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 virus (WNV) in New York, a virus not recognized in the Western Hemisphere prior to 1999, prompted the review and enhancement of existing guidelines to ensure that surveillance, prevention, and control activities were appropriate for WNV. From New York, WNV spread rapidly westward and by 2004 had been detected in 48 of the United States, including California. In addition to WNV, 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).

Background

Mosquito-borne viruses belong to a group of viruses commonly referred to as arboviruses (for **ar**thropod-**bo**rne). Although 12 mosquito-borne viruses are known to occur in California, only WNV, western equine encephalomyelitis virus (WEE) and St. Louis encephalitis virus (SLE) are significant causes of human disease. WNV is having a serious impact upon the health of humans, horses, and wild birds as it becomes established statewide. In 2004, there were 830 WNV human infections with 28 deaths, and 540 horse cases (230 died or were euthanized). Consequently, the California Arbovirus Surveillance Program emphasizes forecasting and monitoring the temporal and spatial activity of WNV, WEE, and SLE. These viruses are maintained in 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 *Culex* mosquitoes, such as 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 *Cx. quinquefasciatus*, play an important role in WNV, 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 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 WNV viruses affects elderly people most seriously. WEE and WNV can be an important disease in horses and emus, and kills a wide variety of endemic and imported birds. There are WEE and WNV 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 WNV and to request appropriate laboratory tests. Public health officials need to be alerted if a mosquito-borne viral disease is detected, especially if the public health risk is high.

Surveillance

Surveillance includes the monitoring of climatic factors, estimating immature and adult mosquito abundance, and assessing virus activity by testing mosquitoes, sentinel chickens, wild birds (including dead birds for WNV), horses, and humans for evidence of infection. Surveillance must focus not only on mosquito-borne viruses known to exist in California, but be sufficiently broad to also detect newly introduced viruses.

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 WNV, surveillance efforts emphasize the testing of this species. Other species that should be tested, especially for WNV and WEE, include *Culex quinquefasciatus, Culex pipiens*, 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 Vectorborne Diseases (CVEC). Procedures for processing mosquitoes for virus infection are detailed in Appendix B. The current surveillance system is designed to detect WNV 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 arboviral transmission in bird populations can be accomplished by 1) testing dead birds for WNV, 2) using caged chickens as sentinels and bleeding them routinely to detect viral antibodies (seroconversions), and 3) collecting and bleeding wild birds to detect viral antibodies. 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 WNV. Some agencies conduct their own testing, but send positive samples to DHS for confirmation and official reporting. Because SLE cross-reacts with WNV in antibody testing, sera drawn from SLE or WNV positive chickens are confirmed 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, WNV frequently causes death in North American birds, especially those in the family Corvidae (e.g. crows, ravens, magpies, jays). Dead bird surveillance was initiated by DHS in 2000 to provide early detection of WNV. Dead bird surveillance has been shown to be one of the earliest indicators of WNV activity in a new area,

and in 2004 the dead bird surveillance program detected the presence of WNV before other surveillance elements in 53 of California's 58 counties. Birds that meet certain criteria are tested for WNV through collaboration with many local, state, and federal agencies. In 2004, a total of 93,057 dead birds were reported to DHS' dead bird hotline (1-877-WNV-BIRD) and website http://westnile.ca.gov. Of the 5,728 birds that were tested, 3,232 tested positive for WNV. 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 WNV as horse owners begin vaccinating to protect their horses. If confirmed cases do occur, it is a strong indication that WEE or WNV is active in that region of the State. Veterinarians are contacted annually by DHS and the California Department of Agriculture (CDFA) to advocate equine vaccination and to describe diagnostic services that are available in the event of a suspected case of WEE or WNV encephalitis. Other mosquito-borne viruses may also cause encephalitis in horses; 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 WNV, 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 WNV 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 WNV. For patients with extensive mosquito exposure in which SLE, WEE, and WNV 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 WNV. Positive specimens can be submitted to the VRDL for confirmation. Physicians are required to report viral encephalitis, viral meningitis, and WNV cases to their local health department. Laboratories are also required to report cases of arboviral encephalitis and WNV (Title 17 Sections 2500 and 2505). Cases that are confirmed to be due to WNV, 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 WNV 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 WNV 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 infection rate in mosquitoes
4. Sentinel chicken seroconversions
5. Fatal infections in birds
6. Infections in equids and ratites (e.g. emus and ostriches)
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. 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., dead birds for WNV, 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. SLE and WNV transmission risk increase with increasing temperature. Because equine infections with SLE do not result in disease, equine cases are not included in the SLE risk assessment. Abundance and infection of the *Culex pipiens* complex are included in both SLE and WNV 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 WNV calculations.

Table 1. Mosquito-borne Virus Risk Assessment

WEE Surveillance Factor	Assessment Value	Benchmark	Assigned Value
1. **Environmental Conditions** 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	
2**. Adult *Culex tarsalis and Ochlerotatus melanimon*** (bridge vector) **abundance** Determined by trapping adults, identifying them to species, and comparing numbers to averages previously documented for an area for current time period.	1	*Cx. tarsalis* abundance well below average (<50%)	
	2	*Cx. tarsalis* abundance below average (50-90%)	
	3	*Cx. tarsalis* abundance average (90-150%)	
	4	*Cx. tarsalis* and *Oc. melanimon* abundance above average (150-300%)	
	5	*Cx. tarsalis and Oc. melanimon* abundance well above average (>300%)	
3. **Virus infection rate in *Cx. tarsalis and Oc. melanimon* mosquitoes** Tested in pools of 50. Test results expressed as minimum infection rate (MIR) per 1,000 female mosquitoes tested (or per 20 pools).	1	*Cx. tarsalis* MIR / 1000 = 0	
	2	*Cx. tarsalis* MIR / 1000 = 0 - 1.0	
	3	*Cx. tarsalis* MIR / 1000 = 1.1 - 2.0	
	4	*Cx. tarsalis* MIR / 1000 = 2.1 - 5.0 and/or *Oc. melanimon* MIR/1000 > 0	
	5	*Cx. tarsalis* MIR / 1000 > 5.0 and *Oc. melanimon* MIR/1000 >0	
4. **Sentinel chicken seroconversion** 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 per flock.	1	No seroconversions	
	2	One seroconversion in single flock over broad region	
	3	One to two seroconversions in a single flock in specific region	
	4	More than two seroconversions in single flock or one to two seroconversions in multiple flocks in specific region	
	5	More than two seroconversions per flock in multiple flocks in specific region	
5. **Infections in equines or ratites**	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	
6. **Human cases**	1	No human cases	
	3	One human case in broad region	
	4	One human case in specific region	
	5	More than one human case in specific region	
7. **Proximity to urban or suburban regions** (score only if virus activity detected) Risk of outbreak is highest in urban areas because of high likelihood of contact between humans and vectors.	1	Virus detected in remote area	
	2	Virus detected in rural areas	
	3	Virus detected in small towns	
	4	Virus detected in suburban areas	
	5	Virus detected 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)		**TOTAL**	
		AVERAGE	

