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) and is an integral part of integrated mosquito management programs conducted by local mosquito and vector control agencies. Surveillance and interagency response guidelines have been published previously by the California Department of Public Health formerly known as the California Department of Health Services (Walsh 1987) and the Mosquito and Vector Control Association of California (Reisen 1995). The detection of West Nile virus (WNV) in New York, a virus not recognized in the Western Hemisphere prior to 1999, prompted the review and enhancement of existing guidelines to ensure that surveillance, prevention, and control activities were appropriate for WNV. From New York, WNV spread rapidly westward and by 2004 had been detected in 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, chikungunya and Venezuelan encephalitis viruses. If an existing or introduced virus is detected, it is critical that local and state agencies are prepared to respond in a concerted effort to protect people and animals from infection and disease. The current document describes an enhanced surveillance and response program for mosquito-borne viruses in the State of California. Its contents represent the collective effort of the California Department of Public Health (CDPH), the Mosquito and Vector Control Association of California (MVCAC), and the University of California at Davis (UCD).

Background

Mosquito-borne viruses belong to a group of viruses commonly referred to as arboviruses (for **ar**thropod-**bo**rne). Although 12 mosquito-borne viruses are known to occur in California, only WNV, western equine encephalomyelitis virus (WEE) and St. Louis encephalitis virus (SLE) are significant causes of human disease. WNV is having a serious impact upon the health of humans, horses, and wild birds throughout the state. In 2006, there were 292 WNV human infections with 7 deaths and 58 horse cases (24 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 wild bird-mosquito cycles that do not depend upon infections of humans or domestic animals to persist. Surveillance and control activities focus on this maintenance cycle, which involves primarily *Culex* mosquitoes, such as the western encephalitis mosquito, *Culex tarsalis*, and birds such as house finches and house sparrows.

Immature stages (called larvae and pupae) of *Culex tarsalis* can be found throughout California in a wide variety of aquatic sources, ranging from clean to highly polluted waters. Most such water is associated with irrigation of agricultural crops or urban wastewater. Other mosquito species, such as *Culex pipiens* and *Culex quinquefasciatus*, play an important role in WNV, and possibly SLE, transmission cycles in urban and suburban areas. *Aedes 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 also be important bridge (i.e. bird to mammal) vectors in transmission.

Mosquito control is the only practical method of protecting the human population from infection. There are no known specific treatments or cures for diseases caused by these viruses and vaccines are not available for public use. Infection by WEE virus tends to be most serious in very young children, whereas infections caused by WNV and SLE virus affect the elderly most seriously.

WNV also kills a wide variety of native and non-native birds. There are WEE and WNV vaccines available to protect horses since both viruses can cause severe disease in horses. Mosquito-borne disease prevention strategies must be based on a well-planned integrated pest management (IPM) program that uses real-time surveillance to detect problem areas, focus control, and evaluate operational efficacy. The primary components of an IPM program include education, surveillance, and mosquito control.

Education

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

Surveillance

Surveillance includes the monitoring, visualization and analysis of data on climatic factors, 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 to larval control efforts. Four adult mosquito sampling methods are currently used in California: New Jersey light traps, carbon dioxide-baited traps, gravid (egg-laying) traps, and resting adult mosquito collections. The advantages and disadvantages of these sampling methods, and guidelines for the design, operation, and processing of the traps have been discussed in Guidelines for Integrated Mosquito Surveillance (Meyer et al. 2003) and are summarized in Appendix A.

Mosquito Infections

Early 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 an important vector of WNV, surveillance efforts emphasize the testing of this species. Other species that should be tested, especially for WNV and SLE, include *Culex quinquefasciatus*, *Culex pipiens*, *Culex stigmatosoma*, and for WEE include *Aedes melanimon and Ae. dorsalis.* Female mosquitoes are trapped, usually using carbon dioxide-baited or gravid traps, identified to species and counted into groups [pools] of 50 females each for testing at the Arbovirus Research Unit of the Center for Vectorborne Diseases (CVEC) at UC Davis. Procedures for submitting and processing mosquitoes for detecting virus infection are detailed in Appendix B. The current surveillance system is designed to detect WNV, SLE, and WEE. Although generally less sensitive than sentinel chickens, mosquito infections may be detected earlier in the season than chicken seroconversions and therefore provide an early warning of virus activity. Testing adult mosquitoes for infection is one of the best methods to detect newly introduced mosquito-borne viruses that would not otherwise be expected to be present in the state. Testing mosquito species other than *Culex 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 to bird populations can be accomplished by 1) using caged chickens as sentinels and bleeding them routinely to detect viral antibodies (seroconversions), 2) collecting and bleeding wild birds to detect viral antibodies, and 3) testing dead birds reported by the public for WNV.

In California, flocks of ten chickens are placed in locations where mosquito abundance is known to be high or where there is a history of virus activity. Each chicken is bled every two weeks by pricking the comb and collecting blood on a filter paper strip. The blood is tested at the CDPH Viral and Rickettsial Disease Laboratory for antibodies to SLE, WEE, and WNV. Some agencies conduct their own testing, but send positive samples to CDPH for confirmation and official reporting. Because SLE cross-reacts with WNV in antibody testing, SLE or WNV positive chickens may be confirmed by Western blot or cross neutralization tests. Frequent testing of strategically placed flocks of sentinel chickens provides the most sensitive and cost-effective method to monitor encephalitis virus 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 or viral infection rates are low. Sentinel housing, bleeding instructions, and testing protocols are provided in Appendix C.

Virus activity in wild bird populations can be monitored by bleeding young (hatching year) birds to detect initial virus infection or by bleeding older birds to determine if the prevalence of the virus in the region has changed. New infection can be detected by bleeding banded birds in a capture-recapture scheme. In contrast to the convenience of using sentinel chickens, the repeated collection and bleeding of wild birds generally is too labor intensive, technically difficult, and expensive for most local mosquito control agencies to perform routinely. In addition, the actual place where a wild bird became infected is rarely known, because birds usually are collected during daylight foraging flights and not at nocturnal roosting sites where they are 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 CDPH in 2000 to provide early detection of WNV. Dead bird surveillance has been shown to be one of the earliest indicators of WNV activity in a new area, and in 2006 the dead bird surveillance program detected the presence of WNV before other surveillance elements in 38 of the 54 counties that detected WNV (out of a total of 58 California counties). Birds that meet certain criteria are necropsied at the California Animal Health and Food Safety Laboratory and tested for WNV by RT-PCR at CVEC or by rapid antigen tests by local agencies. In 2006, a total of 46,345 dead birds were reported to CDPH's dead bird hotline (1-877-WNV-BIRD) and website, westnile.ca.gov. Of the 6,535 birds that were tested, 1,446 were positive for WNV. The communication and testing algorithm for the dead bird surveillance program is detailed in Appendix D.

