melanimon</i> MIR/1000 >0	
<b>4. Sentinel chicken seroconversion</b>  Number of chickens in a flock that develop antibodies to WEE virus. If more than one flock is present in a region, number of flocks with seropositive chickens is an additional consideration. Typically 10 chickens / flock.	1	No seroconversions	
	2	One seroconversion in single flock over broad area	
	3	One seroconversion in multiple flocks or multiple seroconversions in a single flock in region	
	4	Two to three seroconversions per flock in multiple flocks in region	
	5	More than three seroconversions per flock in multiple flocks in region	
<b>5. Infections in equines or ratites</b>	1	No cases	
	3	One case in broad region	
	4	One or two cases in specific region	
	5	More than two cases in specific region	
<b>6. Human cases</b>	1	No human cases	
	3	One human case statewide (but not within local jurisdiction or region)	
	5	One or more human cases in region	
<b>7. Proximity to urban or suburban regions</b> (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 activity in remote area	
	2	Virus activity in rural areas	
	3	Virus activity in small towns	
	4	Virus activity in suburban areas	
	5	Virus activity in urban area	
<b>Response Level / Average Rating:</b> Normal Season (1.0 to 2.5) Emergency Planning (2.6 to 4.0) Epidemic (4.1 to 5.0)		<b>TOTAL</b>	
		<b>AVERAGE</b>	

SLE Surveillance Factor	Assessment Value	Benchmark	Assigned Value
<b>1. Environmental Conditions</b> Favorable environmental conditions include above normal temperatures with or without above normal water conditions of rainfall, snow pack, and runoff. Urban mosquitoes breeding in municipal water systems may benefit from below normal rainfall. (La Niña season)	1	Temperature well below average	
	2	Temperature below average	
	3	Temperature average	
	4	Temperature above average	
	5	Temperature well above average	
<b>2. Adult <i>Culex tarsalis</i> or <i>pipiens</i> complex abundance</b> Determined by trapping adults, identifying them to species, and comparing numbers to those previously documented for an area.	1	Vector abundance well below average (<50%)	
	2	Vector abundance below average (50-90%)	
	3	Vector abundance average (90-150%)	
	4	Vector abundance above average (150-300%)	
	5	Vector abundance well above average (>300%)	
<b>3. Virus isolation rate in <i>Culex tarsalis</i> and <i>Cx. pipiens</i> complex mosquitoes</b> Tested in pools of 50. Test results expressed as minimum infection rate (MIR) per 1,000 female mosquitoes tested (or per 20 pools).	1	MIR / 1000 = 0	
	2	MIR / 1000 = 0-1.0	
	3	MIR / 1000 = 1.1-2.0	
	4	MIR / 1000 = 2.1-5.0	
	5	MIR / 1000 > 5.0	
<b>4. Sentinel chicken seroconversion</b> Number of chickens in a flock that develop antibodies to SLE virus. If more than one flock is present in a region, number of flocks with seropositive chickens is an additional consideration. Typically 10 chickens / flock.	1	No seroconversions	
	2	One seroconversion in single flock over broad area	
	3	One seroconversion in multiple flocks in region	
	4	Two to three seroconversions per flock in multiple flocks in region	
	5	More than three seroconversions per flock in multiple flocks in region	
<b>5. Human cases</b>	1	No human cases	
	3	One human case statewide (but not within local jurisdiction or region)	
	5	One or more human cases in region	
<b>6. Proximity to urban or suburban regions</b> (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 activity in remote area	
	2	Virus activity in rural areas	
	3	Virus activity in small towns	
	4	Virus activity in suburban areas	
	5	Virus activity in urban area	
<u>Response Level / Average Rating:</u> Normal Season (1.0 to 2.5) Emergency Planning (2.6 to 4.0) Epidemic (4.1 to 5.0)		<b>TOTAL</b>	
		<b>AVERAGE</b>	

WN Surveillance Factor	Assessment Value	Benchmark	Assigned Value
<b>1. Environmental Conditions</b> Favorable environmental conditions in California unknown. Rural transmission may favor El Niño conditions, whereas urban transmission may favor La Niña conditions.	1	Temperature well below average	
	2	Temperature below average	
	3	Temperature average	
	4	Temperature above average	
	5	Temperature well above average	
<b>2. Adult <i>Culex tarsalis</i> and <i>Cx. pipiens</i> complex abundance</b>  Determined by trapping adults, identifying them to species, and comparing numbers to those previously documented for an area.	1	Vector abundance well below average (<50%)	
	2	Vector abundance below average (50-90%)	
	3	Vector abundance average (90-150%)	
	4	Vector abundance above average (150-300%)	
	5	Vector abundance well above average (>300%)	
<b>3. Virus isolation rate in <i>Culex tarsalis</i> and <i>Cx. pipiens</i> complex mosquitoes</b> Tested in pools of 50. Test results expressed as minimum infection rate (MIR) per 1,000 female mosquitoes tested (or per 20 pools).	1	MIR / 1000 = 0	
	2	MIR / 1000 = 0-1.0	
	3	MIR / 1000 = 1.1-2.0	
	4	MIR / 1000 = 2.1-5.0	
	5	MIR / 1000 > 5.0	
<b>4. Sentinel chicken seroconversion</b> Number of chickens in a flock that develop antibodies to WN virus. If more than one flock is present in a region, number of flocks with seropositive chickens is an additional consideration.	1	No seroconversions in California	
	2	Seroconversion in neighboring state, but not CA	
	3	One seroconversion in single flock over broad area	
	4	One seroconversion in one or more flocks in region	
	5	Two or more seroconversions per flock in one or more flocks in region	
<b>5. Dead bird infection</b> Includes zoo collections.	1	No WN positive dead birds in California	
	2	WN positive dead bird in neighboring state, but not CA	
	3	One confirmed WN positive dead bird in California, but none in specific region	
	4	One confirmed WN positive dead bird reported in specific region	
	5	Multiple confirmed WN positive dead birds and multiple reports of dead birds in region	
<b>6. Equine cases</b>	1	No equine cases	
	3	One equine case in broad region	
	4	One equine case in specific region	
	5	Multiple equine cases in specific region	
<b>7. Human cases</b>	1	No human cases	
	3	One human case statewide (but not within specific region)	
	4	One human case in specific region	
	5	Multiple human cases in specific region	
<b>8. Proximity to urban or suburban regions</b> (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 activity in remote area	
	2	Virus activity in rural areas	
	3	Virus activity in small towns	
	4	Virus activity in suburban areas	
	5	Virus activity in urban area	
<b>Response Level / Average Rating:</b>			
Normal Season (1.0 to 2.5)			<b>TOTAL</b>
Emergency Planning (2.6 to 4.0)			
Epidemic (4.1 to 5.0)			<b>AVERAGE</b>

## Characterization of Conditions and Responses

### Level 1: Normal Season

**Risk rating: 1.0 to 2.5**

CONDITIONS
<ul style="list-style-type: none"> <li>• Average or below average snowpack and rainfall; average seasonal temperatures</li> <li>• Mosquito abundance at or below five year average (key indicator = adults of vector species)</li> <li>• No virus isolations from mosquitoes</li> <li>• No seroconversions in sentinel chickens</li> <li>• No WN infected dead birds</li> <li>• No equine cases</li> <li>• No human cases</li> </ul>
RESPONSE
<ul style="list-style-type: none"> <li>• Conduct routine public education (eliminate standing water around homes, use personal protection measures)</li> <li>• Conduct routine mosquito and virus surveillance activities</li> <li>• Conduct routine mosquito larval control</li> <li>• Inventory pesticides and equipment</li> <li>• Evaluate pesticide resistance in vector species</li> <li>• Ensure adequate emergency funding</li> <li>• Release routine press notices</li> <li>• Send routine notifications to physicians and veterinarians</li> <li>• Establish and maintain routine communication with local office of emergency services personnel; obtain Standardized Emergency Management System (SEMS) training</li> </ul>

