



Karen L. Smith, MD, MPH
Director and State Health Officer

State of California—Health and Human Services Agency
California Department of Public Health



EDMUND G. BROWN JR.
Governor

January 11, 2016

TO: PARTICIPATING AGENCIES IN THE 2016 CALIFORNIA
ARBOVIRUS SURVEILLANCE PROGRAM

SUBJECT: 2016 MOSQUITO POOL AND SENTINEL CHICKEN TESTING

Enclosed are instructions for the 2016 arbovirus surveillance season. Order forms for sentinel chicken sera testing should be completed prior to submitting samples. If you have questions about results or submission issues concerning chicken samples or mosquito pools, please contact Tina Feiszli at (510) 412-6253 or tina.feiszli@cdph.ca.gov

IMPORTANT ANNOUNCEMENTS FOR 2016

Mosquito Pool Supplies

Mosquito pool vials and beads are no longer supplied to local agencies by CDPH.

Vials can be purchased through SPEX SamplePrep: www.spexsampleprep.com
or 800-522-7739.

Catalogue Item #: 3116

Description: Polystyrene grinding vial with slip on cap; 12.7 x 51mm

Grinding beads can be purchased through Fisher Scientific: www.fishersci.com or
800-766-7000.

Catalogue Item #: 11-312C

Description: 5mm solid glass beads

Please contact Tina Feiszli if you have any questions about ordering these supplies. They may be available through other vendors, but please be sure to order the correct vials.

Sentinel chicken pick-up dates

Northern region: Pick-up day is scheduled for **April 21, 2016 at Haley Farms in Modesto.**

Southern region: Pick-up day is scheduled for **May 4, 2016 at Demler Egg Ranch in San Jacinto.**

Chickens can be ordered through MVCAC. Order forms can be found at:

<http://www.mvcac.org/2016-sentinel-chicken-orders/>

Table of Contents

Registration of Agencies and Sites	page 3
Procedures for Processing Mosquitoes	page 4
Mosquito Pools Submitted to UCD – 2016 Form	page 5
Instructions for Sentinel Blood Samples	page 6-8
Sentinel Chicken Blood – 2016 Form	page 9
Southern Schedule Districts	page 10
Northern Schedule Districts	page 11
Southern California Schedule for Chicken Sera	page 12
Northern California Schedule for Chicken Sera	page 13

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 sentinel chicken testing through the California Department of Public Health (CDPH). Agencies will be billed in advance for the number of samples to be tested. For mosquito pool testing by UC Davis, agencies no longer need to order or prepay for testing; MVCAC will bill agencies throughout the season for mosquito pool samples received and tested by UC Davis.

Agencies are responsible for registering and updating information for their surveillance sites online at <http://gateway.calsurv.org>. Accurate geographic coordinates in the form of degrees, minutes, and seconds are especially important for registered sites, because they will be used to generate computer maps that will post test results in real-time. These maps are available on the CalSurv Gateway. To ensure accuracy of map coordinates, it is very useful to include street addresses or street intersections. This provides an excellent cross-check of locations by geocoding. Site registration numbers may refer to single collection sites (e.g., a chicken flock), or may be applied to several sites that fall within a 1/2-mile radius (e.g., a group of CO₂ traps for mosquito pools). Questions concerning site registration (including how to convert decimal forms of coordinates to the degree-minute-second form) should be directed to Jody Simpson: jksimpson@ucdavis.edu

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. **Do not use leftover bands from a previous season.** Please contact Tina Feiszli if additional bands are needed at any time throughout the year. Prior to submitting any sentinel chicken blood samples to CDPH, agencies must ensure that each flock site and accompanying bands are registered online at <http://gateway.calsurv.org>. CDPH will test samples only if they are accompanied by the form "SENTINEL CHICKEN BLOOD – 2016" (MBVS-2) for each flock site, which includes 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 to the flock and match the sample card exactly.**

3. Registration of mosquito sampling sites

Registration of new sites used for the collection of mosquitoes for abundance monitoring or virus testing should be done online at: <http://gateway.calsurv.org>. UC Davis will only test mosquito pools from registered sites and they should be accompanied by a "MOSQUITO POOL SUBMISSION" form (MBVS-3), which includes the registered site code and geographic coordinates. If you are unable to determine the geographic coordinates, please provide the nearest address to the location of each site and its site code.