In 2005, CDPH instituted use of a Dynamic Continuous-Area Space-Time (DYCAST) model to identify areas of increased WNV activity in space and time based on the occurrence of dead bird reports. This model was developed in cooperation with the Center for Advanced Research of Spatial Information at Hunter College, City University of New York. Maps were made available to 17 participating mosquito and vector control agencies via a password-protected website. Local agencies used the maps to help focus surveillance and public education activities, and to help establish mosquito control priority areas for reducing WNV enzootic activity and therefore the risk of human infection. Of those human cases with a known onset date and that could be geo-coded within the participating areas (332), 274 (83%) occurred within the quarter square mile areas that were identified by the DYCAST system to be high WNV activity. One hundred sixty-six (50%) human cases occurred in areas identified in these areas approximately one month prior to onset, indicating that DYCAST may be an effective tool in assisting mosquito control agencies to identify areas of high WNV activity.

In 2006, CDPH developed daily DYCAST maps for the entire state and made them available on the CVEC Surveillance Gateway website; a real-time alert system was also introduced to provide high WNV activity counties with custom reports about WNV transmission. A majority of local agencies reported that they used DYCAST maps to assist in decision-making processes for mosquito larviciding (81%, 35 out of 43 local agencies) and adulticiding (71%, 30 out of 42 local agencies). In 2007, the DYCAST procedure will again be run for the entire state of California, and daily maps will be made available online through the California Vector-borne Disease Surveillance Gateway (http://surv.mvcac.org) from May through August.

Tree Squirrel Infections

In 2004, tree squirrels were included as a WNV surveillance tool, based upon evidence that they were susceptible to WNV and could provide information on localized WNV transmission (Padgett et al. 2007). In conjunction with dead birds, tree squirrels were reported to the California WNV hotline, necropsied at the California Animal Health and Food Safety Laboratory and kidney tissue was tested by RT-PCR at CVEC. In 2006, 375 tree squirrels from 33 counties were reported to the WNV hotline, of which 32 of 138 tree squirrels (23.2%) from 9 counties tested positive for WNV. Tree squirrels will continue to be tested for WNV in 2007 and are included in the submission protocol 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 has unfolded for WNV as horse owners vaccinate to protect their horses. If confirmed cases do occur, it is a strong indication that WEE or WNV has amplified to levels where tangential transmission has occurred in that region of the State. Veterinarians are contacted annually by CDPH and the California Department of Agriculture (CDFA) to advocate equine vaccination and to describe diagnostic services that are available in the event of a suspected case of WEE or WNV encephalitis. Other mosquito-borne viruses may also cause encephalitis in horses, and testing of equine specimens for these other viruses is available (see Appendix E).

Human Infections

Local mosquito control agencies rely on the rapid detection and reporting of confirmed human cases to plan and implement emergency control activities to prevent additional infections. However, human cases of arboviral infection are an insensitive surveillance indicator of virus activity because most human infections cause no, or only mild, symptoms. The focus of human WNV, SLE and WEE surveillance is on severe cases, e.g. encephalitis or aseptic meningitis. In an attempt to increase detection of human SLE, WEE, and WNV cases in California, communication with key hospitals and local health officials has been enhanced. Physicians and health care providers are informed of the availability of WNV and other arbovirus testing through the regional public health laboratory network, which consists of the state Viral and Rickettsial Disease Laboratory (VRDL) and 29 county public health laboratories that are able to conduct WNV testing. Providers may also submit specimens to the California Encephalitis Project, which includes testing for WNV and other arboviruses when indicated. Physicians are required to report cases of viral encephalitis, viral meningitis, or WNV fever to their local health department. Laboratories are also required to report positive results for arboviral encephalitis and WNV (Title 17 Sections 2500 and 2505). Since transmission may occur from blood or transplanted organs, blood banks and organ transplantation programs have also begun screening procedures and report positive results to local health departments. Confirmed WNV, SLE or WEE cases 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

Problems detected by surveillance are mitigated through larval and adult control. Mosquito control is the only practical method of protecting people from mosquito-borne diseases. Mosquito control in California is conducted by over 70 local agencies, including mosquito and vector control districts, environmental health departments, and county health departments. Compounds currently approved for larval and adult mosquito control in California are listed in Appendix H.

Larval Control

Mosquito larvae and pupae control methods are target-specific and prevent the emergence of adult female mosquitoes which are capable of transmitting pathogens , causing discomfort, and ultimately producing another generation of mosquitoes. For these reasons, most mosquito control agencies in California target the immature stages rather than the adult stage of the mosquito. Larval mosquito control has three key components: environmental management, biological control, and chemical control.

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

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

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

Adult Control

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

Response Levels

The California Mosquito-borne Virus Surveillance and Response Plan was developed to provide a semi-quantitative measure of virus transmission risk 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 measured and 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 mosquito vectors
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. For surveillance factor 2 (vector abundance), abundance is scaled as an anomaly and compared to the area average over 5 non-epidemic years in a specific region, such as that within the boundaries of a local mosquito and vector control district. The mosquito virus infection rate should be calculated using the most current data. 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 *Culex tarsalis* abundance, and virus spillover into *Aedes* populations. In contrast, SLE activity appears to be greatest during La Niña conditions of drought and hot summer temperatures. SLE and WNV transmission risk increases 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 this mosquito is an important vector in suburban/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** Environmental risk conditions include above normal rainfall, snow pack, and runoff and cool early season ambient temperature followed by a strong warming trend (El Niño season). www.ipm.ucdavis.edu:/WEATHER/wxretrieve.html	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 Aedes 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 *Ae. melanimon* abundance above average (150-300%)	
	5	*Cx. tarsalis* and *Ae. melanimon* abundance well above average (>300%)	
3. **Virus infection rate in *Cx. tarsalis and Ae. 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 *Ae. melanimon* MIR/1000 > 0	
	5	*Cx. tarsalis* MIR / 1000 > 5.0 and *Ae. 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	
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	

SLE Surveillance Factor	Assessment Value	Benchmark	Assigned Value
1. **Environmental Conditions** Environmental risk 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	
<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	

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: 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	
<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	

Characterization of Conditions and Responses for all viruses

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 Public Health (supplement to California Mosquito-Borne Virus Surveillance and Response Plan). www.westnile.ca.gov/resources.php

Key Agency Responsibilities

Local Mosquito and Vector Control Agencies

- Gather, collate, and interpret regional climate and weather data.
- Monitor abundance of immature and adult mosquitoes.
- Collect and submit mosquito pools to CVEC for virus detection.
- Maintain sentinel chicken flocks, obtain blood samples, and send samples to VRDL.
- Pick-up and ship dead birds for necropsy and WNV testing, or test oral swabs from American crows locally via rapid antigen screening assays.
- Update CDPH weekly of all birds that are independently reported and/or tested by VecTest, RAMP or immunohistochemistry (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.
- Communicate regularly with neighboring agencies