### Level 2: Emergency Planning

**Risk rating: 2.6 to 4.0**

CONDITIONS
<ul style="list-style-type: none"> <li>• Snowpack and rainfall and/or temperature above average</li> <li>• Adult mosquito abundance greater than 5-year average (150% to 300%)</li> <li>• One or more virus isolations from mosquitoes (MIR / 1000 is &lt;5)</li> <li>• One to three chicken seroconversions per flock of 10 birds</li> <li>• One WN positive dead bird in California or in specific region</li> <li>• One or two equine cases statewide</li> <li>• One human case statewide</li> <li>• Viral activity in small towns or suburban area</li> </ul>
RESPONSE
<ul style="list-style-type: none"> <li>• Review epidemic response plan</li> <li>• Enhance public education (include messages on the signs and symptoms of encephalitis; seek medical care if needed; inform public about pesticide applications if appropriate)</li> <li>• Enhance information to public health providers</li> <li>• Conduct epidemiological investigations of cases of equine or human disease</li> <li>• Increase surveillance and control of mosquito larvae</li> <li>• Increase adult mosquito surveillance</li> <li>• Increase number of mosquito pools tested for virus</li> <li>• Conduct localized chemical control of adult mosquitoes</li> <li>• Contact commercial applicators in anticipation of large scale adulticiding</li> <li>• Review candidate pesticides for availability and susceptibility of vector mosquito species</li> <li>• Ensure notification of key agencies of presence of viral activity, including the local office of emergency services</li> </ul>

### Level 3: Epidemic Conditions

**Risk rating: 4.1 to 5.0**

CONDITIONS
<ul style="list-style-type: none"><li>• Snowpack, rainfall, and water release rates from flood control dams and/or temperature well above average</li><li>• Adult vector population extremely high (&gt;300%)</li><li>• Virus isolates from multiple pools of mosquitoes (MIR / 1000 &gt; 5.0)</li><li>• More than three seroconversions per flock of ten birds in multiple flocks</li><li>• Multiple confirmed WN positive dead birds and multiple reports of dead birds in region</li><li>• More than two equine cases in specific region</li><li>• One or more human cases in region</li><li>• Virus detection in urban or suburban areas</li></ul>
RESPONSE
<ul style="list-style-type: none"><li>• Conduct full scale media campaign</li><li>• Alert physicians and veterinarians</li><li>• Conduct active human case detection</li><li>• Conduct epidemiological investigations of cases of equine or human disease</li><li>• Continue enhanced larval surveillance and control of immature mosquitoes</li><li>• Broaden geographic coverage of adult mosquito surveillance</li><li>• Accelerate adult mosquito control if appropriate</li><li>• Coordinate the response with the local Office of Emergency Services or if activated, the Emergency Operation Center (EOC)</li><li>• Initiate mosquito surveillance and control in geographic regions without an organized vector control program</li><li>• Request public health exemptions from FIFRA (40 CFR 166) and emergency tolerance exemptions (40 CFR 176)</li><li>• Determine whether declaration of a local emergency should be considered by the County Board of Supervisors (or Local Health Officer)</li><li>• Determine whether declaration of a “State of Emergency” should be considered by the Governor at the request of designated county or city officials</li><li>• Ensure state funds and resources are available to assist local agencies at their request</li><li>• Determine whether to activate a Standardized Emergency Management System (SEMS) plan at the local or state level</li><li>• Continue mosquito education and control programs until mosquito abundance is substantially reduced and no additional human cases are detected</li></ul>

## **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 for virus detection.
- Maintain sentinel chicken flocks, obtain blood samples, and send samples to laboratory.
- Pick-up and ship dead birds for WN testing.
- Update DHS weekly of all birds that are independently reported and/or tested (email: [arbovirus@dhs.ca.gov](mailto:arbovirus@dhs.ca.gov)).
- Immediately notify the dead bird hotline (1-877-WNV-BIRD) of any birds that test positive via the VecTest or RAMP rapid screening tests.
- 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.

### 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 Health Services

- Collate adult mosquito abundance data submitted by local agencies; provide summary of data to local agencies.
- Maintain a WN virus information and dead bird reporting hotline, 1-877-WNV-BIRD, and a WN virus website. <http://westnile.ca.gov/>
- Coordinate submission of specimens for virus testing.
- Maintain database of all specimens tested.
- 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.
- Immediately notify local vector control agency and public health officials when evidence of viral activity is found.
- Conduct epidemiological investigations of cases of equine and human disease.
- Coordinate and participate in a regional emergency response in conjunction with California Office of Emergency Services.
- 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.

### 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 virus.
- Provide a panel of tests for identification of viruses from human, equine, bird, or arthropod vectors.
- Maintain an interactive website 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 WN and testing facilities available at UCD Center for Vector-borne 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 and screen dead birds for WN testing.
- Conduct necropsies and testing on dead crows and other birds.
- Submit bird tissues to UCD for testing.

### Local Health Departments

- Test human specimens for WN.
- Refer human specimens to DHS for further testing.
- Notify local medical community, including hospitals and laboratories, if evidence of viral activity present.
- Collect dead birds and ship carcasses to testing laboratories when needed.
- Participate in emergency response.
- Conduct epidemiological investigations of cases of human disease.
- Assist in public education.

### Governor's Office of Emergency Services

- Coordinate the local, regional, or statewide emergency response under epidemic conditions in conjunction with DHS 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.

### 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.

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## Appendix A: Guidelines for Adult Mosquito Surveillance

The objective of Appendix A is to standardize adult mosquito sampling and reporting procedures to provide comparable and interpretable surveillance results among collaborating mosquito control agencies in California. This section summarizes information from Guidelines for Integrated Mosquito Surveillance in California that recently has been adopted by the Mosquito and Vector Control Association (MVCAC) (Meyer et al. 2003). The MVCAC approach stratifies the use of different sampling methods in rural, small town, and urban environments for each of the major biomes of California and provides 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<sub>2</sub>-baited trap, 3) gravid trap, and 4) adult resting collections. Studies comparing trap design and efficiency for surveillance purposes have been published recently (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	
<b>Pros</b>	<b>Cons</b>
<ul style="list-style-type: none"> <li>All female metabolic states and males collected</li> <li>Minimal collection effort (can be run nightly without service)</li> <li>Long history of use in California</li> </ul>	<ul style="list-style-type: none"> <li>Selective for phototactic nocturnally active mosquitoes</li> <li>Ineffective with competing light sources</li> <li>Sorting time excessive because of other insects in traps</li> <li>Specimens dead; less use for virus detection</li> <li>Collects comparatively few specimens</li> </ul>
CDC/EVS CO <sub>2</sub> Trap	
<b>Pros</b>	<b>Cons</b>
<ul style="list-style-type: none"> <li>Samples biting population</li> <li>Collects large numbers of virus vector species</li> <li>Specimens alive; suitable for virus detection</li> <li>Without light, collects mostly mosquitoes thus reducing sorting time</li> <li>Battery operated, portable</li> </ul>	<ul style="list-style-type: none"> <li>Collects &gt;50% nullipars (have never oviposited)</li> <li>Must be set and picked-up daily</li> <li>Dry ice cost high; availability can be a problem</li> <li>Does not collect males or blooded and gravid females</li> </ul>
Gravid Trap	
<b>Pros</b>	<b>Cons</b>
<ul style="list-style-type: none"> <li>Collects females that have bloodfed; may have higher infection rate</li> <li>Specimens alive; suitable for virus detection</li> <li>Extremely sensitive for <i>Cx.p. quinquefasciatus</i> in urban habitat</li> <li>Bait inexpensive</li> <li>Battery operated, portable</li> </ul>	<ul style="list-style-type: none"> <li>Collects only foul-water <i>Culex</i></li> <li>Bait has objectionable odor</li> <li>Must be set and picked-up daily</li> </ul>

Resting Catches	
<p><b>Pros</b></p> <ul style="list-style-type: none"> <li>• All metabolic states collected</li> <li>• Minimal equipment needed</li> <li>• Specimens alive; suitable for virus detection</li> <li>• Blooded and gravid specimens can be tested to improve sensitivity of virus surveillance</li> </ul>	<p><b>Cons</b></p> <ul style="list-style-type: none"> <li>• Quantification difficult due to:               <ol style="list-style-type: none"> <li>1. shelter size and type</li> <li>2. collector efficiency</li> </ol> </li> <li>• Labor intensive; difficult to concurrently sample a large number of sites</li> </ul>

### **New Jersey (American) Light Trap (NJLT)**

#### **Trap specifications and components (Mulhern 1953)**

1. Ten inch diameter trap tube of sufficient length to accommodate motor, fan, cone screen, and killing jar. A lockable screen cage or holding strap should be added to the bottom of the trap to prevent tampering with the killing jar.
2. A 4- or 5-bladed 9.0-inch diameter fan.
3. Sealed, heavy-duty type refrigerator motor suspended by three support brackets for added stability; air discharge  $\cong$  450-500 cu. ft/min.
4. Hood or cone with a two- or three-point chain attachment for trap hanging.
5. One-quarter inch mesh hardware cloth over the mouth of the trap tube to preclude entry by large moths and other debris.
6. Timer or photoelectric eye to turn trap on/off. The photoelectric eye is preferred to prevent disruptions of trapping time that may occur with a timer due to power outages.
7. 25w, 110v, frosted light bulb.
8. Exterior trap color is insignificant, but underside of hood should be painted white to increase light intensity (Barr et al. 1963).
9. Killing jar with warning label containing a dichlorvos “no-pest” strip should be replaced every three months. A pint or quart jar could be used depending on the number of insects caught.

