



Cone bioassay on IRS treated surfaces in experimental huts

Contributions from IHI, KCMUCo

WHOPES guidelines – Guidelines for testing mosquito adulticides for indoor residual spraying and treatment of mosquito netsⁱ

For IRS, confirmation of the insecticidal action of target dose of an insecticide applied can be carried out by spraying it on wall surfaces made of locally-used building materials and testing the bioefficacy and residual effect in cone tests. Batches of 10 non-blood-fed susceptible female mosquitoes, 2–5 days old, are put in a WHO cones and exposed for a standard exposure time (30 minutes or as stated in the study protocol) on the four walls and ceiling of each hut.

Glossary

Knockdown (KD) is sub-lethal incapacitation where mosquitoes are unable to maintain normal posture and/or unable to fly immediately after exposure to a chemical although recovery may occur during the holding period. It is measured as proportion relative to the control. For the purposes of this SOP, knocked down (KD 60) mosquitoes are classified asⁱⁱ:

- Any mosquito that cannot stand (e.g. has 1 or 2 legs)
- Any mosquito that cannot fly in a coordinated manner
- A mosquito that lies on its back, moving legs and wings but unable to take off
- A mosquito that can stand and take off briefly but falls down immediately

For the purposes of this SOP, dead mosquitoes (24 hour mortality) are classified as:

- Mosquitoes that show no sign of life or movement
- Mosquitoes that cannot stand

1. Procedures

a. Preparation of test systems

- i. Prepare the holding room where mosquitoes will be held pre and post-exposure. Ensure the room is at 27 ± 2 °C and $80 \pm 10\%$ RH. Record temperature and humidity.
- ii. Request mosquitoes from the insectary, as described in protocol. The standard for WHO Phase II IRS cone assays is 2-5 days old non-blood-fed susceptible females (e.g. *An. gambiae* s.s. Kisumu or other susceptible species/strain).
- iii. Label the holding cups for mosquitoes with the study code, mosquito species name, date of exposure, insecticide treatment code target dose, location of cones and initial of technician.



- iv. Transfer 10 female mosquitoes into each paper cup. Put the cups in holding racks inside a cool box for transporting to the huts. Add cotton wool soaked with 10% glucose (or other sugar; see SOP Preparation of sugar-soaked cotton wool for feeding adult mosquitoes in holding cages or during transportation between the laboratory and field) solution to the netting on top of the cups. Place a data logger into the box and transport mosquitoes to the huts (see SOP Transportation of mosquitoes)
 - v. Keep cups in the cool box until the cones are attached to the hut walls.
- b. Exposure of mosquitoes in cones
- i. Randomly assign the huts to treatments/trail arms according to Latin Square design. Start with the control hut, ensure you are using the correct designated aspirator for each hut (and/or each AI).
 - ii. Put on clean nitrile gloves.
 - iii. Attach all necessary cones to the wall with 2-4 strips of masking tape, or pins, on the sides. The study protocol will dictate where and how many cones to fix to the walls/ceiling/other surfaces. For each round of testing, cones are positioned in a previously unused position. Study Director uses a randomly-generated list of coordinates for the position of each cone for each wall. Fix the cones to the surface in a way that the cone is securely attached, and mosquitoes will not be able to escape under the lip of the bioassay cone. Cotton wool may be needed to plug the holes on the cones.
 - iv. Once the cones have been attached, gently aspirate 10 mosquitoes into the first cone and plug the cone with a plastic plug.
 - v. Start the stop-watch. Record starting time in the cone bioassay form.
 - vi. Move to the next successive cone and aspirate in the next batch of 10 mosquitoes. Record starting time.
 - vii. Continue to each cone in turn at 2-3 minute intervals until all cones contain mosquitoes.
 - viii. After 30 minutes of exposure gently aspirate the mosquitoes from the first cone into a labelled cup, using a battery-operated aspirator or manual aspirator with HEPA filter.
 - ix. At every 2-3 minutes intervals remove mosquitoes from the other cones in the same order in which they were introduced, this ensuring that each batch of mosquitoes is exposed for 30 minutes.



- x. Place cups containing mosquitoes back into the cool box. Where possible, use separate racks for each treatment and place into separate, labelled cool boxes to avoid contamination
- xi. Put on a new pair of gloves before handling sugar solution and place cotton wool moistened with 10% glucose solution onto the netting covering each of the cups in the holding racks/cool boxes. Place a wet towel on top of the cups to maintain humidity during transportation back to the holding room. Record the temperature and humidity during transit to the holding room using a data logger.

c. Post Exposure Period

- i. Take holding cups containing mosquitoes back to the temperature and humidity-controlled holding room and ensure glucose-soaked cotton wool is still intact. Hold mosquitoes at $27\text{ }^{\circ}\text{C} \pm 2^{\circ}\text{C}$ and $80 \pm 10\%$ RH.
- ii. Score mosquitoes for knock down 60 minutes after the end of the test (i.e. 90 minutes after the start of exposure) using the data form.
- iii. Ensure that there are enough technicians to record knock down while cone tests of other experimental huts are ongoing.
- iv. Once all bioassays have been completed, ensure that all bioassay cones are removed from the huts and put into a plastic bin with a sealed lid for transport back to the laboratory for cleaning. Use a 10% bleach solution to soak the cones and rinse twice with tap water.
- v. To reduce risk of contamination, use a new pair of gloves when moving from one experimental hut to another, and use a new/ different aspirator when conducting cone bioassays in huts with different treatments and with untreated huts.
- vi. At 24 hours after exposure (or as specified in the study protocol), record mortality in mosquitoes. Enter data in the data forms.
- vii. If required, mosquitoes should be preserved according to the relevant SOP.

ⁱ http://whqlibdoc.who.int/hq/2006/WHO_CDS_NTD_WHOPES_GCDPP_2006.3_eng.pdf

ⁱⁱ *Definition of knock-down and mortality for adult mosquitoes*. Report of the 15th WHOPES Working Group Meeting, 2012 (available at : http://apps.who.int/iris/bitstream/10665/75304/1/9789241504089_eng.pdf).