Cone bioassay of IRS Treated Blocks

Contributions from KCMUCo

WHOPES guidelines- Testing mosquito adulticides for indoor residual spray and treatment of mosquito nets

At least 40 mosquitoes per block need to be tested, in four replicates of 10 mosquitoes. The non-blood-fed susceptible female mosquitoes aged 2–5 days are introduced into plastic cones held at an angle of 45° for an exposure period of 30 minutes or as specified in the study protocol. Substrates are maintained at 30° ± 2°C, 80% ± 10% RH between each bioassay. After exposure, females are placed in 150 ml cups (10 individuals per cup), with sugar solution provided, and maintained in a climatic chamber for 24 hours or as otherwise stated in the study protocol at 27 °C ± 2 °C and 80% ± 10% RH. The percentage mortality after 24 hours, or as otherwise stated in the study protocol, is recorded.

Purpose

Assays of IRS-sprayed blocks are carried out to determine toxicity to test systems (mosquitoes), expressed as knockdown, mortality and duration of effective action at pre-determined time points post spraying. The methodology uses a standard exposure time of 30 minutes at 27±2°C and 80+10%RH but this can vary for different AIs and different protocols. Knockdown is recorded at 60 minutes post exposure and mortality typically after 24 hours.

For non-pyrethroid insecticides and/or insecticides with a mode of action that does not target the nervous system, a different exposure time and/or an extended observation period post-exposure may be required. In addition, other outcome measures may be included such as effect on fecundity post-exposure. Always refer to the relevant study protocol for the required conditions of exposure, length of exposure and observation periods post-exposure. The procedures for conducting cone assays should be closely followed so that tests are performed under the same conditions and standards, and data can be compared between different studies.

1. **Location and identification of Test Items**
   1.1. Transfer the pre-sprayed blocks required for the assay to the appropriate test room for the AI under evaluation.
   1.2. Record the test item codes on the form.

2. **Preparation of Test Systems**
   2.1. The type (sex, age, strain) and total number of mosquitoes to be used in the assay are described in the protocol.

2.2. The holding cages are likely to have male mosquitoes as well as some females that are not fit for the assay. Only fit female mosquitoes should be used in the cone assay. Do not use those that are very small, those obviously missing one or more legs or wings, or those that are unable to fly in a coordinated manner.
2.3. Label all the cups that are to be used in the assay. Label the outside of the cup with the study code, date of exposure, test item (including dosage), substrate type, test system, exposure length and replicate number. Cover the top of the cup with a piece of uncontaminated netting, secure it with elastic band. Pierce at the middle with a scalpel and insert a small piece of cotton wool.
2.4. Remove fit female mosquitoes from the holding cages on the morning of the test using a manual aspirator and transfer 10 mosquitoes at a time (unless otherwise stated in the protocol) into paper cups (10 in each cup). Put the cups into the cup holding racks for carrying to the test room. Do this before you have entered the test room for the first time that day to avoid accidentally transferring any insecticide from the test room to the holding room.

3. **Preparation of Test Room and Materials**
3.1. The day before the assay, ensure that all of the cones and the aspirators have been cleaned.
3.2. On the morning of the assay, confirm that all benches are clean and empty.
3.3. An hour before the acclimation period in the Test Room ensure that the environmental conditions are in the range of 27±2°C and 80±10%RH unless otherwise specified in the protocol.
3.4. Make sure there are post-assay release cages in the Test Room for the release (and recovery) of mosquitoes from the cones at the end of the assay. This eliminates the need to aspirate directly from the cones which can be harmful to health. There must be at least 1 cage for each AI (labelled with the AI name) and one control cage. Cages should ideally be 30x30cm to allow the technicians to put both hands inside simultaneously when manipulating cones inside the cages.

4. **Acclimation (pre-exposure) period**
4.1. Mosquitoes should be transferred to the Test Room in cups and held for a 1 hour acclimation period before starting the cone bioassays.
4.2. Acclimation can either be on the test bench or in the incubator, provided environmental conditions are within the acceptable ranges. Check what is specified in the protocol.
4.3. Record the temperature, humidity, the equipment code of the data logger used and the time that the acclimation period started.
4.4. Record the test item, test system, exposure conditions, outcome measures on a raw data form during the acclimation period.

4.5. At the end of the acclimation period again record the temperature, humidity, the equipment code of the data logger used and the time that the acclimation period ended.

4.6. During the acclimation hour, set up masking tape labels and paper labels with the different test item codes/control across the test bench.