#### **Operation**

At a minimum, trap should be located in each principal municipality of a district or have a distribution of one trap/township (36 sq. mi.) to sample the adult mosquito population within the boundaries of the district’s responsibility. Correct placement of the NJLT is a critical factor in its performance as an effective surveillance mechanism for measuring the relative abundance of phototactic mosquitoes. Place the traps at six-foot height. This can be done by using a metal 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 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.

Traps should be operated from week 13 to week 43 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<sub>2</sub>-baited trap**

#### **Trap design and components**

Currently there are two types of CO<sub>2</sub>-baited traps being used in California: CDC trap and the EVS trap (Pfuntner, 1979), which is a modification of the first. Both trap types are baited either with an insulated container holding 1-2 kg of dry ice or with a cylinder containing compressed CO<sub>2</sub> gas with a regulator that releases 0.5 - 1.0 liters/minute. The dry ice container or the carbon dioxide gas cylinder should be properly labeled as to their contents. Both trap styles use a screened collection bag or a modified gallon ice cream carton with tubular surgical stockinet attached to the bottom of the motor housing unit to retain the collected mosquitoes.

The CDC trap uses:

1. A 3.5" diameter plexiglass cylinder housing a 6v DC motor and a 4-blade fan.
2. Rechargeable 6v battery power source.
3. Aluminum rain shield (optional).

The EVS trap:

1. Uses a 5" diameter PVC cylinder housing a 4.5-6.0v DC motor, and a 2-blade fan.
2. Uses three 1.5v D cell batteries in series as a power source.
3. Lacks an aluminum rain shield above the trap housing.

### **Operation**

Carbon dioxide-baited traps can be used for abundance monitoring or capturing mosquitoes for virus testing. A six foot tall standard should be used to standardize trap placement. Trap location should be fixed and standardized for population and virus infection rate monitoring.

Knowledge of the host-seeking patterns of the target species is essential in determining CO<sub>2</sub>-baited trap placement in the habitat and will enhance the 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<sub>2</sub>-baited traps could be hung on standards on the up-wind side of the source for *Cx. tarsalis* and *Anopheles freeborni* collections. *Ochlerotatus* (formerly *Aedes*) *melanimon* and *Oc. nigromaculis* are mammal feeders and typically hunt 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 readily affect catch size. The mini-light attracts other phototaxic insects that may hinder sorting and/or damage female mosquitoes in the collection container while repelling members of the *Cx. pipiens* complex. The CO<sub>2</sub>-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 should be placed within 100-200m radius of the sentinel flock site.

Maintenance of the traps should be performed regularly. Rechargeable 6v batteries should be charged after each night's run and rechargeable 1.5v batteries should be checked on a battery tester to determine the amount of power left to run the trap motors. Rechargeable 1.5v batteries need to be drained completely before being recharged to maintain full power capacity. Alkaline batteries need to be replaced after every use. The motors, fan blades, and interior of the trap housing should be cleaned on a regular basis.

### **Processing**

Mosquitoes collected for arbovirus surveillance should be processed according to the procedures outlined in Appendix B. Ten pools of a species (*Cx. tarsalis*, *Cx. pipiens*, *Cx. quinquefasciatus*, *Ochlerotatus melanimon*, and *Oc. 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 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 are killed, identified under a dissecting microscope, and counted.

### **Reiter/Cummings gravid traps**

#### **Trap design and components**

The Reiter/Cummings gravid traps consist of a rectangular trap housing 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. The trap housing contains the motor assembly and collection chamber for gravid mosquitoes. The revised Reiter gravid trap (Reiter 1987) utilizes a 6v powered motor using three D cell batteries, whereas the Cummings modified gravid trap (Cummings 1992) uses a 9V motor and four D cell batteries. Both can be operated using a 6V gel cell battery. Both traps place the collection chamber on the inlet side of the motor so that the fan blades will not damage collected mosquitoes. The inlet height should be two inches above the surface of the hay-infusion medium to create a proper vortex.

The oviposition attractant consists of a fermented infusion made by mixing Timothy or alfalfa hay, Brewer's yeast and water. The mixture should sit at room 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, principally for surveillance of *Culex pipiens* complex populations. 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, 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 should be recorded on the DHS Adult Mosquito Occurrence Report Summary Form and faxed to (510) 412-6263. 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. Compare mosquito abundance from collections with the number of service requests from the public to determine the tolerance of neighborhoods to mosquito abundance.
3. Monitor arbovirus vector abundance and minimum infection rates.
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<sub>2</sub> traps and resting adult collections; such data are critical to evaluating the vector potential of the population.

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## 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 SLE or WEE virus titer (Kramer et al. 1990). TEA should be used either outdoors or under a chemical hood. Collections can be knocked down 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 will be extremely important as diagnostics transition from virus isolation to sensitive RNA methods of viral detection. Count and discard dead and dried mosquitoes. Lots of 50 females (minimum of 12 females) per pool of each vector species from each collection site are then counted into individual screw-cap cryovials fitted with O-rings to prevent contact with CO<sub>2</sub> during transport and storage. 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-1-04; where 04 refers to year 2004. **VERY IMPORTANT: POOLS MUST BE ACCOMPANIED BY "MOSQUITO POOLS SUBMITTED FORM MBVS-3" AND CAN ONLY BE TESTED FROM REGISTERED SITES (USE FORM MBVS-1 TO REGISTER COLLECTION SITES - see Appendix C).**

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 with dry ice in an insulated container or in an ultralow temperature freezer. Pools are shipped frozen on dry ice to CVEC for testing by real time multiplex RT-PCR. Each pool is screened for WN, SLE, WEE, and CE-group viruses by the multiplex assay, with positives confirmed by a singleplex RT-PCR and/or virus isolation on Vero cell culture. Care must be taken not to allow pools to defrost during storage or shipment, because each thaw and freeze kills approximately half the virus, 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.



**Reference:**

Kramer, L.D., S.B. Presser, E.J. Houk and J.L. Hardy. 1990. Effect of the anesthetizing agent triethylamine on western equine encephalomyelitis and St. Louis encephalitis viral titers in mosquitoes (Diptera: Culicidae). *J. Med. Entomol.* 27:1008-1010.



### Appendix C: Procedures for Maintaining and Bleeding Sentinel Chickens

1. Procure hens in April when 18 weeks of age to ensure minimal mortality during handling. Hens at this age have not yet begun to lay eggs, but 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 1x1 inch welded wire. The site for each coop must first be registered using FORM MBVS 1 submitted to UC Davis. Coops should be at least two feet off the ground to reduce predator access, facilitate capture of the birds for bleeding, and allow the free passage of the feces through the wire floor to the ground. A single, hinged door should be placed in the middle of the coop, so that the entire coop is accessible during chicken capture. After construction, the lumber and roof should be protected with water seal. A self-filling watering device should be fitted to one end of the coop and a 25 lb. feeder suspended in the center for easy access. In exchange for the eggs, a local person (usually the home owner, farm manager, etc.) should check the birds (especially the watering device) and remove the eggs daily. If hung so the bottom is about four inches above the cage floor and adjusted properly, the feeder should only have to be refilled weekly (i.e., 100 lb. of feed per month per flock of ten birds). Therefore, if proper arrangements can be made and an empty 55 gallon drum provided to store extra feed, sentinel flocks need only be visited biweekly when blood samples are collected.
3. Band each bird in the web of the wing using metal hog ear tags and appropriate pliers. This band number, the date, and site registration number must accompany each blood sample sent to the laboratory for testing.
4. Bleed each hen from the distal portion of the comb using a standard lancet used for human finger "prick" blood samples. The bird can be immobilized by wedging the wings between the bleeder's forearm and thigh, thereby leaving the hand free to hold the head by grabbing the base of the comb with the thumb and forefinger. 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 end of the pre-numbered filter paper strip (opposite from the number) to the wound. Collect several drops in this fashion to completely soak a pre-marked 3/4 inch long portion of the 3/8 inch wide filter paper strip. Place the numbered 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. Staple the completely dry filter paper strips through the number along the top end of a 5x8 inch card, leaving the blood soaked end free so that the laboratory staff can readily remove a standard punch sample. Write the name of the flock and the date onto the card and place it and a single flock specific data sheet into a zip lock plastic 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.** Samples from each bleeding date then can be placed into a mailing envelope and sent to:

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

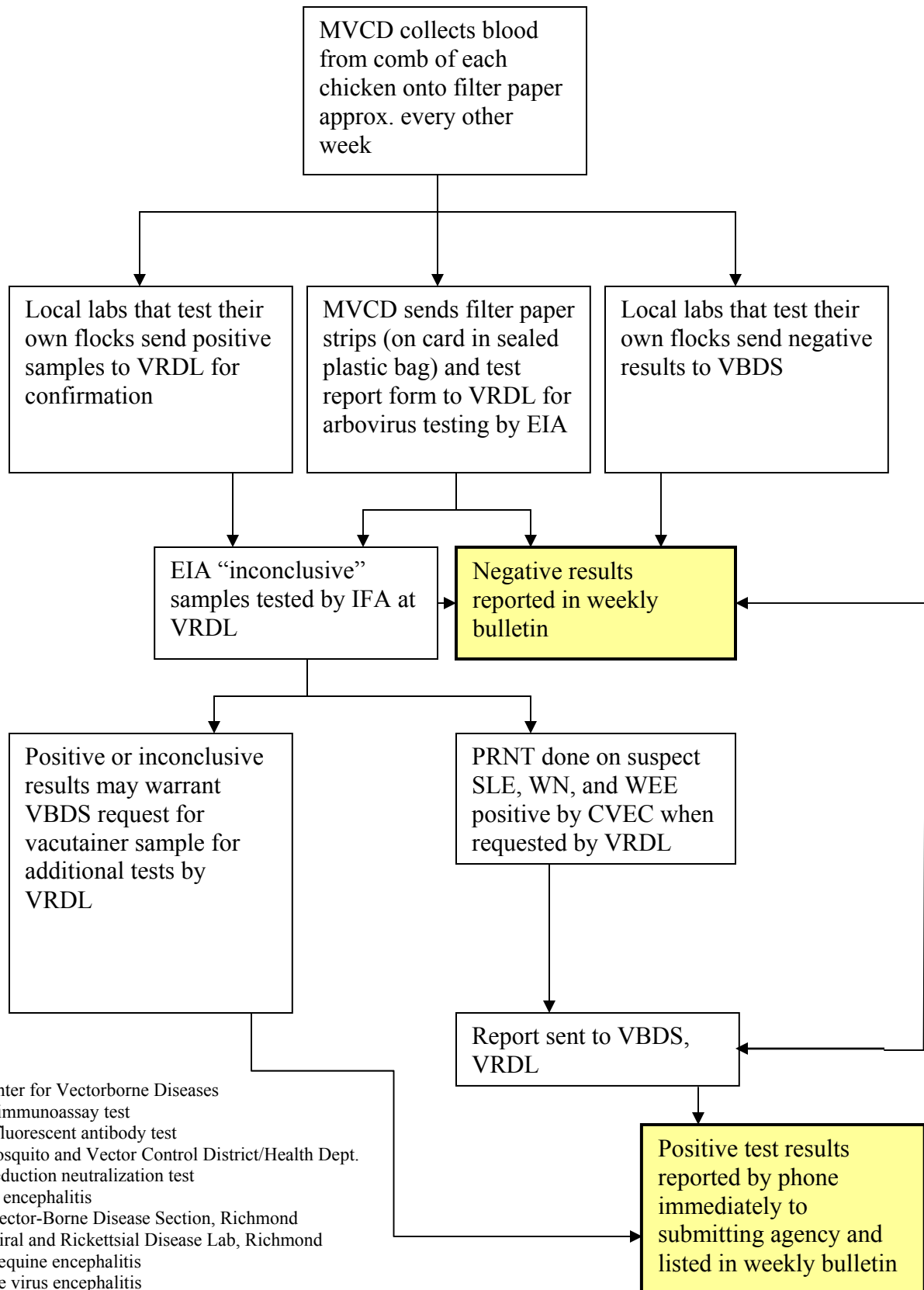
Specimens should be mailed to arrive by Friday afternoon for testing to start the following Monday.

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 200  $\mu$ l of diluent. Specimens are allowed to soak overnight and the eluate tested for WEE, SLE, and WN IgG antibody using ELISA. Positive specimens are confirmed the following day using an indirect fluorescent antibody test. SLE or WN positives are confirmed by cross-neutralization tests.

### 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 WN) and WEE



- Key:**
- CVEC: UCD Center for Vectorborne Diseases
  - EIA: Enzyme immunoassay test
  - IFA: Indirect fluorescent antibody test
  - MVCD: Local Mosquito and Vector Control District/Health Dept.
  - PRNT: Plaque reduction neutralization test
  - SLE: St. Louis encephalitis
  - VBDS: CDHS Vector-Borne Disease Section, Richmond
  - VRDL: CDHS Viral and Rickettsial Disease Lab, Richmond
  - WEE: Western equine encephalitis
  - WN: 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 through the Mosquito and Vector Control Association (MVCAC) for testing of sentinel chicken blood samples and mosquito pools. MVCAC will bill the agency for the number of samples to be tested, register the agency, assign an agency code, and notify VRDL and UC Davis of the names and codes for each registered agency.

As part of an agreement on coordination of surveillance for mosquito-borne viruses, VRDL will accept and test sentinel chicken blood samples only from those California agencies that have placed orders through MVCAC. UC Davis will accept and test mosquito pools only from those agencies that have placed orders through MVCAC.

### **2. Registration of sentinel flock sites and wing band numbers**

Prior to submitting any sentinel chicken blood samples to VRDL, each agency must register each new flock site with UC Davis using the “SURVEILLANCE SITE REGISTRATION” form MBVS-1 (revised 05/11/04). Blood samples sent to VRDL must be accompanied by the form “SENTINEL CHICKEN BLOOD – 2004” (MBVS-2, revised 12/9/2002) for each flock site.

Fill out a MBVS 2 form for each site and include a four digit numeric code for the site along with the wing band numbers of chickens placed at that site. Also include the date the chickens were bled. VRDL will cross check the agency and site code numbers before testing the samples.

VRDL will test samples only if they are accompanied by the appropriate 2004 form which includes the registered agency code (assigned by MVCAC), the registered site code (assigned by you), and, for blood samples, the wing band numbers assigned to that site.

### **3. Registration of mosquito sampling sites**

Registration of new sites used for collection of mosquitoes for virus testing may be accomplished by faxing a copy of the “SITE SURVEILLANCE REGISTRATION 2004” form to (530) 754-6360 (UC Davis) or e-mailing it to [bfeldridge@ucdavis.edu](mailto:bfeldridge@ucdavis.edu) at the same time the pools are shipped to UC Davis. UC will test the pools provided that adequate information is provided on the “MOSQUITO POOL SUBMISSION” form (MBVS-3, revised 12/19/01), including your agency code, your site code for the site and geographic coordinates. If you are unable to determine the geographic coordinates, please provide a map to UC Davis showing the location of each site and its site code.

The geographic coordinates will be used to generate computer maps that will show all registered sites and test results for each site each week. Also, as part of a collaborative effort, UCD will be generating up-to-date maps from the weekly results for posting at <http://vector.ucdavis.edu/>.

**4. Questions?** Please contact Al Hom, Vector-Borne Disease Section at (510) 412-6254 or [arbovirus@dhs.ca.gov](mailto:arbovirus@dhs.ca.gov).

**SURVEILLANCE SITE REGISTRATION 2004**  
**Please fax to 530-754-6360 or email to bfeldridge@ucdavis.edu**

**AGENCY CODE:** \_\_\_\_\_  
 (MUST BE 4 LETTERS, NO NUMBERS)

**SITE CODE:** \_\_\_\_\_  
 (MUST BE 4 NUMBERS, NO LETTERS)

\_\_\_\_\_  
 Agency Name

\_\_\_\_\_  
 County in which site located

Latitude \_\_\_\_\_ - \_\_\_\_\_ - \_\_\_\_\_  
 (Degrees, minutes, seconds)

Longitude \_\_\_\_\_ - \_\_\_\_\_ - \_\_\_\_\_  
 (Degrees, minutes, seconds)

Elevation (feet) \_\_\_\_\_

Address (if you have one) \_\_\_\_\_

Note: Please do not record coordinates in decimal form. If you need help in converting decimal coordinates to the degrees-minutes-seconds form, please send an inquiry by e-mail to Bruce Eldridge or Chris Barker.