SLE Surveillance Factor	Assessment Value	Benchmark	Assigned Value
1. **Environmental Conditions** 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. Temperature data link: http://www.ipm.ucdavis.edu:/WEATHER/wxretrieve.html	1	Avg daily temperature during preceding month <56 ^{o} F	
	2	Avg daily temperature during preceding month 57-65^{o} F	
	3	Avg daily temperature during preceding month 66-74^{o} F	
	4	Avg daily temperature during preceding month 75-83 ^{o} F	
	5	Avg daily temperature during preceding month >83 ^{o} F	
2. **Adult *Culex tarsalis* or *pipiens complex* abundance** Determined by trapping adults, identifying them to species, and comparing numbers to those previously documented for an area for current time period.	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%)	
3. **Virus infection rate in *Culex tarsalis* and *Cx. pipiens complex* mosquitoes** 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	
4. **Sentinel chicken seroconversion** 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 per flock.	1	No seroconversions	
	2	One seroconversion in single flock over broad region	
	3	One to two seroconversions in a single flock in specific region	
	4	More than two seroconversions in single flock or one to two seroconversions in multiple flocks in specific region	
	5	More than two seroconversions per flock in multiple flocks in specific region	
5. **Human cases**	1	No human cases	
	3	One human case in broad region	
	4	One human case in specific region	
	5	More than one human case in specific region	
6. **Proximity to urban or suburban regions** (score only if virus activity detected) Risk of outbreak is highest in urban areas because of high likelihood of contact between humans and vectors.	1	Virus detected in remote area	
	2	Virus detected in rural areas	
	3	Virus detected in small towns	
	4	Virus detected in suburban areas	
	5	Virus detected in urban area	
Response Level / Average Rating: Normal Season (1.0 to 2.5) Emergency Planning (2.6 to 4.0) Epidemic (4.1 to 5.0)		**TOTAL**	
		AVERAGE	

WNV Surveillance Factor	Assessment Value	Benchmark	Assigned Value
1. **Environmental Conditions**. Rural transmission may favor El Niño conditions, whereas urban transmission may favor La Niña conditions. Temperature data link: http://www.ipm.ucdavis.edu:/WEATHER/wxretrieve.html	1	Avg daily temperature during preceding month <56 ^{o} F	
	2	Avg daily temperature during preceding month 57-65^{o} F	
	3	Avg daily temperature during preceding month 66-74^{o} F	
	4	Avg daily temperature during preceding month 75-83 ^{o} F	
	5	Avg daily temperature during preceding month >83 ^{o} F	
2. **Adult *Culex tarsalis* and *Cx. pipiens complex* abundance** Determined by trapping adults, identifying them to species, and comparing numbers to those previously documented for an area for current time period.	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%)	
3. **Virus infection rate in *Culex tarsalis* and *Cx. pipiens* complex mosquitoes** 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	
4. **Sentinel chicken seroconversion** Number of chickens in a flock that develop antibodies to WNV. If more than one flock is present in a region, number of flocks with seropositive chickens is an additional consideration. Typically 10 chickens per flock.	1	No seroconversions	
	2	One seroconversion in single flock over broad region	
	3	One to two seroconversions in a single flock in specific region	
	4	More than two seroconversions in single flock or one to two seroconversions in multiple flocks in specific region	
	5	More than two seroconversions per flock in multiple flocks in specific region	
5. **Dead bird infection** Includes zoo collections.	1	No WNV positive dead birds	
	2	One WNV positive dead bird in broad region	
	3	One WNV positive dead bird in specific region	
	4	Two to five WNV positive dead birds in specific region	
	5	More than five WNV positive dead birds and multiple reports of dead birds in specific region	
6. **Equine cases**	1	No equine cases	
	3	One equine case in broad region	
	4	One or two equine cases in specific region	
	5	More than two equine cases in specific region	
7. **Human cases**	1	No human cases	
	3	One human case in broad region	
	4	One human case in specific region	
	5	More than one human case in specific region	
8. **Proximity to urban or suburban regions** (score only if virus activity detected) Risk of outbreak is highest in urban areas because of high likelihood of contact between humans and vectors.	1	Virus detected in remote area	
	2	Virus detected in rural areas	
	3	Virus detected in small towns	
	4	Virus detected in suburban areas	
	5	Virus detected in urban area	
Response Level / Average Rating: Normal Season (1.0 to 2.5) Emergency Planning (2.6 to 4.0) Epidemic (4.1 to 5.0)		**TOTAL**	
		AVERAGE	

Characterization of Conditions and Responses

Level 1: Normal Season

Risk rating: 1.0 to 2.5

CONDITIONS
• Average or below average snowpack and rainfall; average seasonal temperatures • Mosquito abundance at or below five year average (key indicator = adults of vector species) • No virus infection detected in mosquitoes • No seroconversions in sentinel chickens • No WNV infected dead birds • No equine cases • No human cases
RESPONSE
• Conduct routine public education (eliminate standing water around homes, use personal protection measures) • Conduct routine mosquito and virus surveillance activities • Conduct routine mosquito larval control • Inventory pesticides and equipment • Evaluate pesticide resistance in vector species • Ensure adequate emergency funding • Release routine press notices • Send routine notifications to physicians and veterinarians • Establish and maintain routine communication with local office of emergency services personnel; obtain Standardized Emergency Management System (SEMS) training

Level 2: Emergency Planning

Risk rating: 2.6 to 4.0

CONDITIONS
• Snowpack and rainfall and/or temperature above average • Adult mosquito abundance greater than 5-year average (150% to 300%) • One or more virus infections detected in mosquitoes (MIR / 1000 is <5) • One or more seroconversions in single flock or one to two seroconversions in multiple flocks in specific region • One to five WNV positive dead birds in specific region • One or two equine cases in region • One human case in region • Virus detection in small towns or suburban area
RESPONSE
• Review epidemic response plan • Enhance public education (include messages on the signs and symptoms of encephalitis; seek medical care if needed; inform public about pesticide applications if appropriate) • Enhance information to public health providers • Conduct epidemiological investigations of cases of equine or human disease • Increase surveillance and control of mosquito larvae • Increase adult mosquito surveillance • Increase number of mosquito pools tested for virus • Conduct localized chemical control of adult mosquitoes • Contact commercial applicators in anticipation of large scale adulticiding • Review candidate pesticides for availability and susceptibility of vector mosquito species • Ensure notification of key agencies of presence of viral activity, including the local office of emergency services

