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

Advantages and Disadvantages of Mosquito Sampling Methods:

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

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

New Jersey (American) Light Trap (NJLT)

Operation

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

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

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

Processing

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

CDC style CO_2-baited trap

Operation

Carbon dioxide-baited traps can be used for abundance monitoring or capturing mosquitoes for virus testing. Traps should be hung from a 6-foot tall standard (approximately 4 feet above ground level) to standardize trap placement for population and virus infection rate monitoring. Knowledge of the host-seeking patterns of the target species is essential in determining CO_2-baited trap placement in the habitat to enhance catch size and therefore sampling sensitivity. *Culex tarsalis* primarily bloodfeed on birds and hunt along vegetative borders and tree canopies where birds roost and nest. *Culex erythrothorax* are best collected within wetland areas near

dense stands of tules and cattails. In large, open breeding sources such as rice fields, CO_2-baited traps could be hung on standards on the up-wind side of the source for *Culex tarsalis* and *Anopheles freeborni* collections. *Aedes melanimon* and *Aedes nigromaculis* are mammal feeders and typically seek hosts over open fields.

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

Processing

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

Reiter/Cummings gravid traps

Trap design and components

The Reiter/Cummings gravid traps consist of a rectangular trap housing [plastic tool box] with an inlet tube on the bottom and an outlet tube on the side or top. The rectangular housing is provided with legs to stabilize the trap over the attractant basin containing the hay-infusion mixture. (Cummings 1992). The oviposition attractant consists of a fermented infusion made by mixing hay, Brewer's yeast and water. The mixture should sit at ambient temperature for a minimum of three to four days prior 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 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-1-11; where 11 refers to year 2011. Data on each pool can be entered directly in electronic format through the California Vectorborne Disease Surveillance Gateway (http://gateway.calsurv.org/). POOLS MUST BE ACCOMPANIED BY "MOSQUITO POOLS SUBMITTED FORM MBVS-3" AND CAN ONLY BE TESTED FROM REGISTERED SITES. Surveillance sites should be registered online at: **http://gateway.calsurv.org/.** Faxed registration forms (MBVS-1) will be accepted from agencies without adequate internet access.

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

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

not to allow pools to defrost during storage or shipment, because each freeze-thaw cycle may result in a 10-fold decrease in viral titer, and all virus will be lost if the specimens sit at room temperature for extended periods. Address shipment to: Center for Vectorborne Diseases, University of California, VetMed3A room 4206, 1 Shields Ave, Davis CA 95616. Pools received by Wednesday will be tested and reported through the Gateway the same week.