Mosquito and Vector Control Association of California

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

California Department of Public Health

- Collate adult mosquito abundance data submitted by local agencies; provide summary of data to local agencies.
- Maintain a WNV information and dead bird reporting hotline, 1-877-WNV-BIRD, and a WNV website: www.westnile.ca.gov
- Coordinate submission of specimens for virus testing.
- Provide supplies for processing mosquito pool and sentinel chicken diagnostic specimens
- Test sentinel chicken sera for viral antibodies.
- Maintain data including registration of collection sites, entry of mosquito abundance and pool data, and sentinel chicken sera data through the Surveillance Gateway [http://gateway.calsurv.org/]
- Test human specimens for virus.
- Distribute a weekly bulletin summarizing surveillance test results.
- Send weekly surveillance results to the UC Davis interactive website.
- Provide statewide, daily DYCAST human risk maps, available through the California Vectorborne Disease Surveillance Gateway (http://gateway.calsurv.org/).
- Provide analysis of DYCAST risk data and notification to local agencies when appropriate
- Immediately notify local vector control agency and public health officials when evidence of viral activity is found.
- Conduct epidemiological investigations of cases of human disease.

- Coordinate and participate in a regional emergency response in conjunction with California 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 endemic and introduced viruses.
- Provide a proficiency panel of tests for identification of viruses from human, equine, bird, or arthropod vectors.
- Maintain an interactive website [http://gateway.calsurv.org/] for dissemination of mosquito-borne virus information and data.
- Maintain inventory of antigens, antisera, and viruses to detect the introduction of exotic viruses.
- Provide confirmation of tests done by local or state agencies.

California Department of Food and Agriculture

- Notify veterinarians and veterinary diagnostic laboratories about WEE and WNV and testing facilities available at UCD Center for Vectorborne Disease Research.
- Provide outreach to general public and livestock and poultry producers on the monitoring and reporting of equine and ratite encephalitides.
- Facilitate equine and ratite sample submission from the field.
- Conduct epidemiological investigations of equine cases.

California Animal Health and Food Safety Laboratory

- Identify 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 CDPH 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 American crows via rapid assay or RT-PCR as resources allow.
- Participate in emergency response.
- Conduct epidemiological investigations of cases of human disease.
- Report WNV cases to CDPH.
- Conduct public education.

Governor's Office of Emergency Services

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

Federal Centers for Disease Control and Prevention

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

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Appendix A: Guidelines for Adult Mosquito Surveillance

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

The four sampling methods currently used by mosquito control agencies are: 1) New Jersey (American) light trap, 2) CDC or EVS style CO_2-baited trap, 3) gravid trap, and 4) adult resting collections. Collection location sites should be geocoded and registered using the Surveillance Gateway [http://gateway.calsurv.org/]. Studies comparing trap design and efficiency for surveillance purposes have been published (Reisen et al. 2000; Reisen et al. 2002). These guidelines describe: 1) a comparison of the sampling methods, 2) equipment design, 3) operation, 4) specimen processing, 5) data recording and analysis, and 6) data usage.

Advantages and Disadvantages of Mosquito Sampling Methods:

New Jersey Light Trap	
Pros	**Cons**
• All female metabolic states and males collected • Minimal collection effort (can be run nightly without service) • Long history of use in California	• Selective for 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 blood meal; may have higher infection rate than CO_2 trap • 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 be in the immediate vicinity of sentinel flocks to diminish attractancy competition. Traps should be placed away from street and security lights that may diminish attractancy of the trap bulb.

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 to enhance catch size and therefore sampling sensitivity. *Culex tarsalis* primarily bloodfeed on birds and hunt along vegetative borders and tree canopies where birds roost and nest. *Culex erythrothorax* are best collected within wetland areas near dense stands of tules and cattails. In large, open breeding sources such as rice fields, CO_2-baited traps could be hung on standards on the up-wind

side of the source for *Culex tarsalis* and *Anopheles freeborni* collections. *Aedes melanimon* and *Aedes nigromaculis* are mammal feeders and typically 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 affect catch size. The mini-light should be removed, because it attracts other phototaxic insects that may hinder sorting and/or damage female mosquitoes in the collection container and may repel members of the *Culex pipiens* complex. The CO_2-baited trap should not be placed in immediate proximity to the sentinel chicken flock because it will compete with, and therefore lessen, exposure of the sentinel birds, but may be placed within 100-200 m radius of the sentinel flock site.

Processing

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

Reiter/Cummings gravid traps

Trap design and components

The Reiter/Cummings gravid traps consist of a rectangular trap housing [plastic tool box] with an inlet tube on the bottom and an outlet tube on the side or top. The rectangular housing is provided with legs to stabilize the trap over the attractant basin containing the hay-infusion mixture. (Cummings 1992). The oviposition attractant consists of a fermented infusion made by mixing hay, Brewer's yeast and water. The mixture should sit at ambient temperature for three to four days to allow fermentation and increase attractancy. New solutions should be made at least biweekly to maintain consistent attractancy.

Operation

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

Processing

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

Adult resting collections

Trap design and operation

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

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

Processing

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

Data recording and analysis

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

Data usage

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

1. Assess control efforts.
2. Monitor arbovirus vector abundance and infection rates.

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

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Appendix B: Procedures for Processing Mosquitoes for Arbovirus Detection

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

2. Anesthetize mosquitoes by cold, carbon dioxide, or triethylamine (TEA). TEA is recommended because specimens are permanently immobilized with minimal mortality and with no loss of virus titer. TEA should be used either outdoors or under a chemical hood. Collections can be anesthetized outdoors using a few drops of TEA, the specimens transferred to Petri dishes, and then taken into the laboratory for processing. If refrigerated and kept humid, mosquitoes will remain alive in covered Petri dishes for one or two days without additional anesthesia. If mosquitoes are frozen before processing, sorting to species and enumeration must be done on a chill table to prevent virus loss.

3. Sort mosquito collections to species under a dissecting microscope at 10X to ensure correct identification and to make sure that extraneous mosquito parts (i.e., legs, wings) or other small insects such as chironomids or *Culicoides* are not inadvertently included in the pools. This is extremely important because diagnostics have transitioned from virus isolation to sensitive RT-PCR methods of viral detection. Count and discard dead and dried mosquitoes. Lots of 50 females (minimum of 12 females) per pool of each vector species from each collection site are then counted into individual polystyrene vials with snap caps containing two 5mm glass beads. Recommended sampling effort is ten pools of 50 females of each species from each site per week to detect minimum infection rates (MIRs) ranging from 0 to 20 per 1,000 females tested. Vials with pools should be labeled sequentially starting with #1 each year after the site code; e.g., KERN-1-06; where 06 refer to year 2006. Data on each pool can be entered directly in electronic format through the California Vectorborne Disease Surveillance Gateway (http://gateway.calsurv.org/). POOLS MUST BE ACCOMPANIED BY "MOSQUITO POOLS SUBMITTED FORM MBVS-3" AND CAN ONLY BE TESTED FROM REGISTERED SITES. Surveillance sites should be registered online at: **http://gateway.calsurv.org/** Faxed registration forms (MBVS-1) will be accepted from agencies without adequate internet access.

