

February 20, 2024

TO: PARTICIPATING AGENCIES IN THE 2024 CALIFORNIA
ARBOVIRUS SURVEILLANCE PROGRAM

SUBJECT: 2024 MOSQUITO POOL AND SENTINEL CHICKEN TESTING

Enclosed are instructions for the 2024 arbovirus surveillance season. If you have questions about results or have submission issues concerning chicken samples or mosquito pools, please contact Hannah Romo at (510) 412-6298 or Hannah.Romo@cdph.ca.gov.

IMPORTANT ANNOUNCEMENTS FOR 2024

Mosquito Pool Supplies

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 Hannah Romo 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 chickens

Pick-up date: Mid-April, specific date and location TBD (Stanislaus County, Turlock/Hilmar area)

The pickup location is different that what was previously used in 2023.

Chicken order forms should be returned to MVCAC by March 1st.

<https://acrobat.adobe.com/id/urn:aaid:sc:US:12d96b7b-2c66-4884-81e8-a958391d5dd5?viewer%21megaVerb=group-discover>

Chicken supplies (wing bands, lancets, and filter paper) will be shipped to local agencies in early spring.

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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. Chickens can be ordered through MVCAC. Mosquito pool testing by the UC Davis Arbovirus Research and Training (DART) laboratory 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 the [CalSurv Gateway](https://gateway.calsurv.org) (<https://gateway.calsurv.org>).

2. Registration of mosquito sampling sites

Registration of new sites used for collection of mosquitoes for virus testing may be done through the [CalSurv Gateway](https://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 pools provided that adequate information is provided on the “MOSQUITO POOL SUBMISSION” form, which includes the registered agency code, registered site code, and geographic coordinates.

Recording the geographic coordinates of all surveillance sites allows users to filter data spatially for analysis, and the locations are used to generate computer maps that show all registered sites and test results. As part of a collaborative effort, the DART laboratory hosts [real-time maps](https://maps.calsurv.org) (<https://maps.calsurv.org>). Local agencies can log in on the mapping website or the [CalSurv Gateway](https://gateway.calsurv.org) to access more detailed maps and enhanced analysis tools.

3. Registration of sentinel chicken flocks 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 the [CalSurv Gateway](https://gateway.calsurv.org). CDPH will only test samples if they are accompanied by the “SENTINEL CHICKEN BLOOD” form for each flock site, which includes the registered agency code, the registered site code, band numbers, and date bled.

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 with cold, carbon dioxide, or triethylamine (TEA). TEA is recommended because specimens are permanently immobilized with minimal mortality and no loss of virus titer (Kramer et al. 1990). 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 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 (e.g., chironomids or *Culicoides*) are not inadvertently included in the pools. 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 5 mm 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 each year with the pool number and year after the agency code (e.g., KERN-1-24, where 24 refers to the year of collection). Number pools consecutively starting with "1" for each calendar year within your agency.

Data on each pool can be entered directly in electronic format through the [CalSurv Gateway](https://gateway.calsurv.org/) (<https://gateway.calsurv.org/>). Pools must be accompanied by the "Mosquito Pool Submission Form" (generated using the CalSurv Gateway) and can only be tested from registered surveillance sites. Surveillance sites should be [registered online](#).

Register the surveillance site code for each pool in the CalSurv Gateway that consists of a designated four-letter agency code followed by six digits to identify the site (e.g., KERN000001). 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 KERN000003, etc.).

4. Freeze pools immediately at -80°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 DART for testing by real-time multiplex RT-PCR. Agencies will receive an automated email 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 West Nile virus (WNV), St. Louis encephalitis virus (SLEV), and western equine encephalomyelitis virus (WEEV) by a multiplex RT-qPCR assay. Positives with Ct scores >35 are confirmed by a singleplex RT-qPCR with a different set of virus species-specific primers and probes. Invasive *Aedes* mosquitoes and other mosquito species can also be tested for chikungunya, dengue, and Zika viruses (CDZ) upon request by a separate multiplex RT-qPCR. If testing for chikungunya, dengue, and Zika viruses is desired, this should be indicated by checking the box for “CDZ Testing” when preparing the online pool submission form in the CalSurv Gateway. Pools can be tested for other *Aedes*-borne viruses such as yellow fever on request. Pools from selected areas are also screened for additional viruses using Vero cell culture with isolates identified by genetic sequencing. 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 mosquito pools to the following address:

ATTN: Anil Singapuri
VM://PMI Room 3336, Vet Med 3A
1285 Veterinary Medicine Mall
University of California, Davis
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 bleeds in the refrigerator. If a chicken tests positive on the first bi-weekly bleed date, the reference sample should be submitted for testing to determine if the seroconversion occurred prior to initial deployment.

INSTRUCTIONS FOR BLOOD COLLECTION ON FILTER PAPER STRIPS:

1. Pre-label half-inch wide filter paper strips with the bleed date and wing band number.
2. Bleed each hen from the distal portion of the comb using a standard lancet used for human finger "prick" blood samples. Use alcohol swabs on comb before bleeding. The comb should be "pricked" with the lancet and blood allowed to flow from the "wound" to form a drop.
3. Collect the blood onto the filter paper strips by touching the opposite end of the pre-labeled strip to the wound. **The blood must completely soak through a $\frac{3}{4}$ inch length portion of the strip.**
4. Air dry the strips. 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 inch card in sequential order, from left to right, by stapling the labeled end towards the top edge of the card. Leave the blood-soaked end free so that laboratory staff can remove a standard punch sample. **See example on page 9.**
6. Write the county, agency code, site code, and date bled onto the card and place it into a Ziploc plastic bag (only one card per bag). It is important that the blood samples do not become dirty, wet, or touch each other. Samples must be accompanied by a "SENTINEL CHICKEN BLOOD FORM" outside the Ziploc bag. Do not staple the form to the bag.

Samples from each collection date can be placed into a mailing envelope and sent to:

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

In the laboratory specimens will be tested for WNV, SLEV, and WEEV antibodies using an enzyme immunoassay. Positive specimens will be confirmed with an indirect fluorescent antibody or western blot test. 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 tube with the site code, band number, and date bled. 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 precautions taken. **Containers must be shipped with a label stating: “Biological Substances, Category B, UN3373”.**

Contact Hannah Romo, VBDS in Richmond (510) 412-6298, concerning any questions about sample submissions or test results.

INSTRUCTIONS FOR SENTINEL BLOOD

Attach filter paper strips to a labeled 5" X 7" card as shown below. Each strip should be labeled with the date bled and band number.

COUNTY	THESE ARE CHICKEN BLOOD SAMPLES								
AGENCY									
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:

Expedited shipping (e.g., Priority mail, UPS) is recommended. Specimens will be tested within 1-3 days upon receipt by the laboratory. Agencies will be notified by email that results have been entered into the CalSurv Gateway, and seroconversions will also be reported in the weekly California Arbovirus Surveillance Bulletin.

2024 Schedule* for Chicken Samples

Mid-April: Pick up chickens in Stanislaus County (specific date and location TBD). Bleed and hold samples in refrigerator for reference if needed. Bleed chickens during highlighted weeks and ship to address below for testing.

April 2024

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 2024

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 2024

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 2024

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 2024

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 2024

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 2024

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 2024

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

December 2024

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				

Legend

- Bleed/ship week
- State Holiday

**Mail samples to: California Dept. of Public Health
 Vector Borne Disease Section, G164 (ATTN: ARBO)
 850 Marina Bay Parkway
 Richmond, CA 94804**

**Suggested scheduled based on tentative mid-April pick-up date. Districts may opt to sample on alternate weeks.*