ALTOSID® P35 TESTING PROTOCOL

Altosid® P35 larvicide is a unique granule formulation that is uniform and of sufficient density to be easily applied to larval habitats using aerial and ground application methods. Altosid® P35 larvicide will control mosquito emergence for 35 days of continuously wet conditions at labeled use rates. (S)-Methoprene will begin releasing and controlling adult emergence immediately after application.

To evaluate the effectiveness of an application, there are a few simple steps to take that are outlined in the following protocol. After an application, observations should be made after first 48-72 hours to determine inhibition of adult emergence. Dip counts taken at various locations at the treated site are a good way to collect pupae. The IGR effect from methoprene will prevent adult emergence for up to 35 days post-treatment. Pupae must be collected from the field and held in the lab to determine inhibition of adult emergence. Collecting larvae is not an effective method for determining inhibition of adult emergence. Mosquito larvae must be in a treated environment upon molting to pupae. Water brought back from the treatment site will lose methoprene and there may not be enough left in the water to cause an effect.

HELPFUL EVALUATION TIPS:

- Choose a site that is readily accessible containing abundant larval populations
- Ensure proper calibration of equipment and uniform application
- Existing pupae present at the site of treatment will not be affected by the IGR, wait 48 hours following the application to evaluate pupae for IGR effect
- Untreated sites may be used as a comparison to treatment sites
- Try to collect 25 pupae per dip for evaluation
- Record data and observations in a lab book or in prepared data sheets
- Take dip counts at random locations within the treatment site, dip count numbers will vary based on site size and is up to the investigator to determine the appropriate sampling size and location
- Altosid® P35 larvicide has a range of application rates, it is suggested that on first evaluation 5 pounds per acre be used

ALTOSID® P35 FIELD TESTING GUIDELINES:

Pre-treatment larval abundance (first and second instar larvae, third and fourth instar larvae, and pupae) should be recorded in both experimental and control sites. The sampling method should be appropriate to the type of breeding habitat, and the appropriate number of samples should be taken from each habitat based on the type and size of the habitat. Larval instars and pupae from each sample are counted and recorded.

Post-treatment larval abundance (all stages) should be monitored 48 hours post application and then weekly using pupae collected from treated and untreated control areas. After the first 48 hours, begin collecting pupae and rearing them out for inhibition of emergence calculations. Inhibition of emergence begins at application, but waiting 48 hours after the initial treatment ensures that all pupae collected have developed in the presence of (S)-methoprene.

Collection of pupae starts at 48 hours, 1 week, 2 week, 3 week, 4 week and 5 week post-treatment, if possible.

Characterization of the habitats in terms of abiotic and biotic factors aids the interpretation of results. Rainfall and any change in water level or other parameters, such as algal bloom, water quality, outflow, temperature or predators in the habitats, should be recorded.

DATA CALCULATIONS:

Use the attached data sheets to record dip counts and pupae collected as follows:

1. **Record conditions at treatment site and any factors that may affect application.**
2. **Record pre-treatment larval populations on the data sheet with 10 random dip counts for the treatment and control areas.**
3. **Begin collecting pupae after the first 48 hours and rearing them for emergence calculations.**
4. **Make pupal collection weekly or when present and check for emergence inhibition until control percentages drop below 70%.**

CALCULATION METHODS:

The efficacy and residual activity of the larvicide is determined from the post-treatment counts of pupae in treated and control sites.

The assessment of an IGR's efficacy is based on the level of inhibition of emergence of adults and the percentage reduction of pupal densities. Pupae are sampled as described on the reverse side.

Adult emergence can be monitored directly in the field by floating sentinel emergence traps in treated and untreated habitats, by pupal isolation, or by sampling and counting pupal skins. Adult emergence may also be assessed by collecting pupae (20–40 per replicate) and bringing them to the laboratory in glass containers with the water from the respective habitats, then transferring them to small cups inside the holding cages. Dead pupae found in the cups should be removed and any morphological abnormalities recorded.

When adult emergence is monitored in the laboratory using pupae collected from treated and untreated habitats, IE% is calculated using the following formula, on the basis of determining adult emergence from the number of pupae isolated:

$$IE(\%) = \left(\frac{C-T}{C}\right) x100$$

Where C = percentage emerging or living in control habitats and T = percentage emerging or living in treated habitats.