Procedures for Processing Mosquitoes for Arbovirus Detection - 2006

1. Collect mosquitoes alive and return them immediately to the laboratory. Females should be offered 5-10% sucrose if held overnight or longer before processing.

2. Anesthetize mosquitoes by carbon dioxide, or triethylamine (TEA). TEA is recommended because specimens are permanently immobilized with minimal mortality and with no loss of SLE or WEE 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.

3. Sort mosquito collections to species under a dissecting microscope at 10X to ensure correct identification and to make sure that other small insects such as chironomids or *Culicoides* are not inadvertently included in the pools. Count and discard dead and dried mosquitoes. Lots of 50 females (minimum of 12 females) per pool of each vector species from each collection site are then counted. Place each mosquito pool in an individual plastic (**not glass**) screw-cap cryovial fitted with O-rings to prevent contact with CO_2 during transport and storage. Recommended sampling effort are 10 pools of 50 females of each species from each site per week to detect minimum infection rates (MIRs) ranging from 2 to 20 per 1,000 females tested. Pools should be labeled sequentially starting with #1 each year after the site code. VERY IMPORTANT: POOLS MUST BE ACCOMPANIED BY "MOSQUITO POOLS SUBMITTED FORM MBVS-3" AND CAN ONLY BE TESTED FROM REGISTERED SITES VIA ONLINE REGISTRATION (http://vector.ucdavis.edu/arbo.html).

   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, i.e., pool #1 may be from KERN0001, and pool #2 may be from KERN0004.

4. Freeze pools immediately at -70°C either with dry ice in an insulated container or in an ultra-low temperature freezer. Pools are shipped frozen on dry ice to the UC Davis Center for Vector-borne Disease Research for testing by an *in situ* enzyme linked **immunosorbent assay (EIA).** Care must be taken not to allow pools to defrost during storage or shipment, because each thaw and freeze kills approximately half the virus, and all virus will be lost if the specimens sit at room temperature.

Pools are shipped frozen on dry ice and must have a dry ice label on outside of container to indicate the weight of the dry ice. **Mosquito pools sent for arrival on Friday must be received at CVEC no later than 3: 00 PM.**

UC Davis
Center for Vector-borne Disease Research
Old Davis Road
Davis, CA 95616

5. Separate protocols will be sent to agencies conducting rapid assays which need to send buffer solution for confirmation testing.

6. Questions concerning test results should be directed to the Vector-Borne Disease Section, California Department of Health Services (DHS) at (510) 412-6252or ahom@dhs.ca.gov. The weekly Arbovirus Surveillance bulletin will report test results and a list of agencies that submitted samples for that week.