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% 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 (Kramer et al. 1990). TEA should be used either outdoors or under a chemical hood. Collections can be knocked down outdoors using a few drops of TEA, the specimens transferred to Petri dishes, and then taken into the laboratory for processing. If refrigerated, mosquitoes will remain alive in covered Petri dishes for 1 or 2 days without additional anesthesia. If mosquitoes are frozen before processing, sorting to species and enumeration must be done on a chill table to prevent virus loss.
3. Sort mosquito collections to species under a dissecting microscope at 10X to ensure correct identification and to make sure that extraneous mosquito parts (i.e., legs, wings) or other small insects such as chironomids or *Culicoides* are not inadvertently included in the pools. This is extremely important because diagnostics have transitioned from virus isolation to sensitive RNA methods of viral detection. Count and discard dead and dried mosquitoes. Pools are comprised of up to 50 females of each vector species from each collection site 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-1-16, where 16 refers to year 2016. **POOLS MUST BE ACCOMPANIED BY A "MOSQUITO POOL SUBMISSION" FORM (MBVS-3) AND CAN ONLY BE TESTED FROM REGISTERED SITES.**

Surveillance sites should be registered online at: <http://gateway.calsurv.org>. List the site code for each pool which consists of a designated four-letter agency code followed by 6 digits identifying the site (e.g., KERN000001). Number pools consecutively starting with 1 for each calendar year. Pool numbers do not need to follow the ordering of site codes: e.g., pool #1 may be from KERN000001, pool #2 may be from KERN000004, pool #3 may be from KERN000058, etc.

4. Freeze pools immediately at -80°C either with dry ice in an insulated container or in an ultra-low temperature freezer. Pools are shipped frozen on dry ice to UC Davis for testing by a real-time multiplex RT-PCR. Agencies will receive an automated notification that results have been entered into the CalSurv Gateway, as well as a summary of positive pools; additionally, positive pools will be reported weekly in the California Arbovirus Surveillance Bulletin. Each pool is screened for WNV, SLEV, and WEEV by a multiplex qRT-PCR, with positives with CT scores > 35 confirmed by a singleplex RT-PCR with a different set of virus species-specific primers and probes. Care must be taken not to allow pools to defrost during storage or shipment, because each freeze-thaw cycle may result in a decrease in viral titer; all virus will be lost if the specimens sit at room temperature for extended periods.

Ship pools to: **Ying Fang, University of California, One Shields Avenue, VM: PMI (3336 Vet Med 3A), Davis, CA 95616**

**INSTRUCTIONS FOR
SENTINEL BLOOD SAMPLES
January 2016**

REMEMBER TO TAKE A REFERENCE BLOOD SAMPLE FROM EACH OF YOUR CHICKENS ON THE DAY YOU PICK THEM UP. STORE THIS SAMPLE IN THE REFRIGERATOR FOR REFERENCE IN THE EVENT THE BIRD TESTS POSITIVE ON THE FIRST BI-WEEKLY BLEED.

Blood samples (dried on filter paper strips) should be stapled onto index cards and must be accompanied by form MBVS-2 (Sentinel Chicken Blood-2016) **outside** the plastic bag. Do not staple the form to the bag.

INSTRUCTIONS:

Bi-weekly dry blood sample submissions:

1. **Ensure all staff involved follow these instructions exactly**
2. Collect blood samples onto half-inch wide filter paper strips. The blood must completely soak through a ¾ inch length portion of the strip. Use alcohol swabs on comb before bleeding and ensure strip is dried in dust free environment.
3. Each strip must include the date bled and the wing band number.
4. Attach strips to a 5" X 7" card in sequential order, from left to right (example on page 8).
5. Place the soaked-end down. Attach samples firmly to card in the same band number sequence that is listed on the form. Samples should not move.
6. Do not cover the date and band information on the strips.
7. Place the following on the index card:
County, Agency code, Site, and Date Bled.
8. Place card with attached samples in a zip lock plastic bag large enough to fit around card.
Do not staple form to bag.
Do not put form in the bag with the samples.
Do not put more than one sample card per bag.

Ship to:

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

In the laboratory specimens will be tested for SLEV, WEEV, and WNV IgG antibodies using an enzyme immunoassay. Positive specimens will be confirmed with an indirect fluorescent antibody and western blot test as needed. Whole blood/serum may be requested to clarify inconclusive results.