Level 3: Epidemic Conditions

Risk rating: 4.1 to 5.0

CONDITIONS
• Snowpack, rainfall, and water release rates from flood control dams and/or temperature well above average • Adult vector population extremely high (>300%) • Virus infections detected in multiple pools of mosquitoes (MIR / 1000 > 5.0) • More than two seroconversions per flock in multiple flocks in specific region • More than five WNV positive dead birds and multiple reports of dead birds in specific region • More than two equine cases in specific region • More than one human case in specific region • Virus detection in urban or suburban areas
RESPONSE
• Conduct full scale media campaign • Alert physicians and veterinarians • Conduct active human case detection • Conduct epidemiological investigations of cases of equine or human disease • Continue enhanced larval surveillance and control of immature mosquitoes • Broaden geographic coverage of adult mosquito surveillance • Accelerate adult mosquito control if appropriate • Coordinate the response with the local Office of Emergency Services or if activated, the Emergency Operation Center (EOC) • Initiate mosquito surveillance and control in geographic regions without an organized vector control program • Determine whether declaration of a local emergency should be considered by the County Board of Supervisors (or Local Health Officer) • Determine whether declaration of a "State of Emergency" should be considered by the Governor at the request of designated county or city officials • Ensure state funds and resources are available to assist local agencies at their request • Determine whether to activate a Standardized Emergency Management System (SEMS) plan at the local or state level • Continue mosquito education and control programs until mosquito abundance is substantially reduced and no additional human cases are detected

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

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

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 WNV testing, or test corvid species via rapid screening assay.
- Immediately notify the dead bird hotline of any birds that test positive via the VecTest or RAMP rapid screening tests.
- Update DHS weekly of all birds that are independently reported and/or tested (email: arbovirus@dhs.ca.gov).
- 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 WNV information and dead bird reporting hotline, 1-877-WNV-BIRD, and a WNV website: http://westnile.ca.gov/
- Coordinate submission of specimens for virus testing.
- 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 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 WNV and testing facilities available at UCD Center for Vectorborne Disease Research.
- Provide outreach to general public and livestock and poultry producers on the monitoring and reporting of equine and ratite encephalitides.
- Facilitate equine and ratite sample submission from the field.
- Conduct epidemiological investigations of equine cases.

California Animal Health and Food Safety Laboratory
- Identify and screen dead birds for WNV testing.
- Conduct necropsies and testing on dead birds.
- Submit bird tissues to UCD for testing.
- Test equine specimens for WNV.

Local Health Departments and Public Health Laboratories
- Test human specimens for WNV.
- 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.
- Test dead birds via rapid assay or PCR as resources allow.
- Participate in emergency response.
- Conduct epidemiological investigations of cases of human disease.
- Report WNV cases to DHS.
- Conduct 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.

Federal Centers for Disease Control and Prevention
- Provide consultation to state and local agencies in California if epidemic conditions exist.
- Provide national surveillance data to state health departments.

References

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

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

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

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

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

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

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

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

Reeves, W.C. 2000. The threat of exotic arbovirus introductions into California. Proc. Calif. Mosq. Vector Control Assoc. 68: (in press).

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

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

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

Appendix A: Guidelines for Adult Mosquito Surveillance

The objective of Appendix A is to standardize 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 guidelines recommend stratifying the use of different sampling methods in rural, small town, and urban environments for each of the major biomes of California and provide a listing of target vector and nuisance mosquito species. The stratified sampling approach monitors vector populations and virus activity in rural enzootic foci, agricultural, or suburban amplification sites, and densely populated urban centers to provide estimates of early, eminent, and current epidemic risk.

The four sampling methods currently used by mosquito control agencies are: 1) New Jersey (American) light trap, 2) CDC or EVS style CO_2-baited trap, 3) gravid trap, and 4) adult resting collections. 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	
Pros	**Cons**
• All female metabolic states and males collected • Minimal collection effort (can be run nightly without service) • Long history of use in California	• Selective for phototaxic nocturnally active mosquitoes • Ineffective with competing light sources • Sorting time excessive because of other insects in traps • Specimens dead; less use for virus detection • Collects comparatively few specimens
CDC/EVS CO_2 Trap	
Pros	**Cons**
• Samples biting population • Collects large numbers of virus vector species • Specimens alive; suitable for virus detection • Without light, collects mostly mosquitoes thus reducing sorting time • Battery operated, portable	• Collects >50% nullipars (have never blood fed or oviposited) • Must be set and picked-up daily • Dry ice cost high; availability can be a problem • Does not collect males or blooded and gravid females
Gravid Trap	
Pros	**Cons**
• Collects females that have bloodfed and digested the meal; may have higher infection rate • Specimens alive; suitable for virus detection • Extremely sensitive for *Cx.p. quinquefasciatus* in urban habitat • Bait inexpensive • Battery operated, portable	• Collects only foul-water *Culex* [mostly *pipiens* complex] • Bait has objectionable odor • Must be set and picked-up daily

Resting Catches	
Pros	**Cons**
• All metabolic states collected • Minimal equipment needed • Specimens alive; suitable for virus detection • Blooded and gravid specimens can be tested to improve sensitivity of virus surveillance	• Quantification difficult due to: 1. Variable shelter size and type 2. Variable collector efficiency • Labor intensive; difficult to concurrently sample a large number of sites

New Jersey (American) Light Trap (NJLT)

Operation

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

Traps should be kept away from smoke or chemical odors that may be repellent to the mosquitoes. Traps should be away from buildings in which animals are housed and not 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 14 to week 44 of the calendar year for districts north of the Tehachapi Mountains and all year long for districts south of the Tehachapi. Ideally, the traps should run for four to seven nights before the collection is retrieved (Loomis and Hanks 1959). The trap should be thoroughly cleaned with a brush to remove spider webs or any other debris that may hinder airflow through the trap. A regular cleaning schedule should be maintained during the trapping season to maintain trap efficiency.

Processing

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

CDC style CO_2-baited trap

Operation

Carbon dioxide-baited traps can be used for abundance monitoring or capturing mosquitoes for virus testing. A six foot tall standard should be used to standardize trap placement for population and virus infection rate monitoring. Knowledge of the host-seeking patterns of the target species is essential in determining CO_2-baited trap placement in the habitat 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_2-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_2-baited trap should not be placed in immediate proximity to the sentinel chicken flock because it will compete with, and therefore lessen, exposure of the sentinel birds, but should be placed within 100-200m radius of the sentinel flock site.

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.

<u>Reiter/Cummings gravid traps</u>

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

Operation

The Reiter/Cummings gravid trap is primarily used in suburban and urban residential settings for surveillance of *Culex pipiens* complex. The trap is placed on the ground near dense vegetation that serves as resting sites for gravid females. Specimens may be retrieved on a one to three day basis.