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

Appendix C: Procedures for Maintaining and Bleeding Sentinel Chickens

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

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

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

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

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

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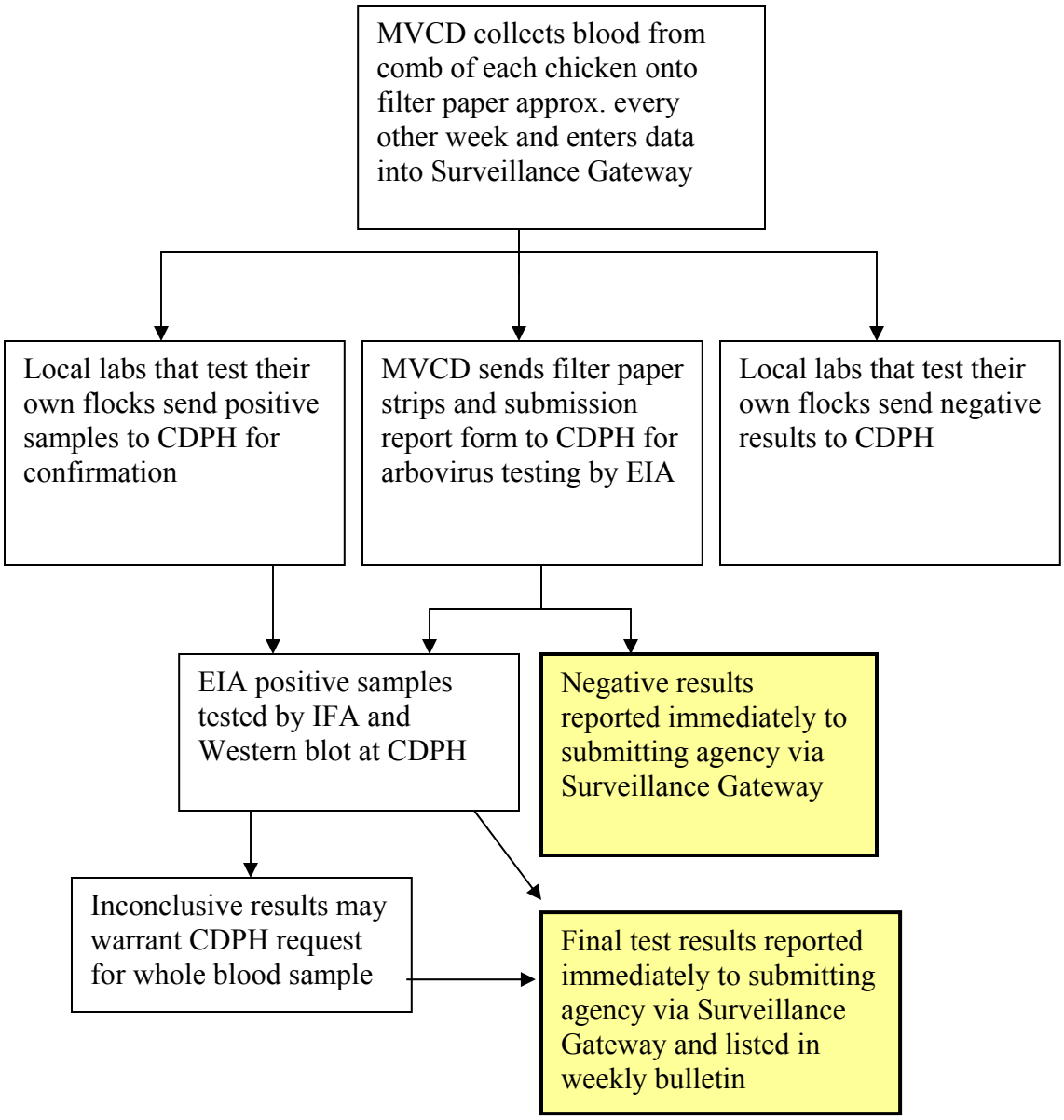
Appendix C

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



Key:

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

Surveillance for Mosquito-borne Viruses
Registration of Agencies and Sites

1. Participation of agencies

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

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

2. Registration of sentinel flock sites and wing band numbers

Agencies must use the unique band numbers assigned to their district by CDPH each year. Prior to submitting any sentinel chicken blood samples to CDPH, each agency must ensure that each flock site and accompanying band numbers are registered online at: http://gateway.calsurv.org/. CDPH will only test samples if they are accompanied by the form "SENTINEL CHICKEN BLOOD – 2011" (MBVS-2) for each flock site, which includes the registered agency code, the registered site code (assigned by local agency), the wing band numbers assigned to that site, and date bled. **Also, the form should indicate any changes made and match the sample card exactly.**

