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EDMUND G. BROWN JR.
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TO: PARTICIPATING AGENCIES IN THE 2017 CALIFORNIA
ARBOVIRUS SURVEILLANCE PROGRAM

SUBJECT: 2017 MOSQUITO POOL AND SENTINEL CHICKEN TESTING

Enclosed are instructions for the 2017 arbovirus surveillance season. 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 2017

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

Southern region: Pick-up day is scheduled for **March 30, 2017 at Demler Egg Ranch in San Jacinto.**

Northern region: Pick-up day is scheduled for **April 20, 2017 at Haley Farms in Modesto.**

Chickens can be ordered through MVCAC. Order forms can be found at:
<http://www.mvcac.org/2017-sentinel-chicken-orders/>

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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. Mosquito pool testing by the Davis Arbovirus Research and Training laboratory (DART) will be billed through the Mosquito and Vector Control Association (MVCAC).

Agencies are responsible for registering and maintaining updated information for their surveillance sites online at: <http://gateway.calsurv.org/>. Accurate geographic coordinates are especially important for registered sites, because they will be used to generate computer maps that will post test results in real-time. DART will host real-time maps in ArcGIS format at <http://maps.calsurv.org/>. In addition to these maps, local agencies can access maps using a Google Maps interface through the California Vectorborne Disease Surveillance Gateway (<http://gateway.calsurv.org>) that provide enhanced analysis tools and detail.

2. Registration of sentinel flock sites and wing band numbers

Prior to submitting any sentinel chicken blood samples to CDPH, agencies must register each flock site and accompanying wing band numbers at <http://gateway.calsurv.org>. 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. CDPH will test samples only if they are accompanied with a submission form for each flock site, which includes the registered site code, the wing band numbers assigned to that site, and date bled. 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>. DART will only test mosquito pools from registered sites and they must be accompanied with a "MOSQUITO POOL SUBMISSION" form.

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 ≤ 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-1-17, where 17 refers to year 2017. **Pools must be accompanied by a "MOSQUITO POOL SUBMISSION FORM" 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. 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.

All pools are tested for WNV, SLEV, and WEEV. Invasive *Aedes* mosquitoes are also tested for chikungunya, dengue, and Zika viruses (CDZ). Other mosquito species can be tested for CDZ upon request. Agencies will receive an email notification that results have been entered into the CalSurv Gateway, as well as a summary of positive pools. Positive pools will also be reported in the weekly California Arbovirus Surveillance Bulletin.

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

INSTRUCTIONS FOR SENTINEL CHICKEN BLOOD SAMPLES

Take a reference blood sample from each chicken on the day you pick them up.

Store the reference bleed samples in the refrigerator. If a chicken tests positive on the first bi-weekly bleed, the reference bleed should be tested to determine if the seroconversion occurred prior to deployment at the surveillance site.

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 $\frac{3}{4}$ inch length portion of the strip. Use alcohol swabs on comb before bleeding and ensure strips are dried in a dust free environment.
3. Each strip must include the date bled and the wing band number.
4. Attach strips to a 5" X 7" index card in sequential order, from left to right (example on page 9).
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. Include the following information on the index card:
County, Agency code, Site, and Date Bled.

Place the index card with attached samples into a ziplock plastic bag. Do not put more than one index card per bag.
8. Include a submission form for each flock. Do not put the form in the ziplock bag.

Ship to:

California Department of Public Health
Vector Borne Disease Section, G164 (**ATTN: R. Payne**)
850 Marina Bay Parkway
Richmond, CA 94804

In the laboratory specimens will be tested for WNV, SLEV, and WEEV 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 Sentinel Blood Submission form 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 #	DATE BAND #	DATE BAND #	DATE BAND #	DATE BAND #	DATE BAND #	DATE BAND #	DATE BAND #	DATE BAND #	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: April 10, 2017

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 1, 2017

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

2017 Southern California Schedule for Chicken Sera

March 30: 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.

April 2017

S	M	T	W	T	F	S
				30	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						

May 2017

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 2017

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 2017

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 2017

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	31		

September 2017

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 2017

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 2017

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**
Vector Borne Disease Section G164 (ATTN: Robert Payne)
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

2017 Northern California Schedule for Chicken Sera

April 20: 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.

April 2017

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

May 2017

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	1	2	

June 2017

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 2017

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 2017

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 2017

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 2017

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 2017

S	M	T	W	T	F	S
	30	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
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