Site is located \_\_\_\_\_ miles in a \_\_\_\_\_ Direction from \_\_\_\_\_  
 (nearest city or town)

**NAME OR NUMERICAL SITE DESIGNATION USED BY AGENCY:** \_\_\_\_\_  
 (e.g., Adohr Farms, or 234A. The use of a name for each site is highly recommended)

**SITE DESCRIPTION:**

Land Use (e.g., forest, farmland, urban, suburban, range land, etc.)

\_\_\_\_\_  
 Habitat (e.g., pond, vernal pool, back yard, river bank, etc.)

\_\_\_\_\_  
 Surroundings (e.g., school, homes, regional park, businesses, industrial sites)

\_\_\_\_\_  
 Other description

**SITE FUNCTIONS:**

**This site is used for (check all that apply – this is important information):**

- Sentinel chickens
- Mosquitoes for virus isolation
- Mosquitoes for abundance report
- Other (describe)

**Trap type(s) used** (e.g., NJLT, CDC-CO<sub>2</sub>, gravid trap, etc.) \_\_\_\_\_

If composite site, number of traps or other collection methods used \_\_\_\_\_

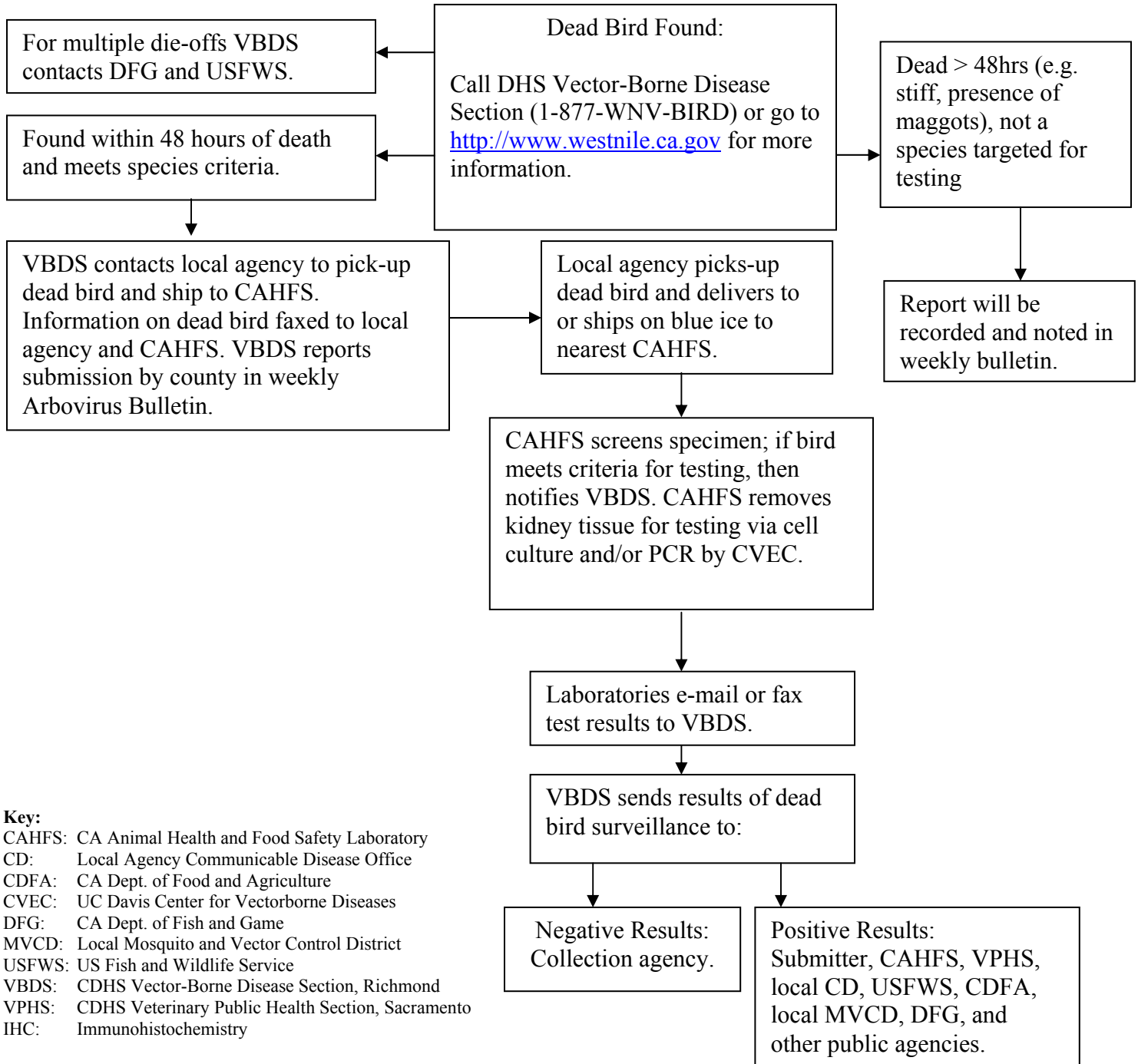
Form MBVS-1 (Revised 5/13/04)





**Appendix D: Procedures for Testing Dead Birds**

In 2000, DHS initiated a dead bird surveillance program in collaboration with other public agencies. DHS annually notifies about 600 agencies, organizations, and veterinarians involved with wildlife, including rehabilitation centers, about the program. Dead birds are reported to DHS, shipped to a California Animal Health & Food Safety Laboratory for screening and removal of kidney tissue, which is then sent to the UC Davis Center for Vectorborne Diseases (CVEC) for WN viral isolation. The general dead bird testing algorithm is provided below.



## Dead Bird Testing Algorithm with Incorporation of VecTest Screening April 2004

No WNV in region (and prior to July 1 in southern California)

- Ship dead birds (corvids, raptors) to CAHFS (no change to current protocol).
- If a local agency is already doing VecTest or RAMP on oral swabs from crows, then crows should still be shipped to CAHFS.
  - Any positive crow should be reported to the DHS hotline immediately. The suspect dead bird should be sent to CAHFS for necropsy and tissue collection, dry swab of oral cavity sent directly to CVEC for confirmatory testing<sup>1</sup>.
  - DHS should be notified by a weekly report of all birds tested locally via VecTest or RAMP. Information should include dead bird number or species, date, and address.
- Local agencies should forward a summary of all dead bird calls received if they are screened at the local level, whether or not the bird subsequently met the criteria for testing.

WNV in region<sup>2</sup> (or post-July 1 in southern California counties where WNV detected in 2003)

- CAHFS will begin taking a dry swab from oral cavity of dead birds; swab to be tested at CVEC via RT-PCR.
- DHS, in consultation with affected local agencies, may recommend:
  - Discontinue shipping crows and initiate VecTest for crows by local agency AND take dry swab to be shipped to CVEC. Report VecTest results weekly to DHS. Note: DHS will provide one VecTest kit per agency subsequent to WN detection.
  - Continue shipping other bird species to CAHFS.
- OR
  - Reduce or suspend testing of birds from a region. The decision to continue, modify, or suspend testing birds in a WNV positive region will depend in part on resource availability and the value of ongoing surveillance data to direct response activities. At the present time, adequate resources are anticipated to be available throughout the season.
- Local agencies conducting VecTest and RAMP should continue to send results to DHS in weekly reports. Immediately report any positive results to WNV hotline
- Local agencies should continue to forward a summary of all dead bird calls received if the calls are screened at the local level, whether or not the bird subsequently met the criteria for testing.

WNV in an adjacent county

- DHS may recommend that local agencies in regions adjacent to WNV activity initiate taking a dry swab of oral cavity on crows and sending the swab to CVEC.
- Agencies should continue to send all dead birds (including crows) to CAHFS.

<sup>1</sup> VecTest and RAMP have a lower sensitivity and specificity than RT-PCR. Thus it is critical that initial WNV positive birds from a region be confirmed by RT-PCR.