3. Registration of mosquito sampling sites

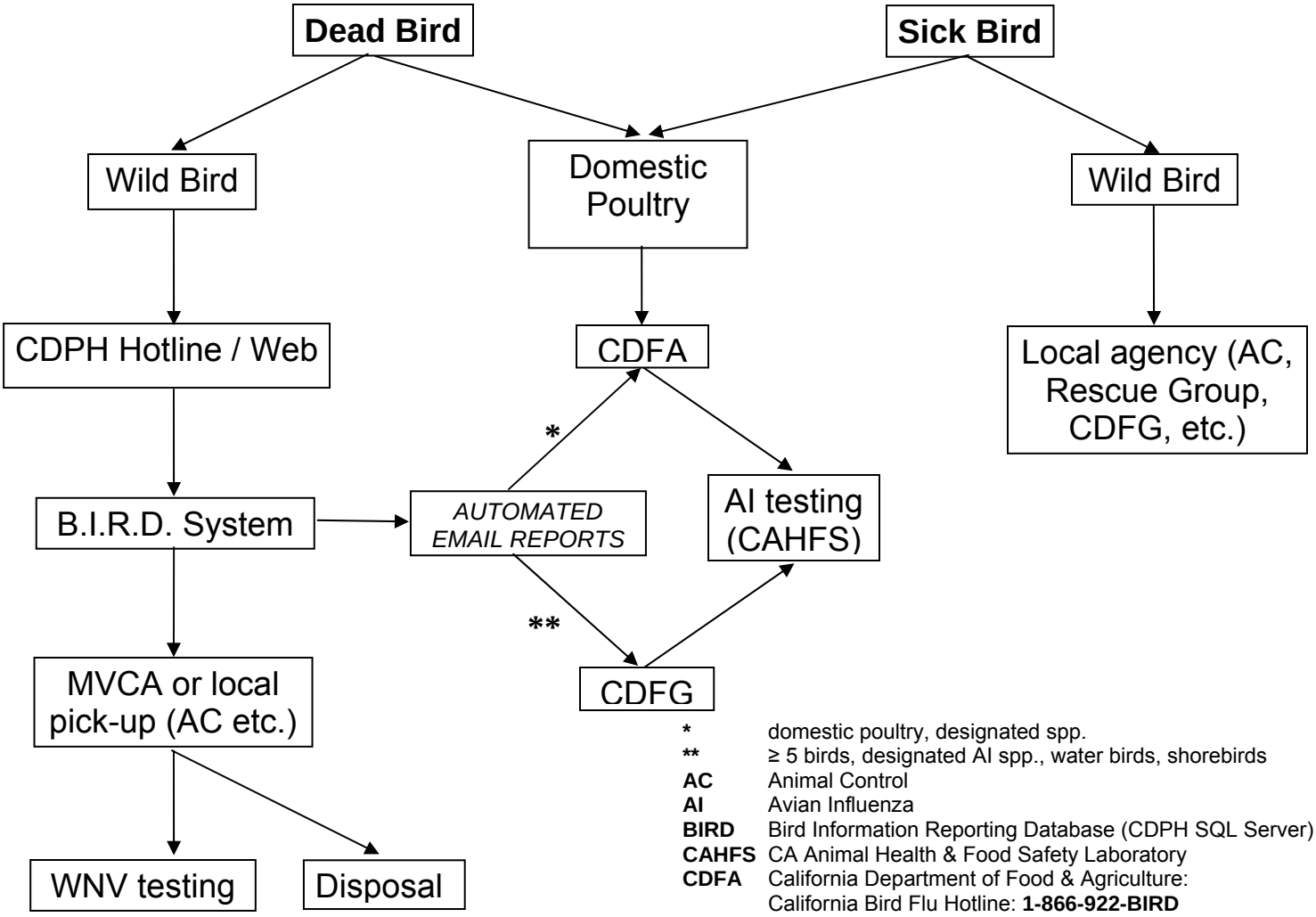
Registration of new sites used for collection of mosquitoes for virus testing may be accomplished by accessing the California Vectorborne Disease Surveillance Gateway http://gateway.calsurv.org/. Since 2010, the CalSurv Gateway has included enhanced spatial capabilities that allow users the option of directly entering geographic coordinates for sites or interactively selecting the location using a new Google Maps-based interface. The laboratory will test the pools provided that adequate information is provided on the "MOSQUITO POOL SUBMISSION" form (MBVS-3, revised 01/12/06), including your agency code, your site code for the site and geographic coordinates.

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

Appendix D: Procedures for Testing Dead Birds and Squirrels

In 2000, CDHS initiated a dead bird surveillance program in collaboration with other public agencies. CDPH annually notifies about 600 agencies, organizations, and veterinarians involved with wildlife, including rehabilitation centers, about the program. The public is also notified about the program through the media and outreach materials. Dead birds and squirrels are reported to CDPH or data entered electronically through the Surveillance Gateway [http://gateway.calsurv.org/] and shipped to the California Animal Health & Food Safety (CAHFS) laboratory at UC Davis for screening and removal of kidney tissue (an oral swab is taken instead if the bird is an American Crow), which is then sent to the UC Davis Center for Vectorborne Diseases (CVEC) for WNV RNA detection via RT-PCR. Beginning in 2010, results from RT-PCR testing at CVEC distinguished between WNV recent and chronic positive birds based on cycle threshold (Ct) values. Chronic positive birds did not likely die from WNV infection and are of limited value for surveillance. Overviews of the dead bird reporting and testing algorithms are provided below.

