

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



Bacillus thuringiensis israelensis (Bti) 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 low-volume larvicide application against *Aedes albopictus* in urban neighborhoods. The document also provides guidelines used in the laboratory to determine the percent mortality of *Ae. albopictus* exposed to *Bacillus thuringiensis israelensis* (Bti). We attempted to develop and document a successful area-wide application of the insecticide Bti and ultimately reduce adult populations of *Ae. albopictus* in urban/suburban residential neighborhoods.

I. Placement of Bioassay Cups

Personnel, Equipment, & Materials

Personnel and Bioassay Jars

1. Use at least a team 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 being granted permission.
2. 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. Note: do not label only the lid make sure the cups themselves are labeled. It is also advisable to add a label with the name of your operation, date, and reasons for the presence of the cup.

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.

Site Selection and Cup Placement

Within the ATM project, 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 was 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) We had approximately 25 city blocks in treatment sites and 47 in the control site (parcels are larger in the control site). We selected 30 parcels (3 cups per parcel x 30 = 90 jars, Fig. 1) in these treatment sites and selected 10 parcels in the control site (3 cups per parcel x 10 = 30 jars). Therefore we used a total of 120 jars to conduct a bioassay in one treatment site and the control site. Jars were placed in the control site each time in order to have negative controls to assess other sources of mortality besides the treatment.
- 2) We conducted 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.
- 3) An application within our study site took approximately 2 hours for a single truck to complete. Preparation time of material took approximately 1.5 hours.
- 4) Place 3 **empty** jars (with their caps underneath each cup) within each parcel, 12-18 hours prior to an area-wide larvicide application: 1) front yard, 2) in the middle of the parcel, usually next to the house, 3) back yard. In order to imitate cryptic habitats, place some of the cups in locations such as: inside recycle bins, under vegetation, in alcoves (a shaded narrow area between two row homes). However, placement of jars in completely covered places such as under a porch or in underground passageways to the backyards is not recommended since these are clearly sites out of bounds to a aerial application of insecticide and will have to be treated differently if they are important sources of *Ae. albopictus* in your area.
- 5) Pick up jars 1-3 hrs post application preferably using the same personnel that placed the cups in the field originally. Fasten caps securely to the jars. If you would 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.
- 6) Pick up **control site** jars prior to treatment site jars in order to prevent contamination if you have only one team. If you have more than one team send one team to control site only.
- 7) Store control and treatment jars in separate coolers with ice packs (cubed ice or blue ice packs work fine). Make sure coolers are clearly marked with "Control" and "Treatment" labels.
- 8) Transport all containers with coolers to laboratory for bioassays.
- 9) Store containers in 0-4°C fridge if you cannot ship them immediately to the laboratory space where the bioassays will be performed.

II. Laboratory Trials

To evaluate the biological activity of a *Bti*, water and ten 2nd and recent 3rd instar laboratory-reared *Ae. albopictus* larvae (reference strains of field-collected mosquitoes) are placed in jars that were collected after area-wide larvicide application.

IMPORTANT NOTE: Before adding water and larvae all containers need to be rinsed under tap water to remove insecticidal material from the outside. Failure to do this will lead to cross contamination between treatment cups and onto control cups. This is especially true when applications are performed after a rain event and mud attaches to the cups. Also in the event of rain, keep any water that may have fallen inside the cups. Just top it off to 250 ml with filtered water. If more than 250 ml are already present (i.e. if the cup was mistakenly placed under a window air-conditioner or a rain spout) then make a note of that but just add the larvae to that water.

Bioassay

1. Add 250 ml of filtered water – you can add tap water if your tap water does not have a lot of chlorine or if you let water sit for 24 hr in open containers.
2. Add 10 2nd and/or early 3rd instar ATM larvae using a plastic Pasteur pipette. Do not let the pipette touch the cup's water surface. If that happens discard and use a fresh one. We chose 10 larvae for *Bti* bioassays because after several trials we concluded that (1) mortality in the control is low since the bioassays only runs for 3 days (72 hours); (2) it is difficult to count efficiently by sight more than 10 live larvae.
3. Count the number of larvae immediately after addition. It is not unusual to see some variation (9-15 larvae). That is fine as long as that number is recorded.
4. Add 40 mg of a 50:50 mix of yeast:lactalbumin. We make a liquid emulsion in water and aliquot equal amounts to all cups as larval food. You can also add finely ground rat-chow but it is harder to keep in suspension to ensure even distribution to all cups.
5. Count the number of live larvae after 24, 48, and 72 hours in both treatment and control cups. Record all numbers carefully (we can provide you with pre-prepared excel files upon request).

Percent Control

To calculate percent control after application of larvicides we used the formula: percent control = $100 - [(T/U)100]$, where T is the post application mean divided by the pre application mean in the treatment site and U is the post application mean divided by the pre application mean in the control (Untreated) site.