Processing

Culex pipiens complex females collected with the gravid trap for arbovirus surveillance should be retrieved daily and the protocol for mosquito pool submission as outlined in Appendix B should be followed. For population monitoring of the *Culex pipiens* complex, collections may

be retrieved every third day. The females are killed, identified and counted before being discarded. Autogenous females may also be attracted to the gravid trap.

Adult resting collections

Trap design and operation

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

Red boxes were developed to standardize collections spatially. Different researchers have used red boxes of varying dimensions. Largest catches are made in semi permanent walk-in red boxes which measure 4' x 4' x 6' (Meyer 1985). Smaller 1' x 1' x 1' foot boxes typically collect fewer specimens, but are readily portable. The entrance of the walk-in red box should be left open, draped with canvas, or closed with a plywood door. The canvas or plywood door should have a 1 or 2 ft gap at the bottom to allow entry of mosquitoes, while affording some protection from the wind and decreasing the light intensity within the box. The box entrance should not face eastward into the morning sun or into the predominant wind direction.

Processing

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

Data recording and analysis

Counts from NJLTs, EVS, and gravid traps should be recorded on the appropriate DHS Adult Mosquito Occurrence Report Summary Forms 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 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_2 traps and resting adult collections; such data are critical to evaluating the vector potential of the population.

References

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Reisen, W. K., H. D. Lothrop, R. E. Chiles, M. B. Madon, C. Cossen, L. Woods, S. Husted, V. L. Kramer, and J. D. Edman. 2004. Invasion of California by West Nile Virus. Emerg. Infect. Dis. [in press].

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

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

Appendix B: Procedures for Processing Mosquitoes for Arbovirus Detection

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

2. Anesthetize mosquitoes by cold, carbon dioxide, or triethylamine (TEA). TEA is recommended because specimens are permanently immobilized with minimal mortality and with no loss of SLE or WEE virus titer. 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 is extremely important because diagnostics have transitioned 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_2 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-1-05; where 05 refer to year 2005. 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 http://westnile.ca.gov for form).

   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 ultra-low temperature freezer. Pools are shipped frozen on dry ice to CVEC for testing by real time multiplex RT-PCR. Each pool is screened for WNV, SLE, and WEE viruses by the multiplex assay, with positives confirmed by a singleplex RT-PCR. Care must be taken not to allow pools to defrost during storage or shipment, because each freeze-thaw cycle results in a 10-fold decrease in viral titer, and all virus will be lost if the specimens sit at room temperature for extended periods. Address shipment to: Center for Vectorborne Diseases, University of California, Old Davis Road, Davis CA 95616.

Appendix C: Procedures for Maintaining and Bleeding Sentinel Chickens

1. Procure hens in April when they are 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 CVEC. 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

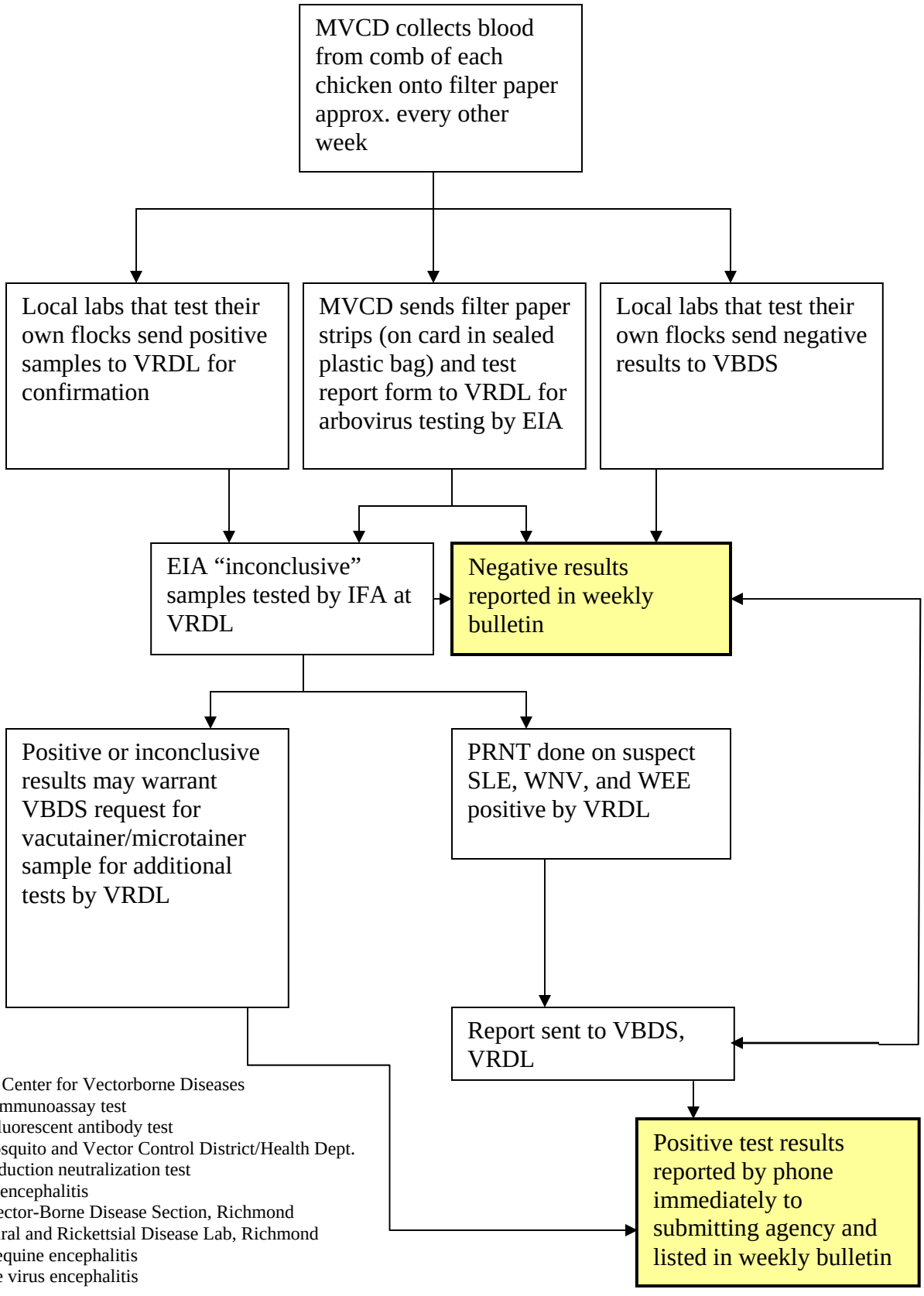
Specimens should be mailed to arrive by Friday afternoon for testing to start the following Monday.