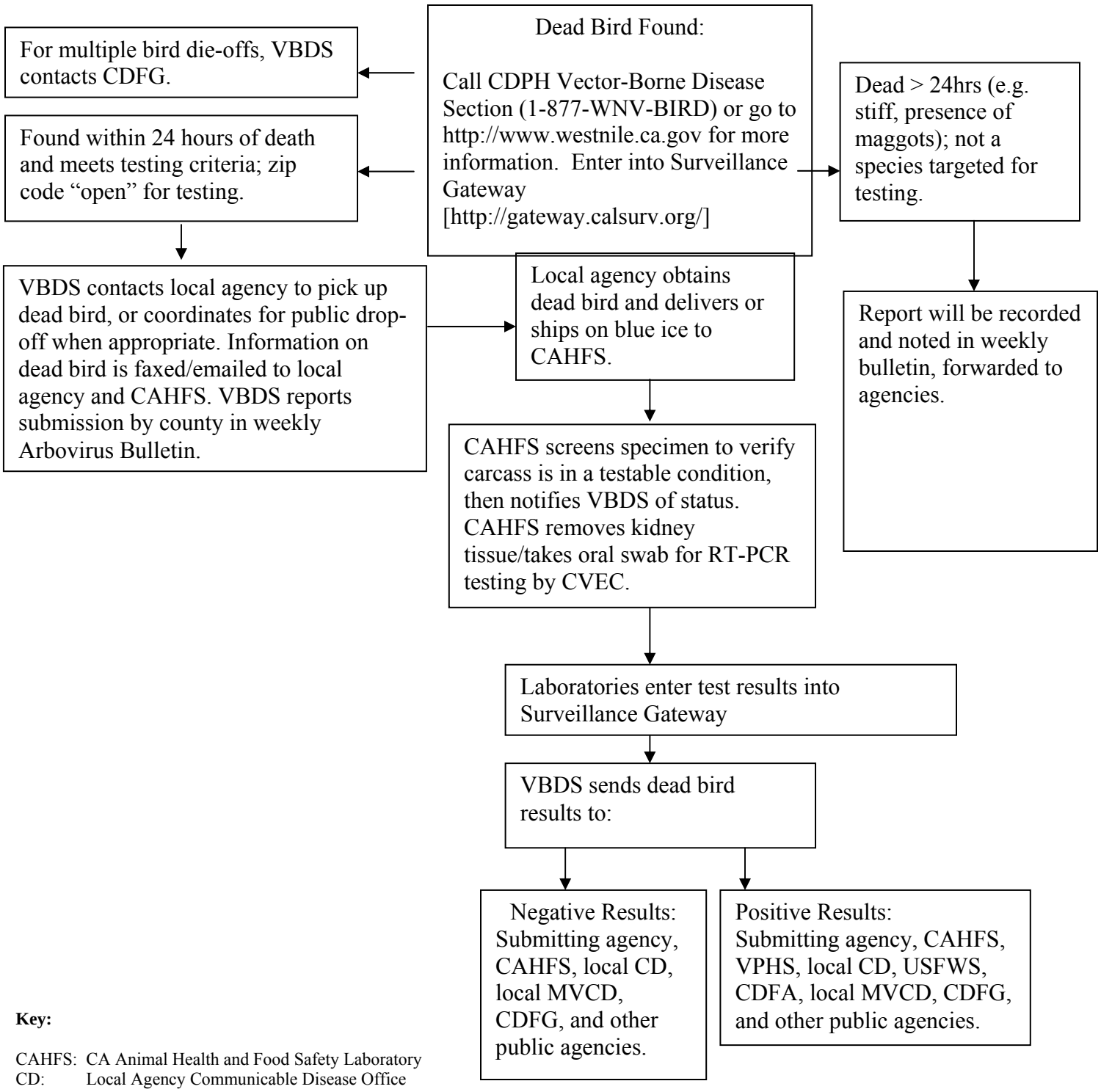
Sick / Dead Bird Reporting Protocol for Public and Local Agencies



*	domestic poultry, designated spp.
**	≥ 5 birds, designated AI spp., water birds, shorebirds
AC	Animal Control
AI	Avian Influenza
BIRD	Bird Information Reporting Database (CDPH SQL Server)
CAHFS	CA Animal Health & Food Safety Laboratory
CDFA	California Department of Food & Agriculture: California Bird Flu Hotline: **1-866-922-BIRD**
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