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

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

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

5. Local agencies that do their own testing should only RAMP® tests, and only after the first positive of the year in their county has been confirmed by the lab at CVEC.

Appendix C: Procedures for Maintaining and Bleeding Sentinel Chickens

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

5. Attach the completely dry filter paper strips to a 5x7 card in sequential order, from left to right by stapling the labeled end towards the top edge of the card, and leaving the blood soaked end free so that the laboratory staff can readily remove a standard punch sample. Write the County, Agency Code, Site, and Date Bled onto the card and place it into a zip lock plastic bag. Do not put more than one sample card per bag. It is important that blooded ends do not become dirty, wet, or touch each other. VERY IMPORTANT: CHICKEN SERA MUST BE ACCOMPANIED BY SENTINEL CHICKEN BLOOD FORM (MBVS- 2) OUTSIDE THE ZIP-LOCK BAG. Do not staple the form to the bag. Samples from each bleeding date then can be placed into a mailing envelope and sent to:

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

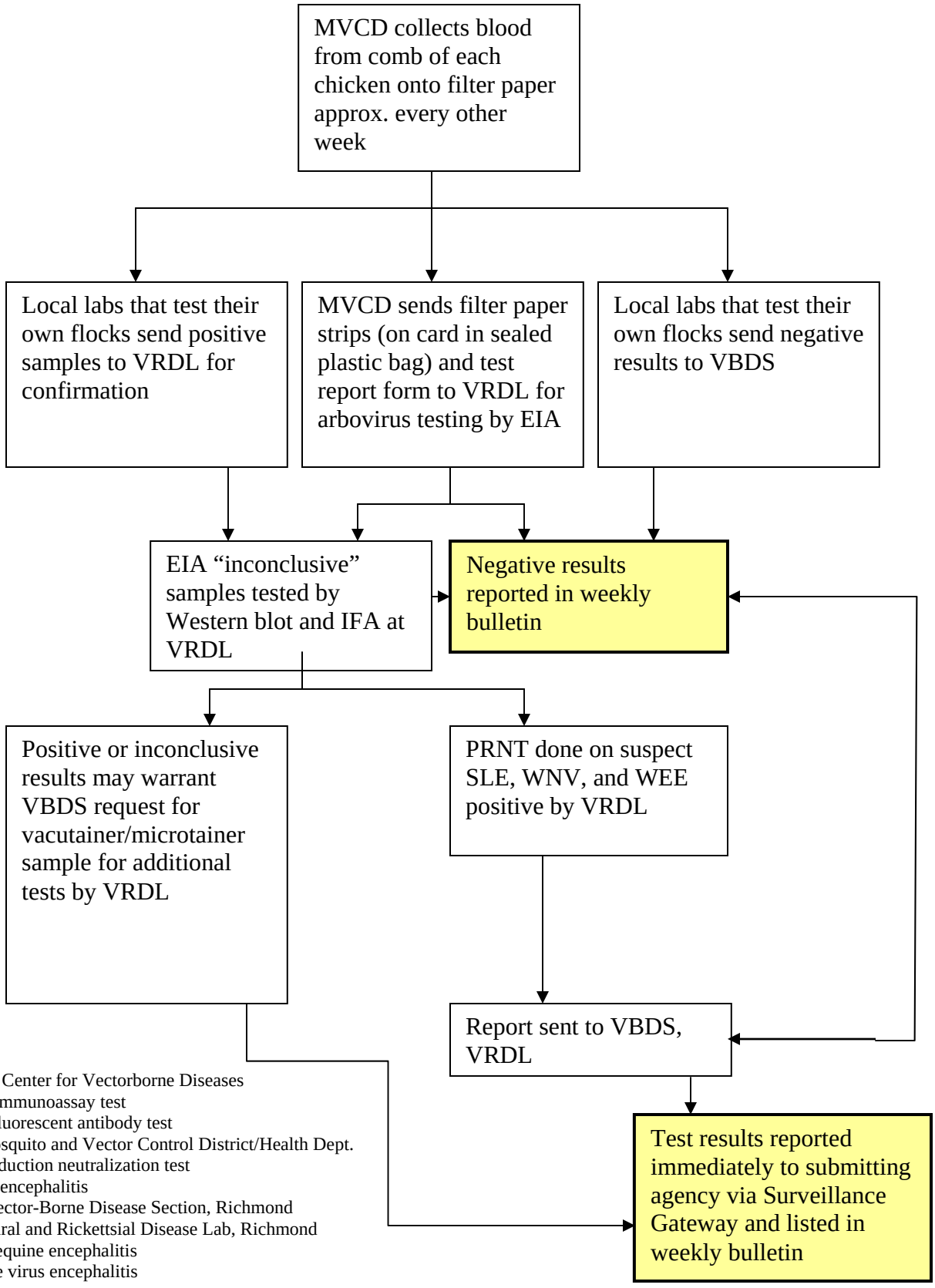
   Specimens should be mailed to arrive no later than Friday afternoon for testing to start the following Monday.

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 150 µl of diluent. Specimens are allowed to soak for 2 hours on a rotator 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 or Western blot. Problematic SLE or WNV positives are confirmed and identified by cross-neutralization tests. Test results are made available online at: http://gateway.calsurv.org/.

