

## Materials Supplied in Kit

<i>Item</i>	<i>Number Needed</i>
Plastic Pipettes	50
Wax-Lined Paper Cups (88 ml / 3 oz)	50
Soup Cups (16 oz)	20
Rubber Bands	50
Fine Mesh Fabric	50
Fish Food (pulverized)	1 Falcon Tube
Eppendorf tube cap	5
Plastic Measuring Cup	1

### **B. Reagents**

Methoprene Diagnostic Doses	100 ml
Bti Diagnostic Doses	100 ml

### **C. Specimens**

180 4<sup>th</sup> instar larvae per site (minimum, testing more improves accuracy)

## Larvicide Bioassay Procedure\*

*\* The minimum number of containers needed for a sufficient sample size when testing a site for resistance is 12. The first 10 should contain the diagnostic doses of the target pesticide, while only acetone should be added to the last two containers as controls.*

- 1) Clear a working area of approximately 1.5 ft<sup>2</sup> and lay out 12 of the **wax-lined paper cups** to be used for the trial.
- 2) Use the **Eppendorf tube cap** to add a consistent amount of **pulverized fish food** into each small wax-lined paper cup.
- 3) Place one small wax cup with fish food inside each of the **soup cups**. Label two containers as the controls, and the others with the pesticide being tested.

- 4) Using one of the **plastic pipettes**, count 15 mosquito larvae into the **plastic measuring cup**. Once the larvae have been counted fill the measuring cup to the 75 ml line with water. Use the plastic pipette to remove 1 ml of water for methoprene trials or 5 ml for Bti trials.
- 5) Pour the larvae and water into the small wax-lined paper cup. Check to make sure no larvae remain in the cylinder. Repeat for each of the 12 containers until they all contain 15 larvae and water.
- 6) Gently shake the bottle of pesticide for at least 30 seconds then add either 1 ml of the diagnostic concentration (see Table A1) for methoprene, or 5 ml for Bti to each small wax-lined cup, except the two control cups.
- 7) Add 1 ml of pure acetone to the control cups for methoprene trials or 5 ml of water for the Bti trials.
- 8) Use the **rubber bands** to attach the **fine mesh fabric** to the top of the soup cups to prevent adults from escaping. Place the containers on a table away from sunlight in a temperature-controlled room.
- 9) For methoprene trials, check the containers every 24 hours for the next 6 days and record the number of adults that have emerged in each container. For Bti trials count the number of larvae alive after 24 hours.
- 10) After the trial is over, discard everything except for the soup cups, which can be reused unless there was spillage.
- 11) If mortality in the control cup is  $> 20\%$  discard this trial and start again.

## Appendices

**Table A1:** These are the diagnostic doses to be used based upon susceptibility curves constructed with susceptible strains for *Culex pipiens* and *Aedes albopictus*. Numbers in yellow are still under development and are in the process of finalization. This document will be updated with the final values will before June 2019.

<b>Species</b>	<b>Methoprene Diagnostic concentration (2x LC-99)</b>
<i>Aedes albopictus</i>	0.1708 µg / ml
<i>Culex pipiens</i>	0.0336 ug / ml
<b>Species</b>	<b>Bti Diagnostic concentration (2x LC-99)</b>
<i>Aedes albopictus</i>	0.025 ITU / ml
<i>Culex pipiens</i>	<i>In development</i>