<sup>2</sup> The size / boundaries of a region will be discussed and determined on a case by case basis. (options: zip code, ecological zone, city, district, county etc)

***Dead Bird Submission Instructions for Local Agencies  
California West Nile Virus (WNV) Dead Bird Surveillance Program  
California Department of Health Services (DHS)  
Division of Communicable Disease Control***

**Dead Bird Reporting and Submission Instructions for Local Agencies**

When your agency receives a call from the public about a dead bird (especially recently dead crows, ravens, magpies, jays, or raptors), or one of your staff members finds a dead bird, please immediately refer them to the DHS Hotline at 1-877-WNV-BIRD (877-968-2473).

**DHS will assess the suitability of the dead bird for testing and contact your agency only if the bird is approved for pickup. The WNV Hotline is monitored 8am-4pm Monday through Friday. Any dead birds sent without prior notification will not be tested.**

Once the dead bird submission is approved, DHS will arrange for the pick-up of the carcass to be shipped from your agency to the nearest California Animal Health and Food Safety Laboratory (CAHFS). Dead birds delivered to CAHFS Turlock and Fresno laboratories will then be transported by CAHFS to Davis. In 2004, all WNV testing will be conducted at the UC Davis Center for Vectorborne Diseases(CVEC). CAHFS will remove specific tissues and forward them to CVEC for viral assay. Shipping and testing expenses will be paid by DHS.

To ensure the proper condition of specimen for testing and to comply with regulations for shipping diagnostic specimens, please follow these instructions.

**Bird Carcasses**

- Only dead birds can be picked up according to our permit.
- Do not touch the carcass with bare hands: wear rubber or latex gloves when picking up and handling it. If gloves are not available, use a plastic bag turned inside out over your hand, and invert the bag to surround the bird.
- Only agencies listed under the permit issued to DHS from the California Department of Fish and Game and U.S. Fish and Wildlife Service are authorized to pick up dead birds. The agencies covered include local mosquito abatement districts, some environmental health departments, and other designated agencies.
- **Collect recently dead birds.** Badly decomposed or scavenged carcasses are of limited diagnostic value. Signs that a bird has been dead for too long (over 48 hours) are the presence of maggots, an extremely light weight carcass, missing

eyes, skin discoloration, skin or feathers rub off easily, strong odor, or a soft, mushy carcass.

- **If upon pick-up the carcass is found to be unacceptable (wrong species or badly decomposed), please collect the bird and dispose of it by placing it inside a double bag (tie or zip lock) and place it in a secure garbage can or dumpster. Immediately call DHS and notify them that the bird will no longer be tested so that we can remove the bird from the “submitted” category.**
- Place each bird carcass into a plastic bag and secure it inside a second plastic bag and zip lock it shut. **Double bagging prevents cross contamination and leakage. There should always be two bags separating the bird from documents/ labels that accompany it during shipping.**
- **Pack the bird carcass with blue ice packs.** An absorbent material, such as newspaper, must be included in the box to prevent any leakage from the box in accordance with shipping regulations.
- **Enclose the shipping document into a SEPARATE ZIP-LOCK BAG. Information includes a return-address label, so your box can be returned, and a copy of the dead bird submission form (with the dead bird number) faxed by DHS.** CAHFS prefers you put this separate zip-lock bag inside the outer bag containing the dead bird.
- Ship the bird carcass in a hard-sided plastic cooler or a styrofoam cooler placed in a cardboard box. If there is space between the Styrofoam cooler and cardboard box, fill the space with wadded newspaper. Unprotected styrofoam containers may break into pieces during shipment. **Notify DHS to arrange for carrier pickup to ship Monday through Thursday. This guarantees arrival at CAHFS before the weekend.**
- Birds that need to be stored over the **weekend** should be put on dry ice or stored at -70°C. Freezing the carcass lessens the quality of tissue samples. **Refrigerating** the carcass is recommended for **overnight storage** (this slows virus deterioration, but does not stop it). **DO NOT store the carcass in a regular freezer** (usually -20°) at any time.
- Label the outside of the package with the words **Diagnostic Specimens ATTN: WNV** above the designated CAHFS address.

#### **For Veterinarians:**

If your laboratory will be performing necropsies, please ship the following tissues in a pooled sample: brain, kidney, heart and spleen. DHS prefers tissues be sent in cryovials on dry ice in a hard plastic or Styrofoam container placed in a cardboard box. If cryovials are not available, use any screw cap tubes. If dry ice is not available, please

use wet ice in shipping container. If using wet ice, please ensure that samples are contained in watertight tubes so that water from melting ice does not leak into tissues. To avoid crushing specimen during shipping, please wrap specimen containers in sufficient padding such as bubble wrap.

Contact information for the California Animal Health and Food Safety Laboratory:

CAHFS Central Laboratory (530) 752-8709  
ATTN: WNV  
Dr. Leslie Woods  
University of California, Davis  
West Health Science Drive  
Davis, CA 95616

CAHFS San Bernardino (909) 383-4287  
ATTN: WNV  
Dr. Deryck Read  
105 West Central Avenue  
San Bernardino, CA 92412

## Appendix E: Procedures for Testing Equines and Ratites

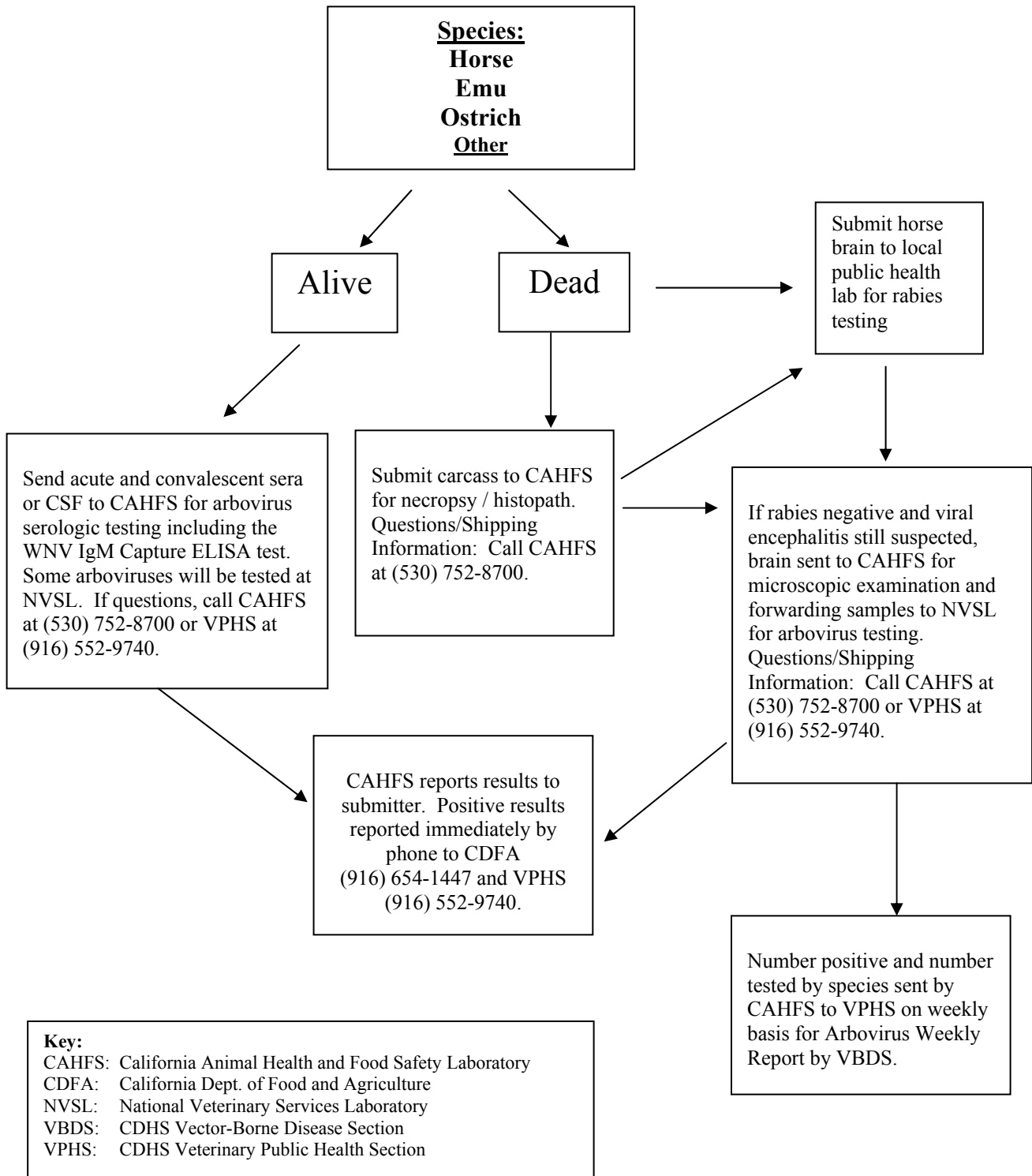
The California Department of Health Services (DHS) and the California Department of Food and Agriculture (CDFA) have a well-established passive surveillance program for equine and ratite encephalomyelitis. Equine encephalomyelitis is legally reportable to CDFA by veterinarians and diagnostic laboratories pursuant to Section 9101 of the Food and Agricultural Code. Venezuelan equine encephalitis and West Nile virus are emergency animal diseases that must be reported to CDFA by telephone within 24 hours.

