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



EDMUND G. BROWN JR.
Governor

January 10, 2018

TO: PARTICIPATING AGENCIES IN THE 2018 CALIFORNIA
ARBOVIRUS SURVEILLANCE PROGRAM

SUBJECT: 2018 MOSQUITO POOL AND SENTINEL CHICKEN TESTING

Enclosed are instructions for the 2018 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 2018

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 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 **February 15, 2018 at Demler Egg Ranch in San Jacinto.**

Northern region: Pick-up day is scheduled for **April 5, 2018 at Haley Farms in Modesto.**

Chickens can be ordered through MVCAC. Order forms can be found at:
<http://www.mvcac.org/amg/wp-content/uploads/2018-Chicken-Order-Form.pdf>

Table of Contents

| | |
|--|-------------------|
| Registration of Agencies and Sites | page 3 |
| Procedures for Processing Mosquitoes | page 4 |
| Mosquito Pools Submitted to DART – 2018 Form | page 5 – 6 |
| Instructions for Sentinel Blood Samples | page 7– 9 |
| Sentinel Chicken Blood – 2018 Form | page 10 |
| Southern Schedule Districts | page 11 |
| Northern Schedule Districts | page 12 |
| Southern California Schedule for Chicken Sera | page 13 |
| Northern California Schedule for Chicken Sera | page 14 |

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 at <http://maps.calsurv.org/>. In addition to the public view of the maps, local agencies can use their Gateway username and password to log in to the same website to access additional details that are available only to Gateway users.

2. Registration of surveillance sites

Registration of new sites used for sentinel chickens or the collection of mosquitoes for abundance monitoring or virus testing should be done online at: <http://gateway.calsurv.org/>. CDPH and DART will only test samples from registered surveillance sites.

3. Registration of sentinel chicken flocks and wing band numbers

Prior to submitting any sentinel chicken blood samples to CDPH, agencies must annually register each flock 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.

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-1-18, where 18 refers to year 2018. Pools must be accompanied by a "MOSQUITO POOL SUBMISSION FORM" that should be printed after entering the records for pools to be submitted at <http://gateway.ca.surv.org>. Pools can only be tested from sites with registered geographic locations.

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). In the rare event that mosquitoes are collected from sites that are not already registered and are used only once (e.g., in response to a disease case notification or for a spot check on abundance in a particular area), pools may be recorded without assigning a unique site code by using your agency's generic "000000" site code, but it is critical that you also record the particular collection site on the Gateway's map for every mosquito collection that uses the generic code. Failure to do so will result in inaccurate records for your agency.

4. 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.
5. Freeze pools immediately at -80°C with either 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 will be 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.

Address mosquito pool shipments to the following address:

ATTN: Ying Fang
University of California
One Shields Avenue
Vet Med: PMI
Room 3336 Vet Med 3A
Davis, CA 95616

For UPS shipments only:

Ying Fang
VM://PMI 3336 Vet Med 3A
1285 Veterinary Medicine Mall
University of California, Davis
Davis, CA 95616

MOSQUITO POOL SUBMISSION FORM-2018

| | |
|--------------|-------|
| Received: | _____ |
| Tested: | _____ |
| Bulletin No: | _____ |

Agency Code _____

Agency _____

Contact Name: _____ Phone: _____

| Site Code | Pool # | Date | Species | Sex | # in Pool | CDZ |
|-----------|--------|------|---------|-----|-----------|-----|
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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 initial deployment.

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 each individual index card with attached samples into a separate ziplock bag (only one card per bag).
8. Include a submission form for each flock. Do not put the form inside 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 precautions 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.

SENTINEL CHICKEN BLOOD - 2018

PLEASE DO NOT PLACE THIS SHEET INSIDE THE ZIPLOCK BAG

VBDS PAGE NUMBER

REGISTERED AGENCY CODE: _____ *SITE CODE _____

Name of Agency: _____

Name of Site: _____ Nearest City or Place: _____

County: _____

DATE BLED : ___/___/___ BLED BY: _____

CONTACT NAME: _____ Telephone (____) ____ - _____

NAME OF ALTERNATE: _____ Telephone (____) ____ - _____

| WING BAND NUMBER IN SEQUENCE | REMARKS OR STATUS ("New" dead, missing, etc.) For new birds added to flock, list the number and state "new" | WEE | SLE | WN |
|------------------------------|---|-----|-----|----|
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Remarks: _____

A COPY OF THIS FORM MUST ACCOMPANY ALL SHIPMENTS OF CHICKEN BLOOD TO VBDS.

Southern Districts

Start Week: April 2, 2018

| 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 Week: April 23, 2018

| 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 |

2018 Southern California Schedule for Chicken Sera

February 15: 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 2018

| 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 2018

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| 13 | 14 | 15 | 16 | 17 | 18 | 19 |
| 20 | 21 | 22 | 23 | 24 | 25 | 26 |
| 27 | 28 | 29 | 30 | 31 | | |

June 2018

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| 17 | 18 | 19 | 20 | 21 | 22 | 23 |
| 24 | 25 | 26 | 27 | 28 | 29 | 30 |

July 2018

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| 15 | 16 | 17 | 18 | 19 | 20 | 21 |
| 22 | 23 | 24 | 25 | 26 | 27 | 28 |
| 29 | 30 | 31 | | | | |

August 2018

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| 19 | 20 | 21 | 22 | 23 | 24 | 25 |
| 26 | 27 | 28 | 29 | 30 | 31 | |

September 2018

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| 30 | | | | | | |

October 2018

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November 2018

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| 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

2018 Northern California Schedule for Chicken Sera

April 5: 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 2018

| S | M | T | W | T | F | S |
|----|----|----|----|----|----|----|
| 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| 8 | 9 | 10 | 11 | 12 | 13 | 14 |
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| 29 | 30 | | | | | |

May 2018

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June 2018

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July 2018

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August 2018

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September 2018

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October 2018

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November 2018

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| 18 | 19 | 20 | 21 | 22 | 23 | 24 |
| 25 | 26 | 27 | 28 | 29 | 30 | |

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