Appendix D

Procedures for Testing Dead Birds: RT-PCR

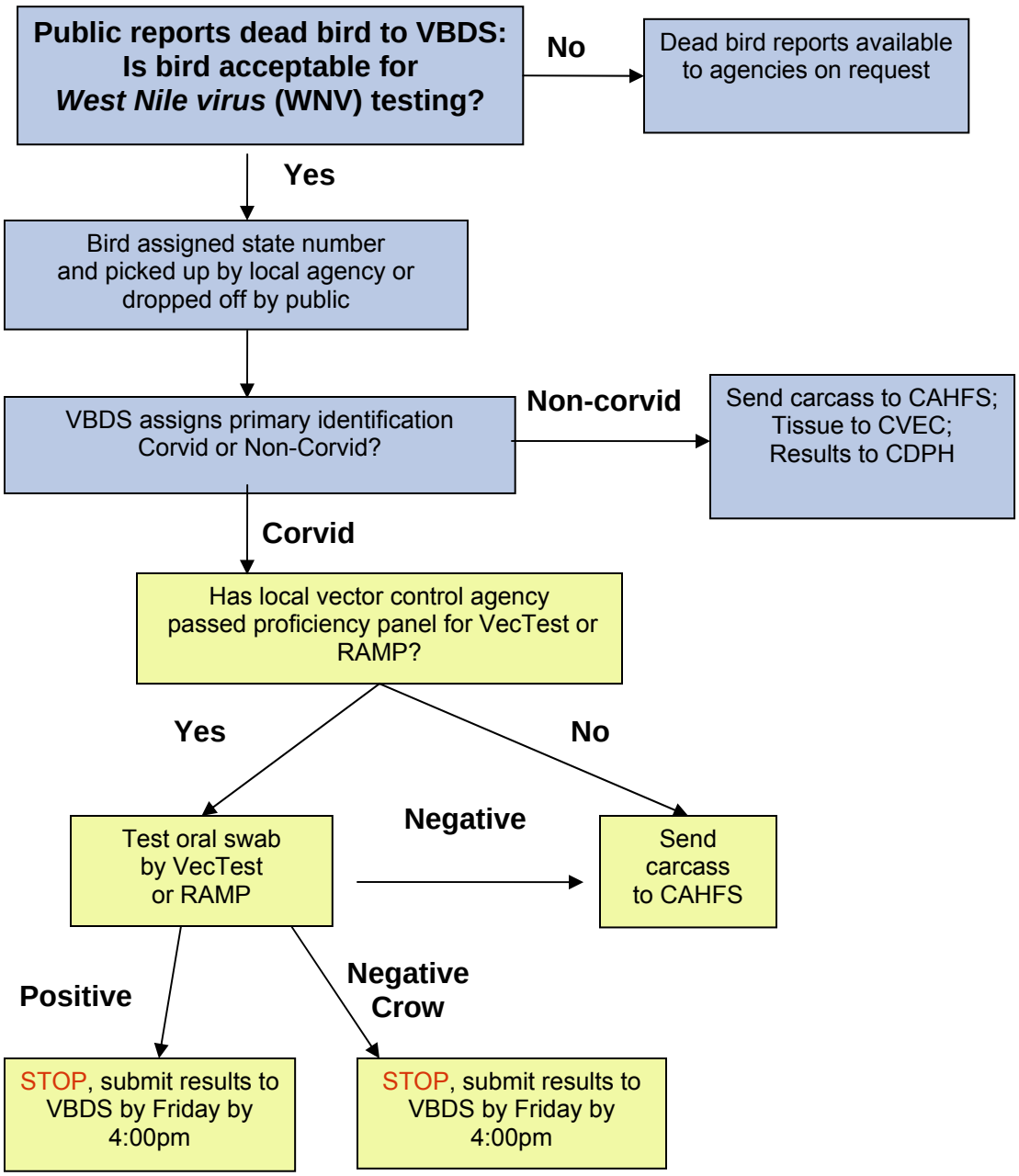


Key:

CAHFS: CA Animal Health and Food Safety Laboratory
CD: Local Agency Communicable Disease Office
CDFA: CA Dept. of Food and Agriculture
CDFG: CA Dept. of Fish and GameCVEC: UC Davis Center for Vectorborne Diseases
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

Appendix D

Procedures for Testing Dead Birds: Rapid Assays



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

VBDS

Local Agencies

Appendix D

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

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

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

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

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

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

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

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

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

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

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

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

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

- Enclose the shipping documents into a SEPARATE ZIP-LOCK BAG. The primary shipping document is a copy of the dead bird submission form which contains the dead bird number and which is located on the Surveillance Gateway [http://gateway.calsurv.org/] or faxed by CDPH. CAHFS prefers that you put this separate zip-lock bag inside the 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: 23219W.**