Whole blood or sera sample submissions:

1. Bleed the chicken(s) requested “for confirmation” into a vacutainer tube.
2. If possible, centrifuge whole blood and aliquot serum into micro-centrifuge tube. Do not put whole blood into micro-centrifuge tube.
3. Clearly label the site code, band number, and date bled on tube. Whole blood samples must be shipped on blue ice (ice packs). Serum aliquots should be shipped on dry ice. Whole blood is not serum. Do not send frozen whole blood.
4. A completely filled out copy of form MBVS-2 must accompany each separate confirmation sample submitted. Indicate on the form that the sample is for “confirmation.”
5. Ship sample(s) in an adequate shipping container with appropriate shipment precaution taken. **Containers must be shipped with a label stating: “Biological Substances, Category B, UN3373”.**

Contact Tina Feiszli, VBDS in Richmond (510) 412-6253 concerning any questions about sample submissions or test results.

INSTRUCTIONS FOR SENTINEL BLOOD

Attach filter paper strips to a 5" X 7" card as shown below. Write the date number on the blank end of each strip and write the identifying information shown

COUNTY	THESE ARE CHICKEN BLOOD
AGENCY	SAMPLES
SITE	
DATE	

PLACE STRIPS IN SEQUENTIAL ORDER AS LISTED ON THE FORM FROM LEFT TO RIGHT

STAPLE THIS END

DATE BAND #									
----------------	----------------	----------------	----------------	----------------	----------------	----------------	----------------	----------------	----------------

Note:

To maximum the turn-around-time for results, expedited shipping is recommended, as samples will be tested upon receipt at the laboratory. Agencies will receive an automated notification that results have been entered into the CalSurv Gateway, as well as a summary of positive bands. Additionally, seroconversions will be reported weekly in the California Arbovirus Surveillance Bulletin.

Southern Districts

Start date: May 16, 2016

AGENCY NAME	CODE	COUNTY
Antelope Valley MVCD	ANTV	LOS ANGELES
City of Long Beach	LONG	LOS ANGELES
City of Moorpark VC	MOOR	VENTURA
Coachella Valley MVCD	COAV	RIVERSIDE
Greater LA Co. VCD	GRLA	LOS ANGELES
L.A. County West VCD	LACW	LOS ANGELES
Northwest MVCD	NWST	RIVERSIDE
Riverside Co. Env. Health	RIVR	RIVERSIDE
San Bernardino Co. VCP	SANB	SAN BERNARDINO
San Diego Co. Env. Health	SAND	SAN DIEGO
San Gabriel Valley MVCD	SGVA	LOS ANGELES
Santa Barbara Co. MVMD	SBCO	SANTA BARBARA
Ventura Co. Env. Health	VENT	VENTURA

Northern Districts

Start Date: May 9, 2016

AGENCY NAME	CODE	COUNTY
Alameda Co. VCD	ACVC	ALAMEDA
Burney Basin MAD	BURN	SHASTA
Butte Co. MAD	BUCO	BUTTE
Colusa MAD	CLSA	COLUSA
Contra Costa MVCD	CNTR	CONTRA COSTA
East Side MAD	EAST	STANISLAUS
Glenn Co. MVCD	GLEN	GLENN
Lake County MAD	LAKE	LAKE
Marin-Sonoma MVCD	MARN	SONOMA
Merced MAD	MERC	MERCED
Nevada County Agr. Dept	NEVA	NEVADA
No. Salinas Valley MVCD	NSAL	MONTEREY
Placer MVCD	PLCR	PLACER
Sacramento-Yolo MVCD	SAYO	SACRAMENTO
Saddle Creek	SCSD	CALAVERAS
San Benito Ag Dept	SBVC	SAN BENITO
San Mateo Co. MAD	SANM	SAN MATEO
Santa Clara Co. VCD	STCL	SANTA CLARA
Santa Cruz Co. MVCD	SCRZ	SANTA CRUZ
Shasta MVCD	SHAS	SHASTA
Solano Co. MAD	SOLA	SOLANO
Sutter-Yuba MVCD	SUYA	SUTTER
Tehama County MVCD	TEHA	TEHAMA

2016 Southern California Schedule for Chicken Sera

May 4: Pick up chickens in San Jacinto. Bleed and hold samples in refrigerator for reference. Bleed chickens during **highlighted weeks** and ship to address below for testing. Samples can be submitted earlier if chickens are acquired prior to May 4.