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 µl of diluent. Specimens are allowed to soak overnight and the eluate tested for WEE, SLE, and WNV IgG antibody using ELISA. Positive specimens are confirmed the following day using an indirect fluorescent antibody test. SLE or WNV 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 WNV) and WEE



Key:

CVEC: UCDavis 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
WNV: West Nile virus encephalitis

Surveillance for Mosquito-borne Viruses
Registration of Agencies and Sites

1. Participation of agencies

Agencies interested in participating in the statewide surveillance program for mosquito-borne viruses should place orders 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. The local agencies are responsible for registering the sites to CVEC, assigning an agency code, and notifying VRDL 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 though MVCAC. CVEC will accept and test mosquito pools only from those agencies that have placed orders though 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 CVEC using the "SURVEILLANCE SITE REGISTRATION" form MBVS-1 (revised 05/13/04). Blood samples sent to VRDL must be accompanied by the form "SENTINEL CHICKEN BLOOD – 2005" (MBVS-2, revised 3/29/2005) for each flock site. All forms are available at http://westnile.ca.gov.

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 2005 form which includes the registered agency code, the registered site code (assigned by the local agency), and, for blood samples, the wing band numbers assigned to that site.

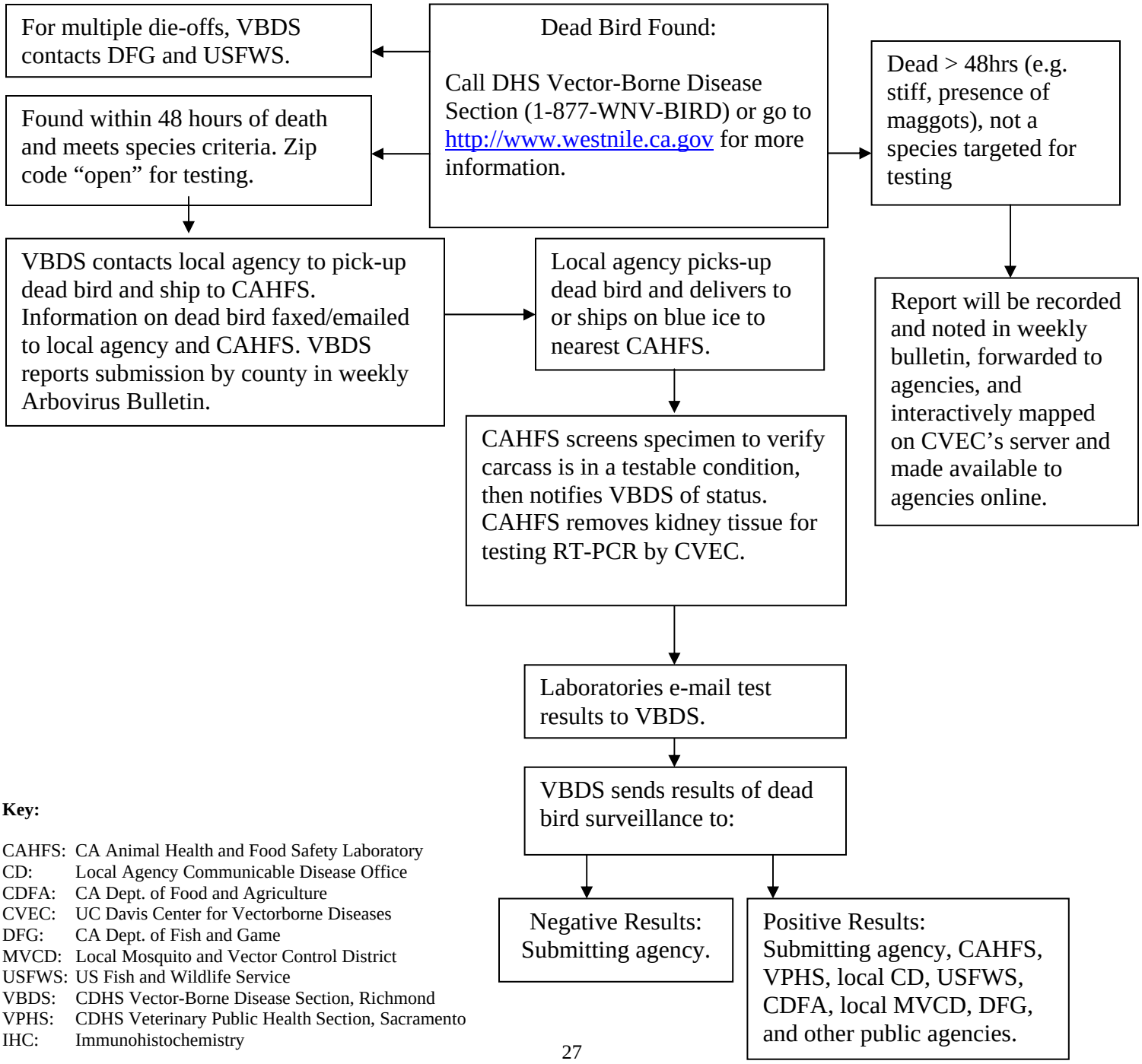
3. Registration of mosquito sampling sites

Registration of new sites used for collection of mosquitoes for virus testing may be accomplished by faxing a copy of the "SITE SURVEILLANCE REGISTRATION 2005" form to (530) 754-6360 (UC Davis) or e-mailing it to bfeldridge@ucdavis.edu at the same time the pools are shipped to CVEC. The laboratory will test the pools provided that adequate information is provided on the "MOSQUITO POOL SUBMISSION" form (MBVS-3, revised 03/29/05), 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 CVEC 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, CVEC will be generating up-to-date maps from the weekly results for posting at http://vector.ucdavis.edu/.