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

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

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

Dead Bird Shipping List

Please verify that your agency has the following items:

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

California Animal Health & Food Safety (CAHFS) laboratories:

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

Appendix E: Procedures for Testing Equines and Ratites

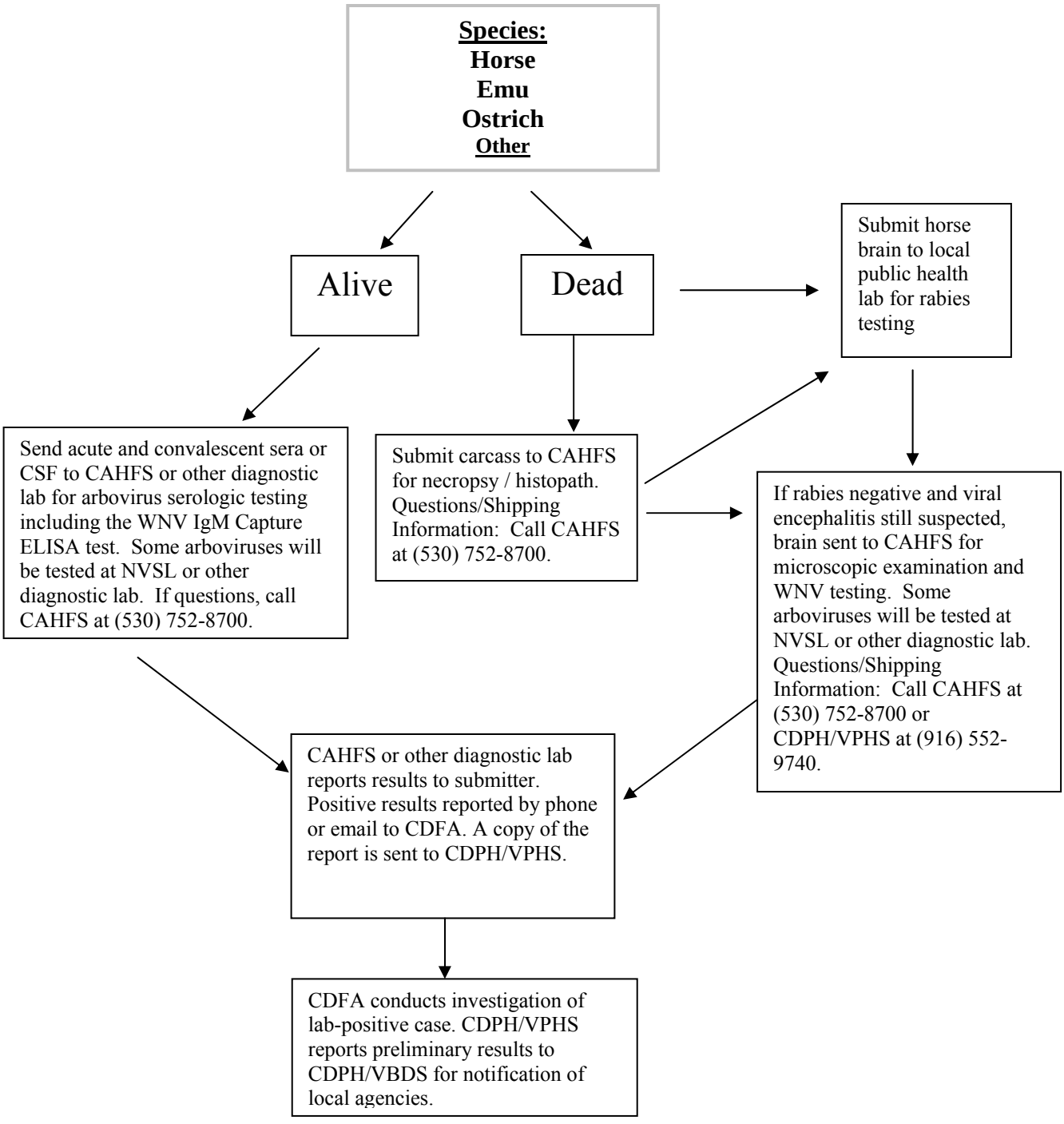
The California Departments of Public Health (CDPH) and Food and Agriculture (CDFA) developed a cooperative passive surveillance program for equine and ratite encephalomyelitis. Primary responsibility for equine and ratite West Nile virus (WNV) surveillance rests with the CDFA. Equine encephalomyelitides are legally reportable to CDFA by veterinarians and diagnostic laboratories pursuant to Section 9101 of the Food and Agricultural Code. Venezuelan equine encephalomyelitis is an emergency animal disease that must be reported to CDFA by telephone within 24 hours. Eastern and western equine encephalomyelitis and WNV are a classified as conditions of regulatory importance and must be reported to CDFA within 2 days.