April 2016

S	M	T	W	T	F	S
					1	2
3	4	5	6	7	8	9
10	11	12	13	14	15	16
17	18	18	20	21	22	23
24	25	26	27	28	29	30

May 2016

S	M	T	W	T	F	S
1	2	3	4	5	6	7
8	9	10	11	12	13	14
15	16	17	18	19	20	21
22	23	24	25	26	27	28
29	30	31				

June 2016

S	M	T	W	T	F	S
			1	2	3	4
5	6	7	8	9	10	11
12	13	14	15	16	17	18
19	20	21	22	23	24	25
26	27	28	29	30	1	2

July 2016

S	M	T	W	T	F	S
					1	2
3	4	5	6	7	8	9
10	11	12	13	14	15	16
17	18	19	20	21	22	23
24	25	26	27	28	29	30
31						

August 2016

S	M	T	W	T	F	S
	1	2	3	4	5	6
7	8	9	10	11	12	13
14	15	16	17	18	19	20
21	22	23	24	25	26	27
28	29	30	31			

September 2016

S	M	T	W	T	F	S
					1	2
3	4	5	6	7	8	9
10	11	12	13	14	15	16
17	18	19	20	21	22	23
24	25	26	27	28	29	30

October 2016

S	M	T	W	T	F	S
						1
2	3	4	5	6	7	8
9	10	11	12	13	14	15
16	17	18	19	20	21	22
23	24	25	26	27	28	29
30	31					

November 2016

S	M	T	W	T	F	S
	31	1	2	3	4	5
6	7	8	9	10	11	12
13	14	15	16	17	18	19
20	21	22	23	24	25	26
27	28	29	30			

Legend:

-  = Bleed/ship week
-  = State Holiday

Mail sample to: **California Dept. of Public Health, Richmond Campus
Specimen Receiving Unit Room B106 (ATTN: ARBO)
850 Marina Bay Parkway
Richmond, CA 94804**

If there are questions, call Tina Feiszli at (510) 412-6253 or e-mail: tina.feiszli@cdph.ca.gov

2016 Northern California Schedule for Chicken Sera

April 21: Pick up chickens in Modesto. Bleed and hold samples in refrigerator for reference. Bleed chickens during **highlighted weeks** and ship to address below for testing. Samples can be submitted earlier if chickens are acquired prior to April 21.

April 2016

S	M	T	W	T	F	S
					1	2
3	4	5	6	7	8	9
10	11	12	13	14	15	16
17	18	19	20	21	22	23
24	25	26	27	28	29	30

May 2016

S	M	T	W	T	F	S
1	2	3	4	5	6	7
8	9	10	11	12	13	14
15	16	17	18	19	20	21
22	23	24	25	26	27	28
29	30	31				

June 2016

S	M	T	W	T	F	S
			1	2	3	4
5	6	7	8	9	10	11
12	13	14	15	16	17	18
19	20	21	22	23	24	25
26	27	28	29	30		

July 2016

S	M	T	W	T	F	S
					1	2
3	4	5	6	7	8	9
10	11	12	13	14	15	16
17	18	19	20	21	22	23
24	25	26	27	28	29	30

August 2016

S	M	T	W	T	F	S
	1	2	3	4	5	6
7	8	9	10	11	12	13
14	15	16	17	18	19	20
21	22	23	24	25	26	27
28	29	30	31			

September 2016

S	M	T	W	T	F	S
					1	2
3	4	5	6	7	8	9
10	11	12	13	14	15	16
17	18	19	20	21	22	23
24	25	26	27	28	29	30

October 2016

S	M	T	W	T	F	S
						1
2	3	4	5	6	7	8
9	10	11	12	13	14	15
16	17	18	19	20	21	22
23	24	25	26	27	28	29

November 2016

S	M	T	W	T	F	S
			1	2	3	4
5	6	7	8	9	10	11
12	13	14	15	16	17	18
19	20	21	22	23	24	25
26	27	28	29	30		

Legend:

- = Bleed/ship week
- = State Holiday

Mail sample to: **California Dept. of Public Health, Richmond Campus
Specimen Receiving Unit Room B106 (ATTN: ARBO)
850 Marina Bay Parkway
Richmond, CA 94804**

If there are questions, call Tina Feiszli at (510) 412-6253 or e-mail: tina.feiszli@cdph.ca.gov