Appendix D: Procedures for Testing Dead Birds

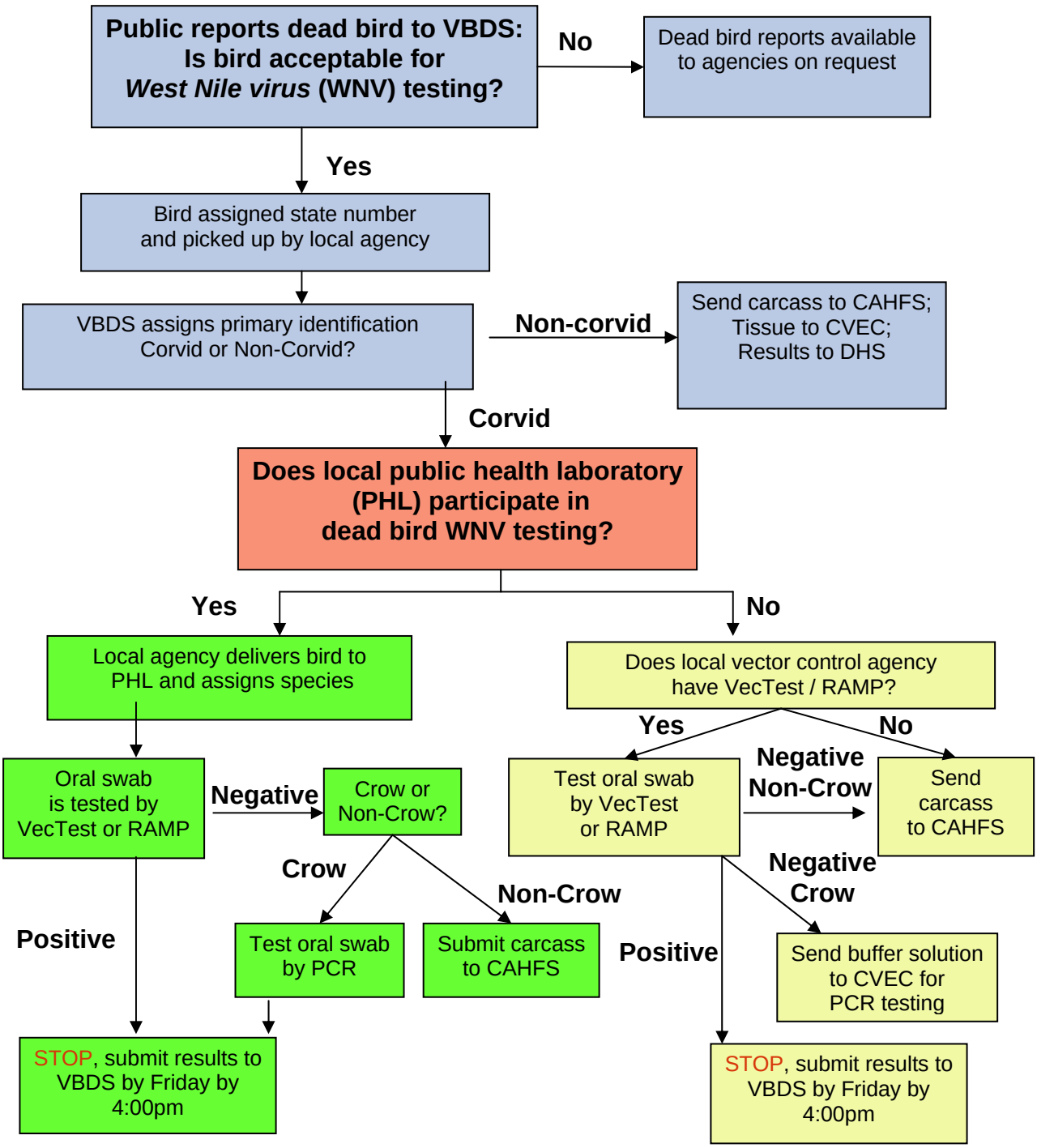
In 2000, DHS initiated a dead bird surveillance program in collaboration with other public agencies. DHS annually notifies about 600 agencies, organizations, and veterinarians involved with wildlife, including rehabilitation centers, about the program. The public is also notified about the program through the media and outreach materials. Dead birds are reported to DHS, shipped to a California Animal Health & Food Safety Laboratory (CAHFS) for screening and removal of kidney tissue, which is then sent to the UC Davis Center for Vectorborne Diseases (CVEC) for WNV RNA detection via RT-PCR. An overview of the dead bird testing algorithm is provided below.



Key:

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

Procedures for Testing Dead Birds: Rapid Assays



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

VBDS
Public Health Labs
Local Agencies

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 and the zip code is "open" for dead bird testing. The WNV Hotline is monitored 8am-4pm Monday through Friday, and seven days a week during the peak months of March to October. Any dead birds sent without prior notification will not be tested.

Once dead bird submission is approved, a bird can be shipped to the nearest California Animal Health and Food Safety Laboratory (CAHFS). Dead birds sent to CAHFS Turlock and Fresno laboratories will then be transported by CAHFS to Davis. In 2005, WNV RT-PCR testing will be conducted at the University of California Center for Vectorborne Diseases (CVEC), as well as at participating public health laboratories (corvid oral swabs only). CAHFS will remove kidney tissues and forward them to CVEC for RT-PCR assay. Shipping and testing expenses will be paid by DHS. VecTest and RAMP rapid screening assays will be conducted by participating local agencies and/or public health laboratories.

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, invert a plastic bag over your hand to handle and bag 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 not of diagnostic value. Signs that a bird has been dead for too long (over 24-48 hours) are the presence of maggots, an extremely lightweight carcass, missing eyes, skin discoloration, skin or feathers that rub off easily, strong odor, or a soft, mushy carcass.

- **If upon pick-up, the carcass is found to be unacceptable (wrong species or badly decomposed), please dispose of bird by placing it inside a bag (tie or zip lock) and discard in a secure garbage can or dumpster.** California Department of Fish and Game and the U.S. Fish and Wildlife Service prefer that your burn or bury the carcass, but disposing of it in a dumpster is acceptable. **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 in our central database.**

- Double bag each bird carcass separately and secure with tie or zip lock... **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.

- Agencies may test corvids via VecTest or RAMP assays. **Notify DHS with results by 4:00 p.m. Friday of each week to have results included in weekly state WNV update.** Reporting forms can be found at http://westnile.ca.gov/publications.htm#forms. **Note: any positive bird must be disposed of as biomedical waste (incineration).** Buffer solution from corvids that test negative should be sent directly to CVEC to be tested by PCR. Buffer solution should be submitted to CVEC in a labeled 2 ml snap cap eppendorf tube, retained inside a 5"x 5"x 2" freezer box with dividers. Box should be placed in a gallon zip lock bag and sent in a padded envelope to CVEC via overnight courier. Samples do not need to be packed on ice.

- **Enclose the shipping document in 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 or contact UPS directly; this guarantees arrival at CAHFS before the weekend.**

- Contact UPS to pick up dead bird either by web **(https://wwwapps.ups.com/pickup/schedule?loc=en_US) or by phone, 1-800-PICK UPS (1-800-742-5877). Select "UPS Next Day Air" and estimate the weight of the**

box (generally 10 lbs for a single large bird packed with ice). For billing, the UPS account number is: 48R89V.

- If you agency uses Golden State Overnight (GSO), please call 1-800-322-5555 and use the DHS account number (**22971**) and the DHS billing zip code (**94804**). It is also possible to access GSO on the web to arrange a pickup (http://www.shipgso.com).