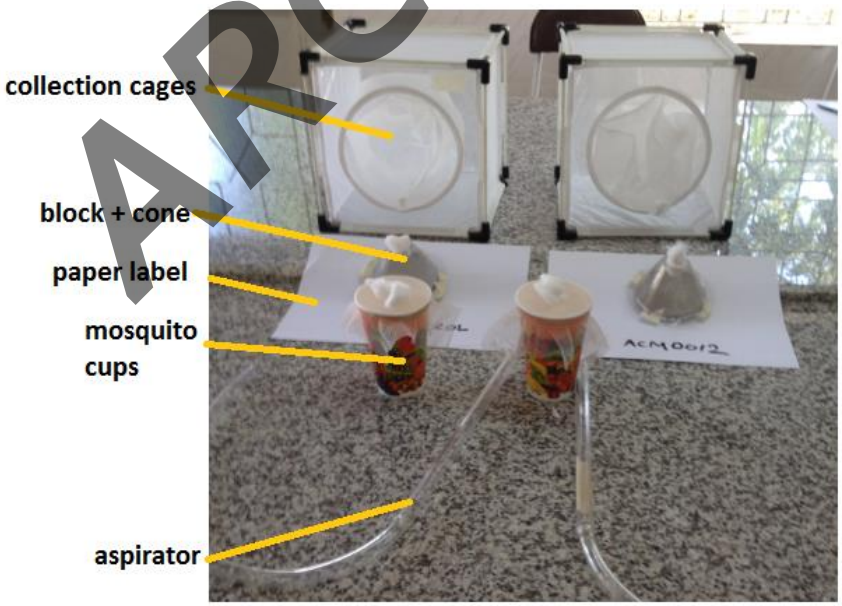
4.7. Ensure the appropriate materials are on the bench or on nearby shelves:

- Clean Aspirators (one for control and one for each treatment).
- Stopwatch
- Marker pen
- Masking tape
- Treated and control blocks
- Cones
- This SOP plus enough copies of necessary forms
- Cup racks (with masking tape labels: control and treatment codes)
- Calibrated data logger with a screen display

4.8. Attach the cones to the treated and control IRS blocks. Use a minimal amount of masking tape to secure the block to the cone and only handle the blocks by their sides. Keep the blocks in the petri dish and make sure the block label is not on the inside of the cone, as that is the untreated side of the block.

4.9. Plug each cone with a plastic bung.

4.10. Once all the cones are ready to use, position the cones at an angle close to 45°. Place the rest of the equipment in front and behind each cone as shown below:



5. Exposure

5.1. Gently transfer 10 mosquitoes (or other number if stated in protocol) into each cone from the corresponding mosquito cups using an aspirator. Ensure that you are using the designated aspirator for each different AI treated block and the control blocks. Do not touch the block with the aspirator.

5.2. Make sure cones are quickly plugged with the plastic bung after transferring the mosquitoes. Hold the plastic bung in one hand whilst introducing the mosquitoes holding the aspirator in your other hand. Do not use unfit mosquitoes (refer section 2.2 above), which might have resulted from aspiration.

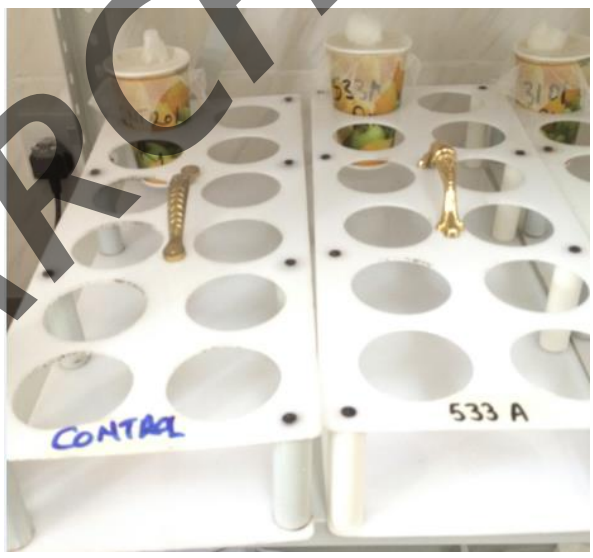
5.3. Once all mosquitoes are in the first cone begin timing using the stopwatch. Note the time on the raw data form. Leave an interval (typically 3 minutes) before repeating the process for the second cone. This interval will give you the time needed to release and recapture the mosquitoes from the first cone at the end of the assay before moving to the next cone in the sequence. Change gloves and aspirator before proceeding if the 2nd cone (2nd block) is of a different AI or concentration, or is a control block. For concentration series of the same AI begin with the lowest concentration and progress to the highest to eliminate the need to change gloves.

5.4. Leave the mosquitoes in the cone to be exposed for the exposure period stated in the study protocol.

5.5. At the end of exposure, place the cone into the corresponding release cage immediately, using both hands. Ensure an elastic band is positioned around the sleeve of the cage to help prevent mosquitoes escaping.

5.6. To remove the mosquitoes from the cone, remove the tape from one end of the block, tilt the cone back from the block, unplug the cone (i.e. remove the cotton wool) and blow into the neck of the cone to gently blow the mosquitoes out. Ensure the sticky side of the tape does not trap any mosquitoes by folding it back away from the block.

5.7. Collect the mosquitoes with the appropriate aspirator and gently transfer them back into the appropriate holding cups. Place 10% glucose solution-soaked cotton wool on top of the cups and place in the racks.



5.8. Repeat the release procedure until all of the mosquitoes have been put back into the original cups, put the cups into the racks and put the racks into a climatic chamber or room set at 27°C ± 2° and 80% RH ± 10%.

5.9. Return the blocks to their acrylic trays and put them back on the shelves in the Block Room.