Reference

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

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



Key:

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 online at: http://gateway.calsurv.org/ 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 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 ensure that each flock site and accompanying band numbers are registered online at: http://gateway.calsurv.org/. Blood samples sent to VRDL must be accompanied by the form "SENTINEL CHICKEN BLOOD – 2006" (MBVS-2) for each flock site. All forms are available at http://gateway.calsurv.org/ or 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 2006 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

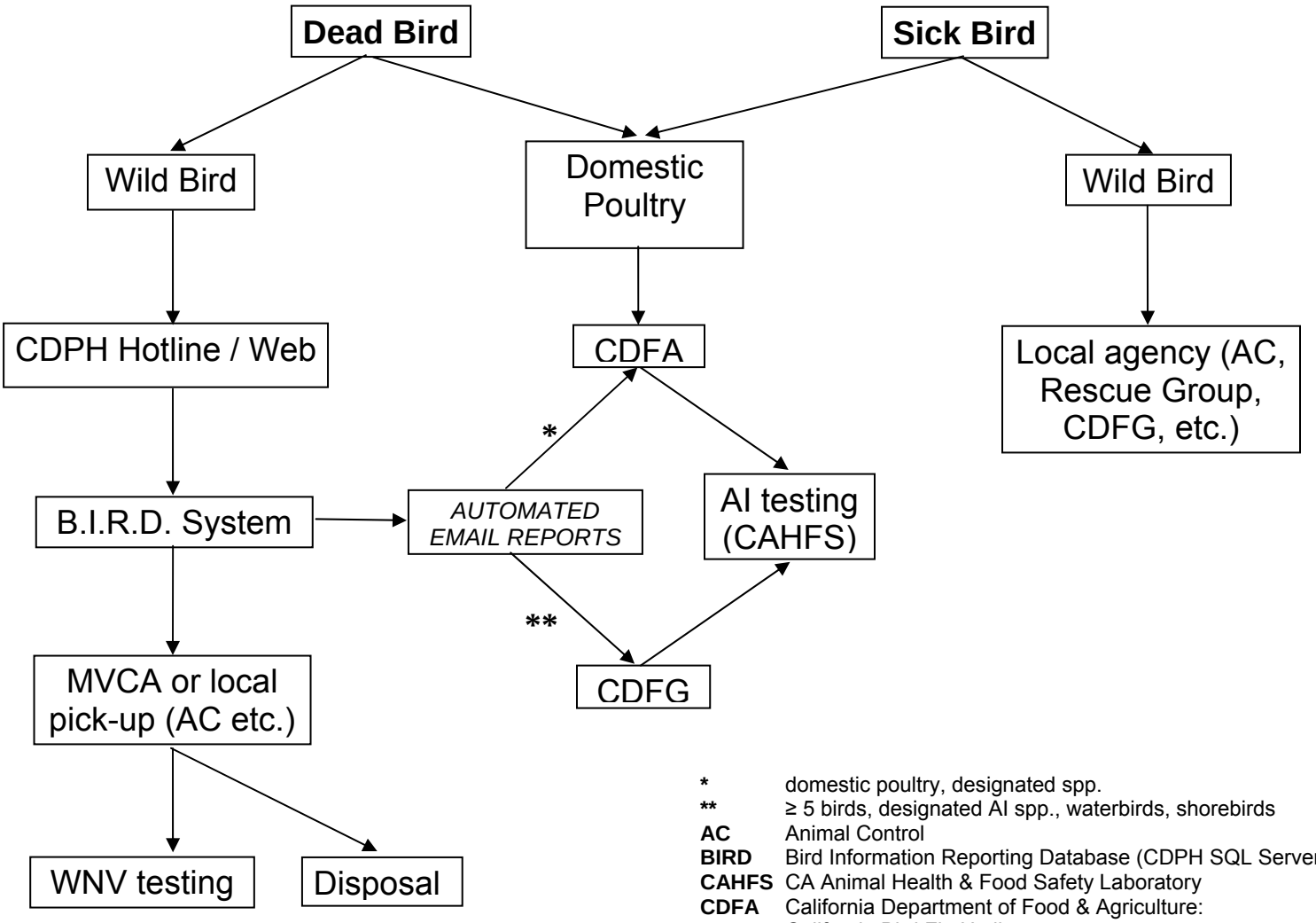
Registration of new sites used for collection of mosquitoes for virus testing may be accomplished by accessing the California Vectorborne Disease Surveillance Gateway (http://gateway.calsurv.org/). The laboratory will test the pools provided that adequate information is provided on the "MOSQUITO POOL SUBMISSION" form (MBVS-3, revised 01/12/06), including your agency code, your site code for the site and geographic coordinates. 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 show all registered sites and test results for each site. Also, as part of a collaborative effort, CVEC will host real-time maps in ArcGIS format at http://maps.calsurv.org. In addition to these maps , agencies can access maps using Google Earth through the California Vectorborne Disease Surveillance Gateway (http://surv.mvcac.org) that provide enhanced functionality and detail.

Appendix D: Procedures for Testing Dead Birds and Squirrels

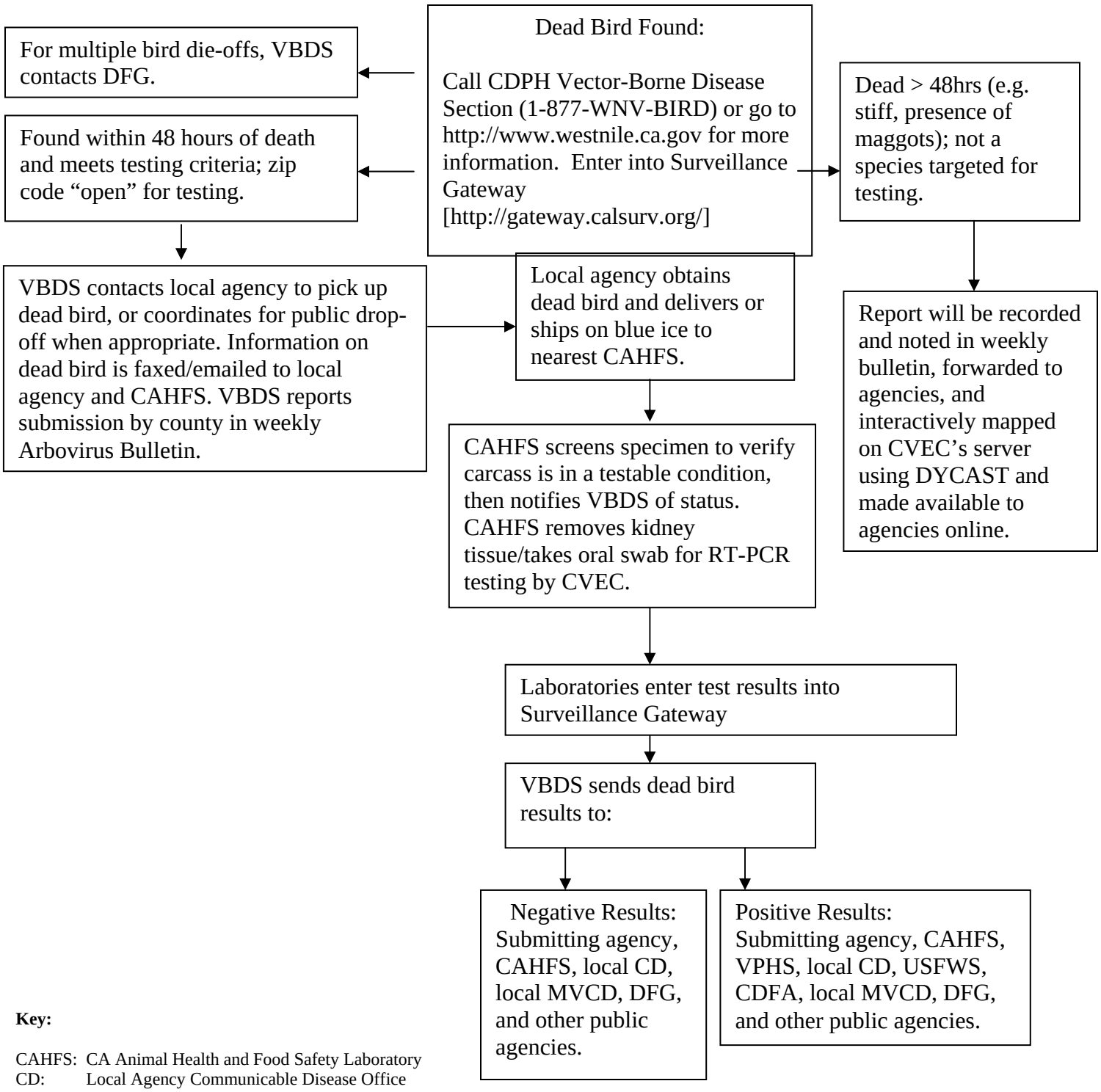
In 2000, CDHS initiated a dead bird surveillance program in collaboration with other public agencies. CDPH annually notifies about 600 agencies, organizations, and veterinarians involved with wildlife, including rehabilitation centers, about the program. The public is also notified about the program through the media and outreach materials. Dead birds are reported to CDPH or data entered electronically through the Surveillance Gateway [http://gateway.calsurv.org/] and shipped to the California Animal Health & Food Safety (CAHFS) laboratory at UC Davis for screening and removal of kidney tissue (an oral swab is taken instead if the bird is an American Crow), which is then sent to the UC Davis Center for Vectorborne Diseases (CVEC) for WNV RNA detection via RT-PCR. Overviews of the dead bird reporting and testing algorithms are provided below.