- Birds that need to be stored over the **weekend** should be put on dry ice or stored at -70ºC. 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 -20º) at any time.

- Label the outside of the package with the words **Diagnostic Specimens ATTN: WNV** above the designated CAHFS address.

Dead Bird Shipping List

Please verify that your district has the following items:

- CAHFS addresses (see below)
- WNV hotline (877-WNV-BIRD) manned 8am-4pm weekdays.
- Crumpled newspapers or another absorbent material
- Rubber or Latex Gloves
- Packing tape
- Dead Bird Shipping Boxes
  - inner zip-lock bag
  - outer zip-lock bag
  - Inner Styrofoam box
  - Outer Cardboard box
- VecTest materials (will be supplied by DHS to agencies that cannot provide their own).
  - VecTest kit
  - Swabs
  - Small White Freezer boxes with dividers

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 92408

CAHFS Fresno (559) 498-7740
ATTN: WNV
Dr. Richard Chin
2789 South Orange Avenue
Fresno, CA 93725

CAHFS Turlock (209) 634-5837
ATTN: WNV
Dr. Bruce Charlton
P.O. Box 1522
Fulkerth & Soderquist Road
Turlock, CA 95381

Appendix E: Procedures for Testing Equines and Ratites

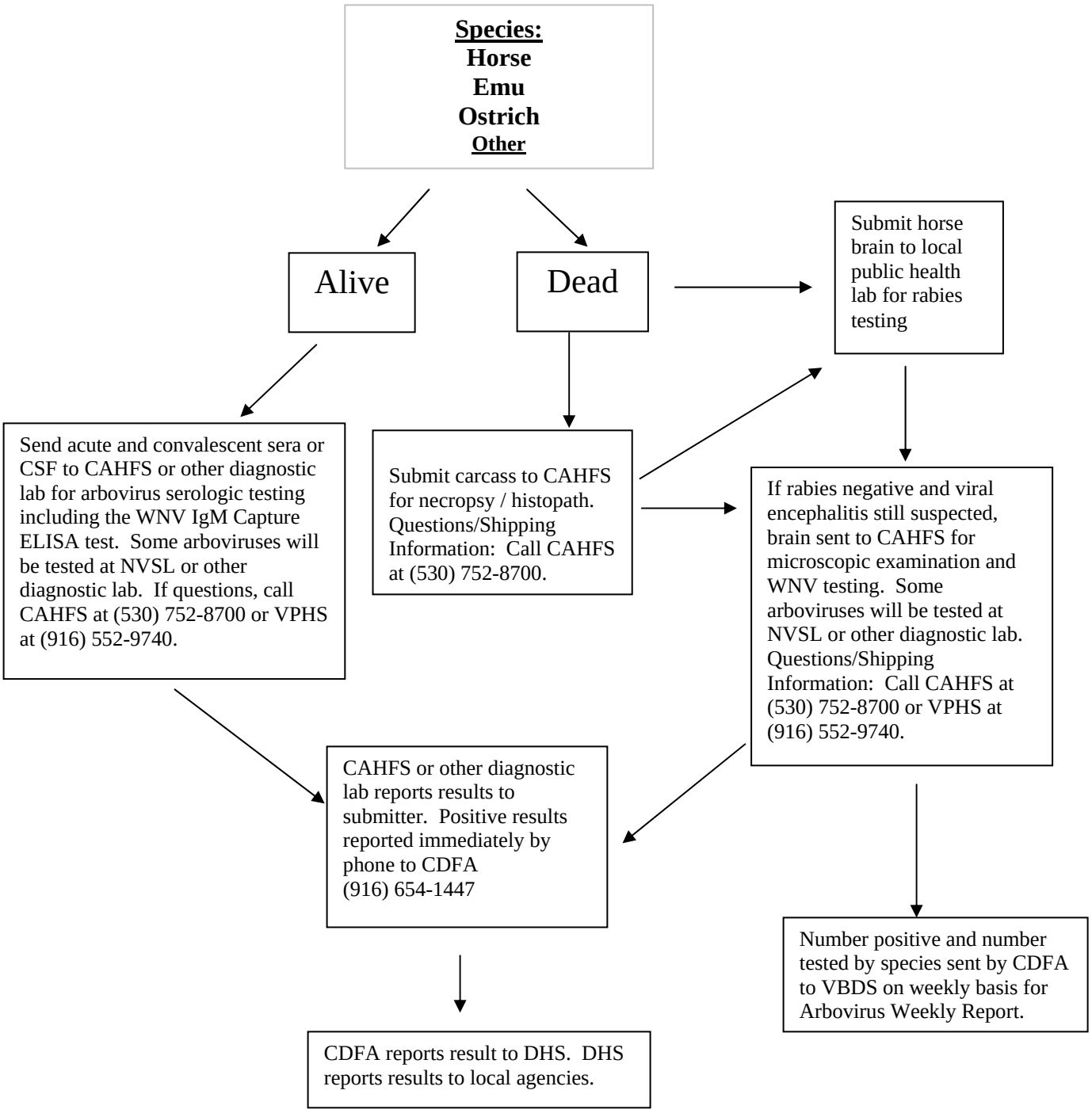
The California Department of Health Services (DHS) and the California Department of Food and Agriculture (CDFA) have a well-established passive surveillance program for equine and ratite encephalomyelitis. Equine encephalomyelitides is legally reportable to CDFA by veterinarians and diagnostic laboratories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

This appendix contains information sent to veterinarians, public health lab directors, local health officers, public health veterinarians, animal health branch personnel, and interested parties every spring to inform them about the California Equine and Ratite Arbovirus Surveillance Program. The mailing includes a case definition for equine encephalomyelitides and instructions for specimen collection and submission for both equine and ratite samples. The information is distributed to approximately 1,200 practitioners, equine organizations, and other interested parties. Specimen submission is coordinated through the California Animal Health and Food Safety Laboratory System's (CAHFS) five regional branches, and other laboratories or individual veterinarians. Equine WNV 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 in some instances, forwarded to the National Veterinary Services Laboratories (NVSL) for further 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 and is included in this appendix.

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 encephalomyelitides.

Additional information on WNV for veterinarians, horse owners, and ratite owners, is available from CDFA/Animal Health Branch (916) 654-1447 and at CDFA's website: http://www.cdfa.ca.gov/ahfss/ah/wnv_info.htm. Information on submission of laboratory samples is available from CAHFS (530) 752-8700 and at CAHFS website: http://cahfs.ucdavis.edu. A brochure containing facts about California WNV surveillance and general information about prevention and control is available from DHS (916) 552-9730 and at DHS' website: http://www.westnile.ca.gov; a special section veterinarians and horse owners is available at: http://westnile.ca.gov/veterinarian.htm.