This appendix contains a copy of the mailing 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 encephalomyelitis and instructions for specimen collection and submission for both equine and ratite samples. The mailing 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) five regional branches, the University of California at Davis (UCD) School of Veterinary Medicine's Veterinary Medical Teaching Hospital, and other laboratories or individual veterinarians. Equine serum 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 forwarding to the National Veterinary Services Laboratories (NVSL) for arbovirus testing. All fatal cases of equine encephalitis are first tested for rabies at the local public health laboratory. An algorithm outlining the protocol for specimen submission and reporting is available for participants in the program.

Outreach is an important component of the program. DHS and CDFA have developed and distributed educational materials concerning the diagnosis and reporting of arboviruses in equines and ratites. DHS and CDFA work closely with equine veterinary referral centers, the California Horse Racing Board, and other interested parties to improve surveillance and reporting of suspect cases of equine and ratite encephalomyelitis.

Additional information on WN virus for veterinarians, horse owners, and ratite owners, is available at CDFA's website: [http://www.cdfa.ca.gov/ahfss/ah/wnv\\_info.htm](http://www.cdfa.ca.gov/ahfss/ah/wnv_info.htm). A brochure containing facts about California WNV surveillance and general information about prevention and control is available at DHS's website: <http://www.westnile.ca.gov>.

## Algorithm for Submission of Specimens from Domestic Animals with Neurologic Symptoms



## STATE OF CALIFORNIA

DEPARTMENT OF FOOD AND AGRICULTURE  
ANIMAL HEALTH AND FOOD SAFETY SERVICES  
ANIMAL HEALTH BRANCH  
1220 N STREET, ROOM A-107  
SACRAMENTO, CA 95814  
(916) 654-1447

DEPARTMENT OF HEALTH SERVICES  
DIVISION OF COMMUNICABLE DISEASE  
VETERINARY PUBLIC HEALTH SECTION  
MS 7308  
P.O. Box 997413  
SACRAMENTO, CA 95899-7413  
(916) 552-9740

April 2004

To: California Veterinarians in Large Animal Practice  
Public Health Laboratory Directors  
Local Health Officers  
Public Health Veterinarians  
Animal Health Branch Personnel  
Interested Parties

**Subject: SURVEILLANCE AND REPORTING OF ARBOVIRAL  
ENCEPHALITIS VIRUSES IN HORSES AND RATITES**

The California Department of Health Services (DHS) and the California Department of Food and Agriculture (CDFA) **will again supplement the costs (as available funding permits) of diagnostic testing on clinically affected horses for arboviral encephalitis viruses.** These include western equine encephalitis (WEE), eastern equine encephalitis (EEE), and West Nile Virus (WNV). These diseases may affect humans, horses, ratites (ostriches, emus, rheas, etc.), and other birds and mammals. Your continued support of the surveillance program in California is important to both human and animal health. **Equine specimen submission instructions are provided in Attachment A.** Ratite specimen submissions should be coordinated through a California Animal Health and Food Safety (CAHFS)\* Laboratory in your area (see attachment).

Veterinarians are often the first to detect the emergence of zoonotic diseases such as WNV. There were 4,426 equine clinical cases of WNV reported in the United States in 2003. Mortality (dead or euthanized horses) was approximately 30%. The first case of California acquired equine WNV was confirmed during October in San Diego County. There was significant detection of WNV in sentinel chickens and mosquito pools in five other counties in Southern California, and the virus has been detected in Southern California birds this year. The Centers for Disease Control and Prevention has predicted that California will be the epicenter for WNV in 2004. A significant level of WEE virus activity in sentinel chickens and mosquitoes has also been observed in recent years. Therefore, continued vigilance on the part of veterinarians, public health officials, and animal keepers is critical.

A comprehensive vaccination and mosquito-control program should be developed in conjunction with a veterinary practitioner if there is any possibility that horses will be exposed to mosquitoes. The original killed virus vaccine is now available as a combination vaccine



Surveillance and Reporting of Arboviral Encephalitis Viruses in Horses and Rattles

Page Two

April 2004

product that includes EEE, WEE, and tetanus. Additionally, a new “recombinant DNA vector virus vaccine” has recently been introduced.

Vaccination provides no benefit after a horse has developed encephalitis. Combination equine encephalitis vaccines should not be used in suspect cases because vaccination titers may interfere with diagnostic tests. Accurate vaccination records should be maintained because it may be important to distinguish between vaccinated and exposed or infected horses. In addition, international shipments of horses with WNV titers may be restricted.

In addition to vaccination, it cannot be overemphasized that the ultimate prevention for WNV, WEE, and EEE includes eliminating or drastically minimizing the mosquito exposure to the horse. This involves the elimination of mosquitoes and their breeding grounds in standing stagnant water. Approved mosquito repellants are also indicated if exposure is unavoidable.

Your participation in this important public health program is greatly appreciated. For more information on WNV and other equine encephalitis viruses, please visit our Web site at <http://www.westnile.ca.gov>. If you require additional information, please contact your CDFA, Animal Health Branch District Office (see attachment) or the Veterinary Public Health Section of DHS at (916) 552-9740.

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Kenneth L. Thomazin, D.V.M.  
Chief  
Animal Health Branch

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Ben Sun, D.V.M., M.P.V.M.  
Acting Chief  
Veterinary Public Health Section

Attachments

cc: Alex Ardans, D.V.M., M.S., Director  
California Animal Health and Food Safety Laboratory System

\* See CAHFS attachment for locations and addresses

## SURVEILLANCE CASE DEFINITIONS FOR WEST NILE VIRUS DISEASE IN EQUINES - 2004

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

### **SUSPECT CLINICAL CASE<sup>4</sup>:**

- Compatible clinical signs

### **EXPOSED EQUID:**

- Detection of IgM antibody to WN virus 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 WN virus infection in a vaccinated horse.
- IgM antibody in equine serum is relatively short-lived; a positive IgM-capture ELISA means exposure to WN virus or rarely a closely related flavivirus (SLE) has occurred, very likely within the last three months.
- Neutralizing antibody, as detected by PRNT, may not be present in equine serum until two weeks or more after exposure to WN virus; it is possible that clinical signs may be present in an equine before a serum PRNT is positive.
- Neutralizing antibody detected in serum by PRNT can indicate past exposure to WN virus; equines naturally exposed to WN virus prior to 2002 may test positive for neutralizing antibody by PRNT.

<sup>1</sup> Preferred diagnostic tissues are equine brain or spinal cord; although tissues may include blood or CSF, the only known reports of WN virus isolation or positive PCR from equine blood or CSF have been related to experimentally infected animals.

<sup>2</sup> 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.

<sup>3</sup> 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)

<sup>4</sup> An equine case classified as a suspect case should, if possible, undergo further diagnostic testing to confirm or rule out WN virus as the cause of the clinical illness.

Protocol for Submission of Laboratory Specimens for  
Equine Neurological Disease Diagnosis and Surveillance  
April 2004

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.

B. Brain

- The local health department and 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 Arboviral 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\*.

2. **Submission forms:** Complete and include the transmittal forms supplied by the CAHFS. See attached sample or download the form from their Website: <http://cahfs.ucdavis.edu> The submittal form for each specimen should be placed in a leak-proof plastic bag and attached to the corresponding container.

3. **Shipment:** Check with the CAHFS Laboratory in your area for assistance with shipping regulations governing the transportation of infectious materials.

\* See CAHFS attachment for locations and addresses

## Appendix F: Protocol for Submission of Laboratory Specimens for Human Disease and Surveillance

**WNV testing is recommended on individuals with the following:**

- A. *Viral Encephalitis*
- B. *Aseptic meningitis* (individuals  $\geq 18$  years of age)
- C. *Acute Flaccid Paralysis/Atypical Guillain-Barré Syndrome/Transverse Myelitis*
- D. *Febrile illness*\*:

- Illness compatible with West Nile fever and lasting  $\geq 7$  days
- Must be seen by a health care provider.