Sick / Dead Bird Reporting Protocol for Public and Local Agencies



*	domestic poultry, designated spp.
**	≥ 5 birds, designated AI spp., waterbirds, shorebirds
AC	Animal Control
BIRD	Bird Information Reporting Database (CDPH SQL Server)
CAHFS	CA Animal Health & Food Safety Laboratory
CDFA	California Department of Food & Agriculture: California Bird Flu Hotline: **1-866-922-BIRD**
CDFG	California Department of Fish & Game http://www.dfg.ca.gov/regions/index.html
CDPH	California Department of Public Health West Nile virus & Dead Bird hotline: **1-877-968-BIRD** website: www.westnile.ca.gov
MVCA	Mosquito & Vector Control Agency

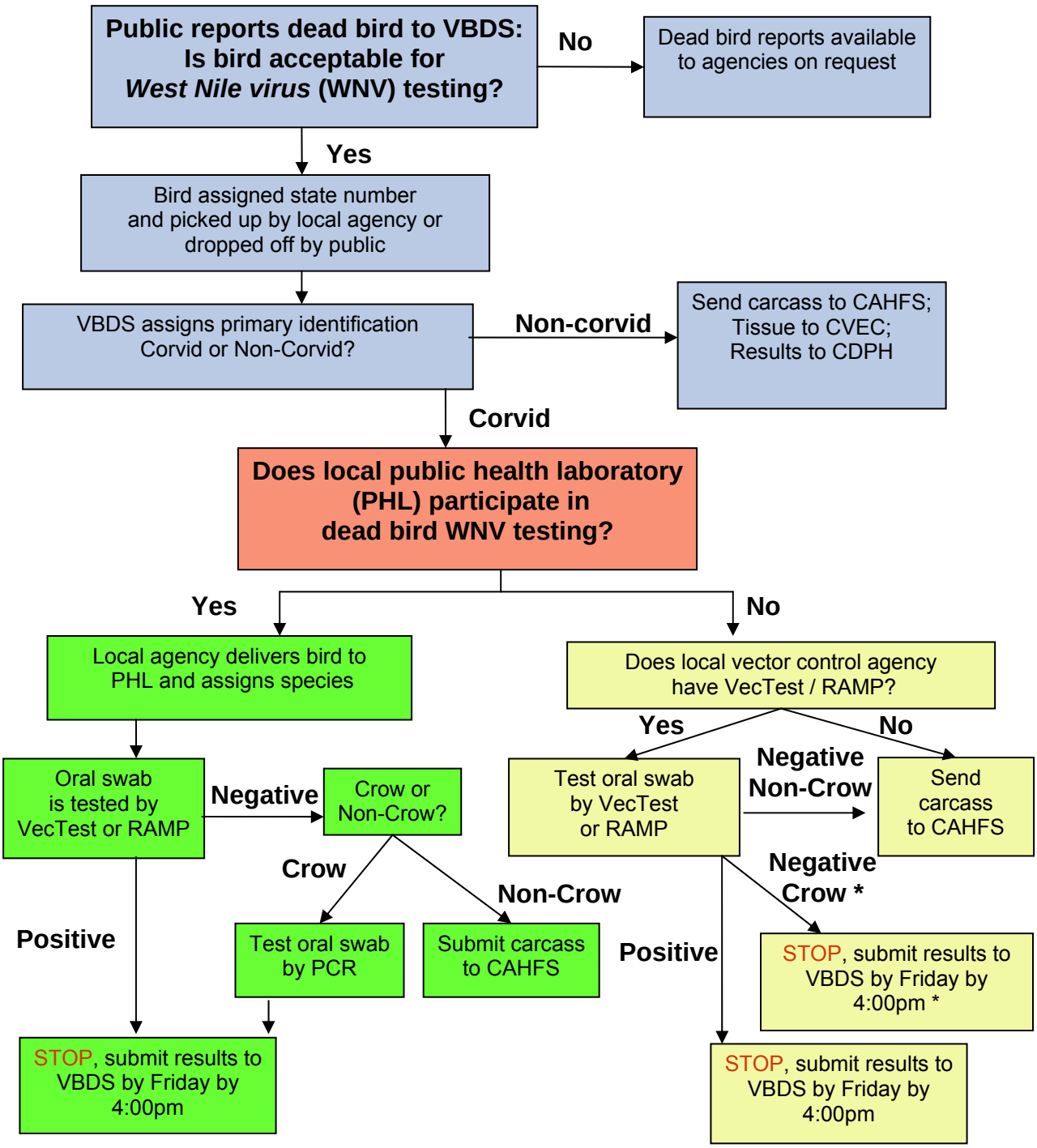
Procedures for Testing Dead Birds: RT-PCR



Key:

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

Procedures for Testing Dead Birds: Rapid Assays



** Please submit carcasses of ALL birds that test negative to CAHFS for further evaluation. Once a county has detected WNV in a dead bird, American Crows will no longer need to be submitted for confirmatory testing.*

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

VBDS
Public Health Labs
Local Agencies

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

When your agency receives a call from the public about a dead bird (especially recently dead crows, ravens, magpies, jays, or raptors) or dead tree squirrel, or one of your staff finds any dead bird, please immediately refer them to the **CDPH West Nile Virus and Dead Bird Hotline at 1-877-968-BIRD (2473)**.