Algorithm for Submission of Specimens from Domestic Animals with Neurologic Symptoms



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

05/02/05
CDHS/VPH

SURVEILLANCE CASE DEFINITIONS FOR WEST NILE VIRUS DISEASE IN EQUINES - 2005

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

CONFIRMED CLINICAL CASE:

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

Plus one or more of the following:

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

SUSPECT CLINICAL CASE[4]:

- Compatible clinical signs

EXPOSED EQUID:

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

Assumptions on which case definition is based:

- Antibody in serum may be due to vaccination or a natural exposure; additional testing must be done to confirm WNV infection in a vaccinated horse.
- IgM antibody in equine serum is relatively short-lived; a positive IgM-capture ELISA means exposure to WNV or rarely a closely related flavivirus (SLE) has occurred, very likely within the last three months.
- Neutralizing antibody, as detected by PRNT, may not be present in equine serum until two weeks or more after exposure to WNV; it is possible that clinical signs may be present in an equine before a serum PRNT is positive.

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[1] Preferred diagnostic tissues are equine brain or spinal cord; although tissues may include blood or CSF, the only known reports of WNV isolation or positive PCR from equine blood or CSF have been related to experimentally infected animals.

[2] The first serum should be drawn as soon as possible after onset of clinical signs and the second drawn at least seven days after the first.
[3] For horses it is recommended that RT-nested polymerase chain reaction assay be used to maximize sensitivity of the test (Emerg. Infect. Dis. 2001 Jul-Aug; 7(4):739-41)
[4]An equine case classified as a suspect case should, if possible, undergo further diagnostic testing to confirm or rule out WNV as the cause of the clinical illness.

Protocol for Submission of Laboratory Specimens for
Equine Neurological Disease Diagnosis and Surveillance
April 2005

Complete information on specimen collection and submission is available on the CDFA website at: http://www.cdfa.ca.gov/ahfss/ah/wnv_lab_submission.htm

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 arbovirus testing.
      - Submission of the intact head is preferable because: 1) brain is better preserved (anatomically and virus titer) when left in the skull during transport, 2) specimens will be ruined if removal is not done correctly, and 3) brain removal in field conditions may increase the risk of exposure to rabies.
      - **The intact head should be chilled (refrigerated, *not* frozen) immediately after removal. Submit it to a CAHFS Laboratory* in your area as quickly as possible.** Prepare a leak-proof insulated transporting container with "cold packs" to keep the specimen at 4^{o} 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 CAHFS. Call 530-752-8700 or visit the CAHFS website at http://cahfs.ucdavis.edu. The submittal form for each specimen should be placed in a leak-proof plastic bag 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.

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

West Nile virus (WNV) testing within the regional public health laboratory network (i.e., the California Department of Health Services Viral and Rickettsial Disease Laboratory and participating local public health laboratories) is recommended on individuals with the following:

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

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

Required specimens:

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

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

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

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

Appendix G: Surveillance Case Definition for West Nile Virus Infection in Humans

(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)

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, myelities 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 out 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. Diflurobenzamide (e.g. Dimilin®25W)
   Use: Impounded tail water, sewage effluent, urban drains and catch basins.
   Limitations: Cannot be applied to wetlands, crops, or near estuaries.

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

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

6. Temephos (e.g. Abate® 2-BG)
   Use: Non-potable water; marshes; polluted water sites
   Limitations: Cannot be applied to crops for food, forage, or pasture. This material is an organophosphate compound and may not be effective on some *Culex tarsalis* populations in the Central Valley.

Adulticides:

1. Organophosphate compounds
   Note: Many *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
Bacillus sphaericus, (Bs)	VectoLex CG	275-77	Valent BioSciences	Granule	Larvae	Biorational
Bacillus sphaericus, (Bs)	VectoLex WDG	73049-57	Valent BioSciences	Water dispersible granule	Larvae	Biorational
Bacillus sphaericus, (Bs)	VectoLex WSP	73049-20	Valent BioSciences	Water soluble packet	Larvae	Biorational
Bacillus thuringiensis var. *israelensis* (Bti)	VectoBac 12AS	73049-38	Valent BioSciences	Liquid	Larvae	Biorational
Bacillus thuringiensis var. *israelensis* (Bti)	VectoBac G	275-50 or 73049-10	Valent BioSciences	Granule	Larvae	Biorational
Bacillus thuringiensis var. *israelensis* (Bti)	VectoBac Tech. Powder	73049-13	Valent BioSciences	Technical powder	Larvae	Biorational
Bacillus thuringiensis var. *israelensis* (Bti)	Aquabac 200G	62637-3	Becker Microbial	Granule	Larvae	Biorational
Bacillus thuringiensis var. *israelensis* (Bti)	Bactimos Briquets	6218-47	Summit	Donut-style briquets	Larvae	Biorational
Bacillus thuringiensis var. *israelensis* (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

Adulticides (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 Arbovirus Surveillance, Mosquito Control, Weather Conditions and Forecasts, and Crop Acreage and Production in California

Website	URL	Available information
California West Nile Virus Website	http://westnile.ca.gov	Up to date information on the spread of West Nile virus throughout California, personal protection measures, online dead bird reporting, bird identification charts, mosquito control information and links, clinician information, local agency information, public education materials.
UC Davis Center for Vectorborne Diseases	http://cvec.ucdavis.edu/	Frequently updated reports and interactive maps on arbovirus surveillance and mosquito occurrence in California.
Mosquito and Vector Control Association of California	http://www.mvcac.org	News, membership information, event calendars, and other topics of interest to California's mosquito control agencies.
California Data Exchange Center	http://cdec.water.ca.gov	Water-related data from the California Department of Water Resources, including historical and current stream flow, snowpack, and precipitation information.
UC IPM Online	http://www.ipm.ucdavis.edu	Precipitation and temperature data for stations throughout California; also allows calculation of degree-days based on user-defined data and parameters.
National Weather Service – Climate Prediction Center	http://www.cpc.ncep.noaa.gov/products/predictions/	Short-range (daily) to long-range (seasonal) temperature and precipitation forecasts. Also provides El Niño-related forecasts.
California Agricultural Statistics Service	http://www.nass.usda.gov/ca/	Crop acreage, yield, and production estimates for past years and the current year's projections. Reports for particular crops are published at specific times during the year – see the calendar on the website.
US Environmental Protection Agency – Mosquito Control	http://www.epa.gov/pesticides/factsheets/skeeters.htm	Describes the role of mosquito control agencies and products used for mosquito control.
US Centers for Disease Control and Prevention – West Nile Virus	http://www.cdc.gov/ncidod/dvbid/westnile/index.htm	Information on the transmission of West Nile virus across the United States, viral ecology and background on WNV, and personal protection measures in various languages.