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

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

Algorithm for Submission of Specimens from Domestic Animals with Neurologic Symptoms



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

SURVEILLANCE CASE DEFINITIONS FOR WEST NILE VIRUS DISEASE IN EQUINES

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

CONFIRMED CLINICAL CASE:

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

Plus one or more of the following:

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

SUSPECT CLINICAL CASE[4]:

- Compatible clinical signs

EXPOSED EQUID:

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

Assumptions on which case definition is based:

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

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

[2] The first serum should be drawn as soon as possible after onset of clinical signs and the second drawn at least seven days after the first.

[3] For horses it is recommended that RT-nested polymerase chain reaction assay be used to maximize sensitivity of the test (Emerg. Infect. Dis. 2001 Jul-Aug; 7(4):739-41)

[4] An equine case classified as a suspect case should, if possible, undergo further diagnostic testing to confirm or rule out WNV as the cause of the clinical illness.

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

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

1. Specimen collection and submission:

A. Blood

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

B. Brain

- The local health department and CDFA/Animal Health District Office should be contacted if rabies is suspected.
- All equine specimens submitted to local public health laboratories for rabies testing and found to be negative, should be sent to CAHFS for arbovirus testing.
- Submission of the intact head is preferable because: 1) brain is better preserved (anatomically and virus titer) when left in the skull during transport, 2) specimens will be ruined if removal is not done correctly, and 3) brain removal in field conditions may increase the risk of exposure to rabies.
- **The intact head should be chilled (refrigerated, *not* frozen) immediately after removal. Submit it to a CAHFS Laboratory* in your area as quickly as possible.** Prepare a leak-proof insulated transporting container with "cold packs" to keep the specimen at 4^{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. It is critical that each specimen submission form be completed in its entirety, including the horse's clinical signs, vaccination history, and location during the two weeks prior to onset. The submittal form for each specimen should be placed in a leak-proof plastic bag. The specimen is collected in a leak proof plastic tube or bag and then placed in to a secondary leak proof plastic bag. The submission form is then attached to the corresponding container and shipped to CAHFS.
3. **Shipment:** Check with the CAHFS Laboratory in your area for assistance with shipping regulations governing the transportation of infectious materials.

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

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

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

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

Required specimens:

- Acute serum: ≥ 2cc serum

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

- Cerebral spinal fluid (CSF): 1-2cc CSF for further testing at CDC (N.B. these results may not be available for several weeks)

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

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

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

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

West Nile virus infection is reportable to local health departments under Title 17 of the California Code of Regulations. Below is the case definition for West Nile virus disease as summarized by the Centers for Disease Control and Prevention (CDC) [available at http://www.cdc.gov/ncidod/dvbid/westnile/clinicians/surveillance.htm#casedef]. Blood donors that test positive for West Nile virus through blood bank screening should also be reported to CDPH, regardless of clinical presentation.

CASE DEFINITION: West Nile Virus

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

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 ranging from febrile headache to aseptic meningitis to encephalitis may occur, and these are usually indistinguishable from similar syndromes caused by other viruses. Arboviral meningitis is characterized by fever, headache, stiff neck, and pleocytosis. Arboviral encephalitis is characterized by fever, headache, and altered mental status ranging from confusion to coma with or without additional signs of brain dysfunction (e.g., paresis or paralysis, cranial nerve palsies, sensory deficits, abnormal reflexes, generalized convulsions, and abnormal movements).

Laboratory Criteria for Diagnosis

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

Case Classification

- *Probable:* An encephalitis or meningitis case occurring during a period when arboviral transmission is likely and with the following supportive serology: 1) a single or stable (less than or equal to twofold change) but elevated titer of virus-specific serum antibodies; or 2) serum IgM antibodies detected by antibody-capture EIA but with no available results of a confirmatory test for virus-specific serum IgG antibodies in the same or a later specimen.
- *Confirmed:* An encephalitis or meningitis case that is laboratory confirmed.