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

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

Members of the public may salvage dead birds found on their property or place of residence. **The public must first call the Dead Bird Hotline and obtain a Dead Bird Number**; a corresponding public salvage submission form will then be faxed to the appropriate agency. The public will be instructed by the hotline staff to double-bag the carcasses and drop them off at the designated agency within 24 hours, between 9 am - 3 pm, Monday – Friday, and **only in areas where local agencies are not picking up dead birds** (e.g., closed zip codes), unless otherwise requested by the local agency. **Note: only dead birds may be brought in by the public to local agencies for shipping. We discourage public salvage of all squirrels because ground squirrels, which could be infected with plague, may be misidentified as tree squirrels.**

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

Once the submission is approved, your agency can ship the carcass to the California Animal Health & Food Safety laboratory at UC Davis (CAHFS Central). Carcasses delivered to CAHFS Turlock, Fresno, or San Bernardino laboratories will be transported by CAHFS to the UC Davis laboratory. CAHFS Central removes specific tissues and forwards the samples to the UC Davis Center for Vectorborne Diseases (CVEC) for WNV testing. Shipping and testing expenses will be paid by CDPH.

NEW!! Carcasses are considered **Category B, Biological Substances.** This replaces the old designation, “Diagnostic Specimen”.

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

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

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

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

- **If upon pick-up the carcass is found to be unacceptable (e.g. a species your agency or CDPH is not accepting or a badly decomposed specimen), please collect the carcass, double-bag it, and dispose of it in a secure garbage can or dumpster.** California Department of Fish & Game prefers that you burn or bury the carcass, but disposing of it in a dumpster is also acceptable. **Please call CDPH immediately and notify us that the animal will no longer be submitted.**

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

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

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

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

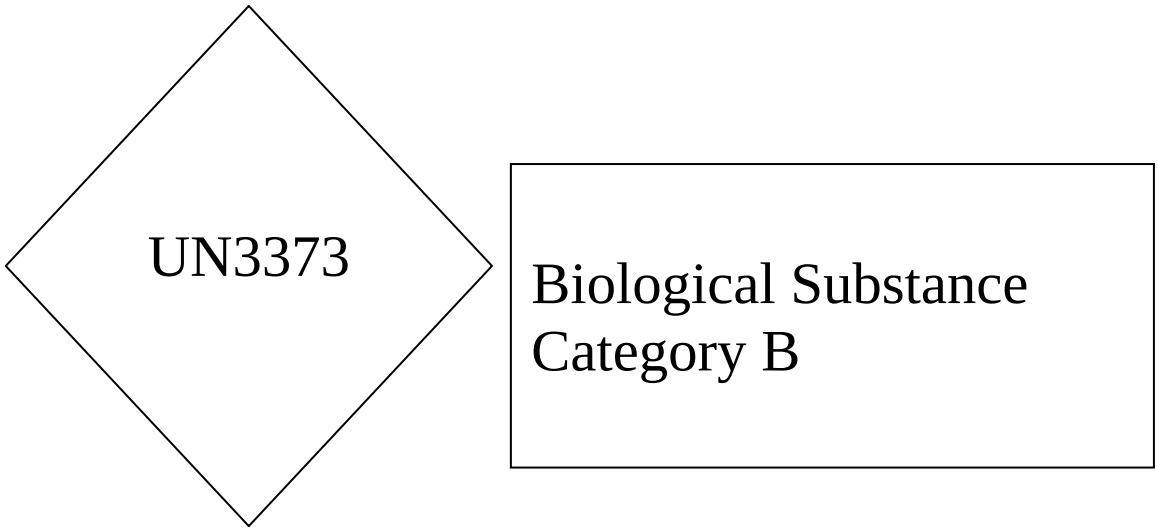
- Contact **UPS** to pick up carcasses either by web (https://wwwapps.ups.com/pickup/schedule?loc=en_US) or by phone **1-800-PICK UPS** (1-800-742-5877)**. Select "UPS Next Day Air"** and **estimate the weight of the box** (generally 10 lbs for a single large bird packed with ice). Please DO NOT UNDER-ESTIMATE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

- If your 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).

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

- Label the outside of the package with the words **ATTN: WNV** above the designated CAHFS address. **Attach the required shipping stickers/labels (Biological Substance, Category B and UN3373) according to the instructions, all provided by CDPH.** (The drawings below are examples of the required shipping stickers/labels. *Please note: these drawings are NOT drawn to scale.*)

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



Dead Bird Shipping List

Please verify that your agency has the following items:

- CAHFS Address (see below)
- UN3373 Stickers
- Biological Substance, Category B Stickers
- GSO or UPS preprinted labels
- WNV hotline number (877-968-BIRD; manned 8am - 5pm, 7 days a week)
- Crumpled newspapers or another absorbent material
- Rubber or Latex Gloves
- Packing tape
- Dead Bird Shipping Boxes
  - inner zip-lock bag
  - outer zip-lock bag
  - inner styrofoam box
  - outer cardboard box
  - blue ice packs

California Animal Health & Food Safety (CAHFS) laboratories:

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

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

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

CAHFS San Bernardino (909) 383-4287 (*FORWARDS CARCASSES TO CENTRAL*)
ATTN: WNV
Dr. Hilu Kinde
105 West Central Avenue
San Bernardino, Ca 92408