*The West Nile fever syndrome can be variable and often includes headache and fever ( $T \geq 38^{\circ}\text{C}$ ). 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.*

- E. *Aseptic Meningitis (individuals  $< 18$  years of age)*\*:
  - After workup for enteroviruses (e.g. CSF PCR, throat or stool isolation)

*\* Identification of human cases is important early in the West Nile virus season to assess the burden of human illness and will be important to target mosquito control and public education activities to reduce exposure risk. Depending on the volume of tests requested and laboratory capacity, the local public health department may discontinue testing of individuals that fall into category (D) and (E) once West Nile virus is well-established in the area.*

### Instructions for Sending Specimens

#### 1. Required

- Acute Serum** -  $\geq 2\text{cc}$  serum collected  $\leq 7$  days after onset
  - Cerebral Spinal Fluid** – 1-2cc CSF *if lumbar puncture is performed*
2. If West Nile is highly suspected and acute serum is negative
- 2<sup>nd</sup> Serum** -  $\geq 2$  cc serum collected 3-5 days after the 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

(Modified from: “CDC. Epidemic/Epizootic West Nile Virus in the United States: Guidelines for Surveillance, Prevention, and Control” at [www.cdc.gov/ncidod/dvbid/westnile/publications.htm](http://www.cdc.gov/ncidod/dvbid/westnile/publications.htm))

### Clinical Description:

Arboviral infections may be asymptomatic or may result in illnesses of variable severity sometimes associated with central nervous system (CNS) involvement. When the CNS is affected, clinical syndromes include aseptic meningitis, myelitides and encephalitis, which are clinically indistinguishable from similar syndromes. Arboviral meningitis is characterized by fever, headache, stiff neck, and pleocytosis in cerebral spinal fluid. Arboviral myelitis is usually characterized by fever and acute bulbar or limb paresis or flaccid paralysis. Arboviral encephalitis is 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.

West Nile fever is a non-specific, self-limited, febrile illness with fever, headache, arthralgias, myalgias, and sometimes accompanied by skin rash or lymphadenopathy. Overlap among the various clinical syndromes is common.

### Case Classification:

A clinically compatible illness, *plus*:

#### Confirmed:

- ❑ Serum enzyme immunoassay (EIA) for virus-specific immunoglobulin M (IgM) and confirmed by demonstration of virus-specific serum immunoglobulin G (IgG) antibodies in the same or a later specimen by plaque reduction neutralization (PRNT), or
- ❑ Fourfold or greater change in virus-specific antibody titer, or
- ❑ Virus-specific immunoglobulin M (IgM) antibodies demonstrated in CSF by antibody-capture EIA, or
- ❑ Isolation of virus from or demonstration of specific viral antigen or genomic sequences in tissue, blood, cerebrospinal fluid (CSF), or other body fluid.

#### Probable:

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

**Please contact CDHS at (510) 307-8606 for questions regarding case classification.**

## 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/pesticides/factsheets/skeeters.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. Difluorobenzamide (e.g. Dimilin®25W)  
Use: Impounded tailwater, 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 *Cx. tarsalis* populations in the Central Valley are resistant to 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)

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, permethrin, resmethrin or sumithrin: e.g. Suspend® SC, Aqua-Reslin®, Scourge® Insecticide, Anvil® 10+10 ULV)

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 (as of 4/20/04)

Active Ingredient	Trade name	EPA Reg. No.	Mfgr.	Formulation	Application	Pesticide classification
<i>Bacillus sphaericus</i> , (Bs)	VectoLex CG	275-77	Valent BioSciences	Granule	Larvae	Biorational
<i>Bacillus sphaericus</i> , (Bs)	VectoLex WDG	73049-57	Valent BioSciences	Water dispersible granule	Larvae	Biorational
<i>Bacillus sphaericus</i> , (Bs)	VectoLex WSP	73049-20	Valent BioSciences	Water soluble packet	Larvae	Biorational
<i>Bacillus thuringiensis</i> var. <i>israelensis</i> (Bti)	VectoBac 12AS	73049-38	Valent BioSciences	Liquid	Larvae	Biorational
<i>Bacillus thuringiensis</i> var. <i>israelensis</i> (Bti)	VectoBac G	275-50 or 73049-10	Valent BioSciences	Granule	Larvae	Biorational
<i>Bacillus thuringiensis</i> var. <i>israelensis</i> (Bti)	VectoBac Tech. Powder	73049-13	Valent BioSciences	Technical powder	Larvae	Biorational
<i>Bacillus thuringiensis</i> var. <i>israelensis</i> (Bti)	Aquabac 200G	62637-3	Becker Microbial	Granule	Larvae	Biorational
<i>Bacillus thuringiensis</i> var. <i>israelensis</i> (Bti)	Bactimos Briquets	6218-47	Summit	Donut-style briquets	Larvae	Biorational
<i>Bacillus thuringiensis</i> var. <i>israelensis</i> (Bti)	Teknar HP-D	73049-404	Valent BioSciences	Liquid	Larvae	Biorational
Monomolecular film	Agnique MMF	2302-14	Henkel Corp.	Liquid	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

### Adukticides (4/20/04)

Active Ingredient	Trade name	EPA Reg. No.	Mfgr.	Formulation	Application	Pesticide classification
Malathion	Fyfanon® ULV	4787-8	Cheminova	Liquid	Adults	OP
Naled	Dibrom® Concentrate	5481-480	AMVAC	Liquid	Adults	OP
Naled	Trumpet™ EC	5481-481	AMVAC	Liquid	Adults	OP
Deltamethrin	Suspend® SC	432-763	Aventis	Liquid	Adults	Pyrethroid
Permethrin	Aqua-Reslin®	432-796	Aventis	Liquid	Adults	Pyrethroid
Permethrin	Biomist® 4+12 ULV	8329-34	Clarke	Liquid	Adults	Pyrethroid
Permethrin	Permanone® Ready-To-Use	432-1182	Aventis	Liquid	Adults	Pyrethroid
Pyrethrins	Pyranone® 25-5	432-1050	Aventis	Liquid	Adults	Pyrethroid
Pyrethrins	Pyrenone® Crop Spray	432-1033	Aventis	Liquid	Adults	Pyrethroid
Pyrethrins	Pyrocide® 7396	1021-1569	MGK	Liquid	Adults	Pyrethroid
Resmethrin	Scourge® Insecticide (4%)	432-716	Aventis	Liquid	Adults	Pyrethroid
Resmethrin	Scourge® Insecticide (18%)	432-667	Aventis	Liquid	Adults	Pyrethroid
Sumithrin	Anvil® 10+10 ULV	1021-1688-8329	Clarke	Liquid	Adults	Pyrethroid

**Appendix I. Websites Related to Weather Conditions and Forecasts, Crop Acreage and Production, Mosquito Control, and Arbovirus Surveillance In California**

<b>Website</b>	<b>URL</b>	<b>Available information</b>
California Data Exchange Center	<a href="http://cdec.water.ca.gov">http://cdec.water.ca.gov</a>	Water-related data from the California Department of Water Resources, including historical and current streamflow, snowpack, and precipitation information.
UC IPM Online	<a href="http://www.ipm.ucdavis.edu">http://www.ipm.ucdavis.edu</a>	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	<a href="http://www.cpc.ncep.noaa.gov/products/predictions/">http://www.cpc.ncep.noaa.gov/products/predictions/</a>	Short-range (daily) to long-range (seasonal) temperature and precipitation forecasts. Also provides El Niño-related forecasts.
California Agricultural Statistics Service	<a href="http://www.nass.usda.gov/ca/">http://www.nass.usda.gov/ca/</a>	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.
West Nile Virus – California Department of Health Services	<a href="http://westnile.ca.gov">http://westnile.ca.gov</a>	Online dead bird reporting, bird identification charts, mosquito control, current information on the location of West Nile virus in California, local agency information, and other related issues.
UC Davis Center for Vectorborne Diseases	<a href="http://cvec.ucdavis.edu/">http://cvec.ucdavis.edu/</a>	Frequently updated reports and interactive maps on arbovirus surveillance and mosquito occurrence in California.
Mosquito and Vector Control Association of California	<a href="http://www.mvcac.org">http://www.mvcac.org</a>	News, membership information, event calendars, and other topics of interest to California’s mosquito control agencies.