Comment

- Because closely related arboviruses exhibit serologic cross-reactivity, positive results of serologic tests using antigens from a single arbovirus can be misleading. In some circumstances (e.g., in areas where two or more closely related arboviruses occur, or in imported arboviral disease cases), it may be epidemiologically important to attempt to pinpoint the infecting virus by conducting cross-neutralization tests using an appropriate battery of closely related viruses. This is essential, for example, in determining that antibodies detected against St. Louis encephalitis virus are not the result of an infection with West Nile (or dengue) virus, or vice versa, in areas where both of these viruses occur.
- The seasonality of arboviral transmission is variable and depends on the geographic location of exposure, the specific cycles of viral transmission, and local climatic conditions.

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

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

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

Additional serological testing is not required. Local health departments should follow up with the donor after two weeks of the date of donation to assess if the patient subsequently became ill. If the donor did become ill as a result of WNV infection, an updated case report form should be sent to CDPH so that the blood donor may be reclassified as a clinical case.

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

Appendix H: Compounds Approved for Mosquito Control in California

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

Larvicides:

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

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

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

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

5. Larviciding oils (e.g. 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.

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

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

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

Adulticides:

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

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

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

Appendix H

PESTICIDES USED FOR MOSQUITO CONTROL IN CALIFORNIA

Larvicides

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

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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

Appendix H

PESTICIDES USED FOR MOSQUITO CONTROL IN CALIFORNIA

Adulticides

Active Ingredient	Trade name	EPA Reg. No.	Mfgr.	Formulation	Application	Pesticide classification
Malathion	Fyfanon® ULV	67760-34	Cheminova	Liquid	Adults	OP
Naled	Dibrom® Concentrate	5481-480	AMVAC	Liquid	Adults	OP
Naled	Trumpet™ EC	5481-481	AMVAC	Liquid	Adults	OP
Prallethrin	Duet Dual Action Adulticide	1021-1795	Clarke	Liquid	Adults	Pyrethroid
Deltamethrin	Suspend® SC	432-763	Aventis	Liquid	Adults	Pyrethroid
Cyfluthrin	Tempo SC Ultra	432-1363	Bayer	Liquid	Adults	Pyrethroid
Permethrin	Aqua-Reslin®	432-796	Bayer	Liquid	Adults	Pyrethroid
Permethrin	Biomist® 4+12 ULV	8329-34	Clarke	Liquid	Adults	Pyrethroid
Permethrin	Permanone® Ready-To-Use	432-1277	Bayer	Liquid	Adults	Pyrethroid
Pyrethrins	Pyrenone® 25-5	432-1050	Bayer	Liquid	Adults	Pyrethroid
Pyrethrins	Pyrenone® Crop Spray	432-1033	Bayer	Liquid	Adults	Pyrethroid
Pyrethrins	Pyrocide® 7396	1021-1569	MGK	Liquid	Adults	Pyrethroid
Resmethrin	Scourge® Insecticide (4%)	432-716	Bayer	Liquid	Adults	Pyrethroid
Resmethrin	Scourge® Insecticide (18%)	432-667	Bayer	Liquid	Adults	Pyrethroid
Sumithrin	Anvil® 10+10 ULV	1021-1688	Clarke	Liquid	Adults	Pyrethroid
Etofenprox	Zenivex E20	2724-791	Wellmark, Intl.	Liquid	Adults	Pyrethroid
Lambda-cyhalothrin	Demand CS	100-1066	Syngenta	Liquid	Adults	Pryethroid

Appendix I: Adult Mosquito Control in Urban Areas

Adult mosquito control via ultra low volume (ULV) application is an integral part of an integrated mosquito management program. This response plan recommends the consideration of adult mosquito control to break local virus transmission cycles and reduce the risk of human infection. The following provides guidelines for local agencies considering ground or aerial ULV control of adult mosquitoes.

Preparatory steps for aerial application contracts

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

Preparatory steps for ground-based applications

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

Implementing an aerial application contract

- Contact commercial applicator and determine availability.

- Review long-term weather forecasts. Ideally applications should be scheduled during periods of mild winds to avoid last minute cancellations.

  Contractor should:

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

  Vector control agency should:

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

Efficacy evaluation for aerial or ground based application

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

Actions at time of application

- Confirm application rate with contractor.

- Confirm treatment block.
- Coordinate efficacy evaluations.

Public notification

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

Basic notification steps

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

Public relations considerations

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

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

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

Reference List

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