Appendix E: Procedures for Testing Equines and Ratites

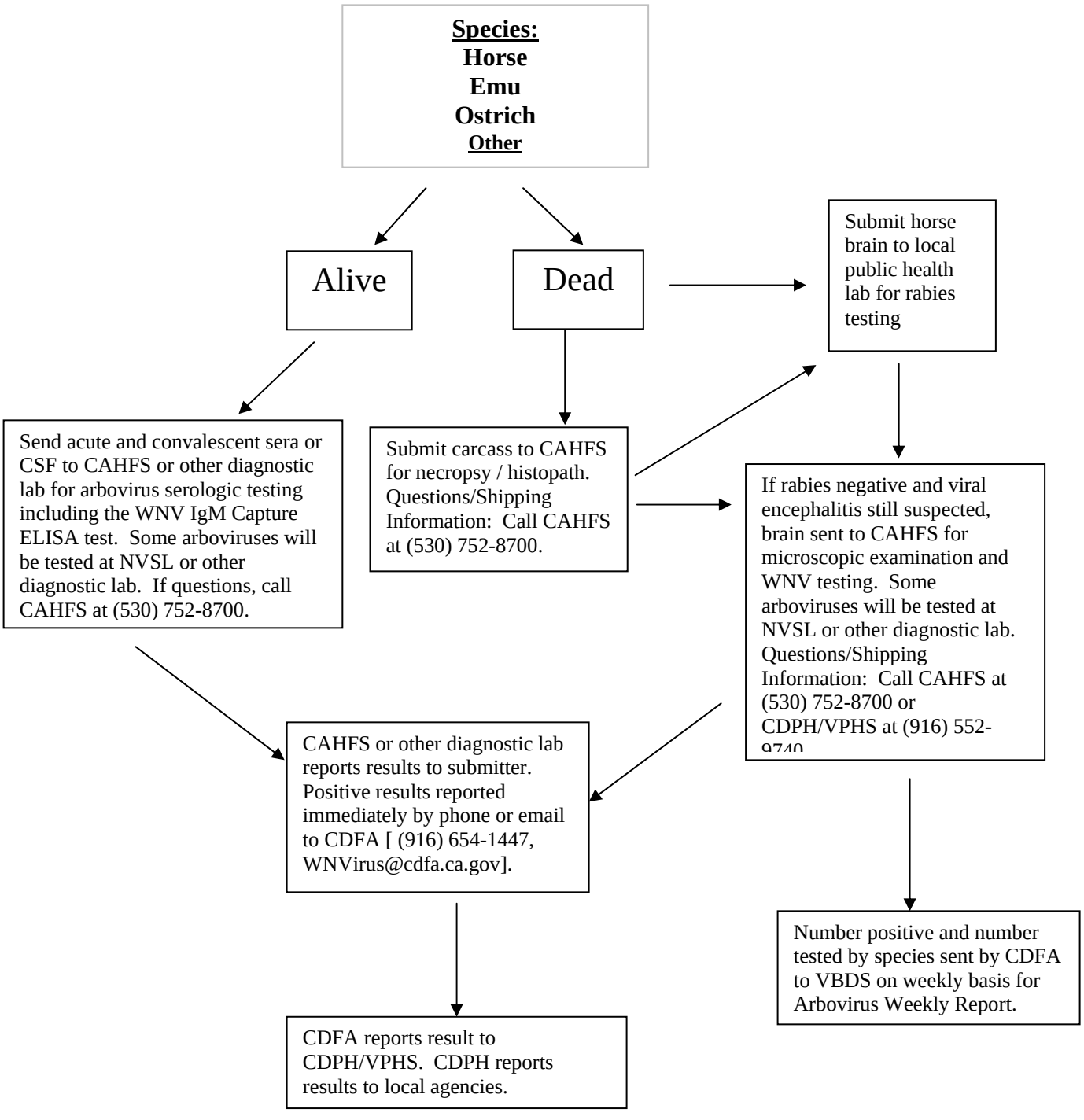
The California Department of Public Health (CDPH) and the California Department of Food and Agriculture (CDFA) have a well-established passive surveillance program for equine and ratite encephalomyelitis. Equine encephalomyelitides is legally reportable to CDFA by veterinarians and diagnostic laboratories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

This appendix contains information sent to veterinarians, public health lab directors, local health officers, public health veterinarians, animal health branch personnel, and interested parties every spring to inform them about the California Equine and Ratite Arbovirus Surveillance Program. The mailing includes a case definition for equine encephalomyelitides and instructions for specimen collection and submission for both equine and ratite samples. The information is distributed to approximately 1,200 practitioners, equine organizations, and other interested parties. Specimen submission is coordinated through the California Animal Health and Food Safety Laboratory System's (CAHFS) 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. CDPH and CDFA have developed and distributed educational materials concerning the diagnosis and reporting of arboviruses in equines and ratites. CDPH 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 the CDFA website: 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: 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: www.westnile.ca.gov; a special section for veterinarians and horse owners is available at: www.westnile.ca.gov/resources.php.

Algorithm for Submission of Specimens from Domestic Animals with Neurologic Symptoms



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

06/12/06
CDPH/VPHS

SURVEILLANCE CASE DEFINITIONS FOR WEST NILE VIRUS DISEASE IN EQUINES - 2007

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.

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 May 2007

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.
- **NOTE**: For WNV, an acute sample only is required since the assay used detects IgM (and vaccine does not interfere). For the other encephalitis viruses, the acute sample should be submitted immediately, and a convalescent sample may be requested later to assist with the interpretation and differentiation of vaccine titers from active infection.

B. Brain

- The local health department and 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*. Protocol for submission of these specimens may be coordinated through the CAHFS Laboratory, and may include sampling for equine herpesvirus, EPM, or other agents associated with clinical neurological presentations.

2. **Submission forms**: Complete and include the transmittal forms supplied by CAHFS. Call 530-752-8700 or visit the CAHFS website at http://cahfs.ucdavis.edu. The submittal form for each specimen should be placed in a leak-proof plastic bag 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 or without additional signs of brain dysfunction. Less common neurological syndromes can include cranial and peripheral neuritis/neuropathies, including Guillain-Barré syndrome.

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

Case Classification:

A clinically compatible illness, *plus*:

Confirmed:

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

Probable:

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

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

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

3. Pyrethroids (synthetic pyrethrin products containing deltamethrin, 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 5/31/06)

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 (5/31/06)

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
Lambda-cyhalothrin	Demand CS	100-1066	Syngenta	Liquid	Adults	Pryethroid

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 Vectorborne Disease Surveillance Gateway	http://surv.mvcac.org	Data management system for California's mosquito control agencies.
California Data Exchange Center	http://cdec.water.ca.gov	Water-related data from the California Department of Water Resources, including historical and current stream flow, snow pack, and precipitation information.
UC IPM Online	http://www.ipm.ucdavis.edu	Precipitation and temperature data for stations throughout California; also allows calculation of degree-days based on user-defined data and parameters.
National Weather Service – Climate Prediction Center	http://www.cpc.ncep.noaa.gov/products/predictions/	Short-range (daily) to long-range (seasonal) temperature and precipitation forecasts. Also provides El Niño-related forecasts.
California Agricultural Statistics Service	http://www.nass.usda.gov/ca/	Crop acreage, yield, and production estimates for past years and the current year's projections. Reports for particular crops are published at specific times during the year – see the calendar on the website.
US Environmental Protection Agency – Mosquito Control	http://www.epa.gov/pesticides/factsheets/skeeters.htm	Describes the role of mosquito control agencies and products used for mosquito control.
US Centers for Disease Control and Prevention – West Nile Virus	http://www.cdc.gov/ncidod/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.