

Area-wide Management of the Asian Tiger Mosquito Truck-mounted Larviciding Standard Operating Procedure¹



Methoprene (Insect Growth Regulator) Bioassays

Introduction:

The purpose of this document is to provide standardized procedures and guidelines to conduct field bioassays in order to evaluate efficacy and penetration of an area-wide application of the insect growth regulator methoprene (Altosid SR-5, SR-20) against *Aedes albopictus* in urban/suburban neighborhoods. The document also provides guidelines used in the laboratory to determine the percent emergence inhibition of *Ae. albopictus* exposed to methoprene. These bioassays were utilized during the effort to develop and document successful area-wide applications of the insecticide methoprene and ultimately reduce adult populations of *Ae. albopictus* in urban/suburban residential neighborhoods.

I. Preparation and Placement of Bioassay Cups

Personnel, Equipment, & Materials

1. Use a team of people to load 2nd/3rd instar larvae into treatment and control cups the morning of the application (this requires careful timing of the application with the availability of lab reared larvae). With a team of 6 experienced individuals 120 cups can be loaded in one hour. Fewer/inexperienced individuals plan on at least two hours.
2. Use teams of two people (placement and record keeping) for cup placements. This increases safety during cup placement, since inspectors are going to enter resident's backyard (parcels) after permission is granted. In more suburban areas that cover larger geographic areas use two teams for the treatment area and one team for the control area.
3. Use 16 oz. Clear Round Wide-Mouth Jars (U-line S99636B, <http://www.uline.com/Product/Detail/S-9936B/Jars-Jugs-Bottles/16-oz-Clear-Round-Wide-Mouth-Jars-Bulk-Pack>). Label each jar with a unique number and record it on a data sheet. This process will be very important to evaluate the final results. It is recommended to use preprinted labels with the agency name, contact number, and "Do Not Touch" on the label.

¹ Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the USDA or other involved parties.

- a. Add 300 ml of de-chlorinated water to each jar.
 - b. Add 40 mg of a 50:50 mix of yeast:lactalbumin or finely ground rat-chow. We make a liquid emulsion in water and aliquot equal amounts to all cups as larval food.
 - c. Add 15 2nd and/or 3rd instar ATM larvae using a plastic Pasteur pipette. We chose 15 larvae for methoprene bioassays because mortality can be higher in the control group due to transport to and from the field and the long amount of time they are held following the application in order to document emergence of the adults.
4. Use teams of two to count and record emergence inhibition in the laboratory as it can take 2 to 4 hours to do this initially.

Site Selection and Cup Placement

Each treatment site was a group of approximately 1,000 parcels, each parcel corresponding to a structure or a house (residential or commercial) and surrounding yard. Initial selection of areas were based primarily on the concentration of past *Ae. albopictus* related mosquito service requests and abundance of *Ae. albopictus* in traps from routine disease and nuisance surveillance monitoring (Unlu et al. 2011).

1. Select 30 parcels (3 cups per parcel x 30 = 90 jars) in your treatment sites and 10 parcels in the control site (3 cups per parcel x 10 = 30 jars). You will use a total of 120 jars to conduct the bioassay in the treatment and control sites. Jars were placed in the control site during each application in order to account for other sources of mortality besides the treatment, such as the handling and transport of the larvae.
2. Conduct truck-mounted larvicide applications in the early morning hours between 1:00 and 5:00 a.m., when human activity and vehicle traffic is at a minimum. An application within a suburban 400 acre site will take approximately 3 to 3.5 hours for a single truck to complete.
3. Place 3 **larvae filled** jars (with their caps underneath each cup) within each parcel, approximately 8 hours (evening) prior to an area-wide larvicide application: 1) front yard, 2) in the middle, between the front and back yard, 3) back yard.
4. Pick up jars the following morning (2-6 hrs post application) preferably using the same personnel which placed the cups in the field originally. Fasten caps securely to the jars. If you are not be able to use the same personnel for jar pick up, detailed notes should be made while placing them in order to guide new inspectors to the location of the jar placed the day before. Record any damaged, tipped over, or missing jars while picking them up. Pick up **control site** jars prior to treatment site jars in order to prevent contamination if you have limited numbers of teams. If you have enough teams send one team to the control site only. Place control site jars in separate containers and label these containers clearly as control.
5. Transport all containers within a temperature controlled environment to prevent excessive larval mortality.

II. Assessing Emergence Inhibition in the Laboratory

Bioassay

1. Clean the outside of all of the jars and then count the number of larvae in each jar immediately upon arrival to the laboratory. It is not unusual to see some variation (11-20 larvae). That is fine as long as that number is recorded.
2. Add 40 mg of a 50:50 mix of yeast:lactalbumin or finely ground rat-chow. We make a liquid emulsion in water and aliquot equal amounts to all cups as larval food.
3. Cover jars with emergence jar lids (small BioQuip Mosquito Breeder tops will fit directly on Uline Jars) or plastic sandwich bags secured with rubber bands.
4. Place bioassay jars in an incubator ($\approx 27^{\circ}\text{C}$). Control jars should be placed in a separate incubator or arranged on the top shelves of the incubator so that no material can fall into controls from the outside of the treatment jars.
5. Count the number of emerged adults, semi-emerged adults, live pupae, dead pupae, live larvae, and dead larvae daily in both treatment and control cups. In some instances dead mosquitoes will be eaten by larvae between recordings but by recording all stages at each counting this can be accounted for. Record all numbers carefully (we can provide you with pre-prepared excel files upon request). Run bioassays until all mosquitoes in the control are dead or have emerged or at least 80% of the controls have emerged (approximately two weeks).
6. Calculate percent emergence using the formula of Mulla et al. (1974): % inhibition of emergence = $1 - 100 (T/C)$, where T is the percent emergence in treated container and C is percent emergence in control container.