

Cone Bioassay

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WHOPES: Guidelines for laboratory and field testing of long-lasting insecticidal nets

Five, susceptible, non-blood-bed, 2-5-day old female Anopheles (species to be stated in the test report) mosquitoes are exposed to each piece of netting (25cm x 25cm) for 3 min under standard WHO cones held at an angle of 45°, after which they are held for 24 h with access to sugar solution. Knock-down is recorded 60 min after exposure and mortality after 24 h. One piece each from four different nets should be tested. Up to four cones at a time may be attached to a piece of netting, and five mosquitoes at one time should be exposed in a cone. This procedure should be repeated until a total of 50 mosquitoes have been exposed to each piece. Results should be reported for each net tested and for the four nets (4 pieces x 10 cone tests x 5 mosquitoes = 200 mosquitoes). Mosquitoes exposed to untreated net pieces are used as controls; they should be tested each day, just before and just after testing treated netting material. If the mortality in controls on any day is <10%, the results for that day should be adjusted by Abbott's formula. If the mortality in controls is >10% on a given day, the results for that day are considered invalid and should be discarded. Bioassays should be carried out at $27 \pm 2^{\circ}\text{C}$ and $75 \pm 10\%$ relative humidity.

The definitions of mortality and knock-down are those recommended by WHOPES. Mosquitoes are considered to be alive if they can both stand upright and fly in a coordinated manner. Mosquitoes that are moribund or dead are classified and recorded as knocked down at 60 min and as dead at 24 h. A mosquito is moribund if it cannot stand (e.g. has one or two legs), cannot fly in a coordinated manner or takes off briefly but falls immediately. A mosquito is dead if it is immobile, cannot stand or shows no signs of life.

Procedure:

1. Preparation of test room and materials

- a. Label paper cups with the protocol code, date of exposure, test item, exposure length, and replicate number
- b. Ensure the cones, aspirators, frame and acrylic cone holding panel have all been cleaned in 10% bleach and rinsed twice with clean water.
- c. Place a clean bench guard on top of the bench, and fix with masking tape.
- d. An hour before the acclimation period, switch on the humidifier and heater as necessary to $27 \pm 2^{\circ}\text{C}$ and $75 \pm 10\%$.

2. Acclimation (pre-exposure) period

- a. Remove the glucose-soaked cotton wool from the mosquito cage approximately 1 hour before starting all testing process.
- b. Remove or reduce if possible males from the cage to be used for testing.
- c. Transfer the cage of mosquitoes to be tested to the test room. Acclimation can be either on the test bench or in the incubator depending on protocol specification.
- d. Aspirate 5 female mosquitoes into each paper cup. Choose female mosquitoes that are fit, appropriately sized, and able to fly consistently. Do not choose mosquitoes that are small, missing one legs or wings, or that are unable to fly in a coordinated manner.
- e. Allow mosquitoes to acclimatize for 1 hour
- f. Record the temperature, humidity, logger ID number, and time acclimation started on the form.

3. Preparation for the cone bioassay

(The setup of the test is done during the acclimation period)

- a. Put on gloves and lab coat
- b. Cover the paper cups with the untreated net
- c. Label paper cups (or disposable cups) with the protocol code, date of exposure, test item, exposure length, and replicate number.
- d. Prepare the frames on the bench. Ensure there is 1 control frame.
- e. Ensure the equipment is prepared and clean: Aspirators (1 for each AI, 1 for control), frame, stopwatch/timer, marker pens, masking tape, cup racks.
- f. Net pieces to be tested should be packed individually in foil
- g. Prepare all the net pieces. Starting with the control, unwrap the net piece from the foil and position the net piece on the frame. Tape the net in place or use bulldog clips to hold it in place. Label the area on the frame with the corresponding net piece ID or protocol ID.
- h. Set up the frame at 45° using a raised platform.
- i. Plug the cone with plastic bung.
- j. Record the temperature, humidity, logger ID, and time acclimation period ended on the form.

4. Exposure

- a. Aspirate 5 female mosquitoes from a cup into the negative control cone and quickly plug the cone with the plastic bung. Ensure that correctly labelled aspirator is used to avoid cross-contamination.
- b. Once all of mosquitoes are in the first cone, start the timer 1.
- c. Wait for 1 minute and aspirate 5 mosquitoes into the next cone.
- d. Repeat this procedure until mosquitoes have been introduced into all cones, changing gloves and aspirator if exposing mosquitoes to different treatments.

- e. 3 minutes after mosquitoes have been introduced into the first cone, aspirate the mosquitoes back into the labelled paper cup (or disposable cup).
- f. Write the time at which the exposure was ended for each cone and read the knock down 60 minutes later (see section 6 for knockdown assessment criteria).
- g. Continue until all the samples have been tested
- h. Place glucose-soaked wool on top of the cups after recording knockdown and place in racks.

5. Post Exposure

- a. Return net pieces to corresponding aluminum foil, wrap and put in the +4°C refrigerator or designated storage following testing.
- b. Decontaminate all insecticide-contaminated material according to the relevant SOP.
- c. Dispose of bench guard in a biohazard bag.

6. Recording knock down and mortality

- a. Knock down. Mosquitoes are scored as being alive if they can both stand upright and fly in a coordinated manner. Mosquitoes that are moribund or dead are classified and recorded as knocked down at 60 minutes.
- b. Mortality. Mosquitoes that are moribund or dead are classified and recorded as dead at 24 hours (or later time point for treatments giving delayed mortality).
- c. A mosquito is moribund if it cannot stand (e.g. has one or two legs), cannot fly in a coordinated manner or takes off briefly but falls immediately. A mosquito is dead if it is immobile, cannot stand or shows no sign of life.

7. Data analysis

- a. If knockdown and mortality in the negative control is < 5%

$$\text{KD60 (\%)} = \frac{\text{Total number of knocked down mosquitoes}}{\text{Total number of mosquitoes tested}} \times 100$$

$$\text{Observed mortality (\%)} = \frac{\text{Total number of dead mosquitoes}}{\text{Total number of mosquitoes tested}} \times 100$$

- b. If knockdown and mortality in the negative control is >5% but <10% correct using Abbots formula. Control mortalities of <5% require no correction.



Abbots formula:

$$\text{Corrected mortality} = \frac{(\% \text{ observed mortality} - \% \text{ control mortality})}{(100 - \% \text{ control mortality})} \times 100$$

- c. When mortality is >10%, the results should be discarded except for when there is delayed mortality when results may be retained up until the time at which control mortality exceeds 10%.

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