# Monitoring and managing insecticide resistance in *Aedes* mosquito populations

Interim guidance for entomologists

World Health Organization

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# Introduction

The use of safe and efficacious insecticides against the adult and larval populations of mosquito vectors is one of the most effective ways to rapidly interrupt transmission of Zika virus, as well as other viruses transmitted by *Aedes* mosquitoes such as chikungunya and dengue.

Insecticide resistance monitoring in field populations of *Aedes* is required to determine the levels, mechanisms and geographical distribution of resistance in order to select appropriate insecticides for vector control. Evidence-based decisions will ensure that effective insecticides are selected and used. Changes in insecticide susceptibility status should also direct policy and operational decisions.

Insecticide resistance monitoring is an essential part of entomological surveillance. Together with information on adult mosquito density, larval and pupal indices, ecology and habitats, and efficacy of vector control interventions, appropriate responses to prevent and control Zika virus and other mosquito-borne viruses can be developed.

This document summarizes WHO test procedures for the detection of insecticide resistance in *Aedes* larvae and adults including insect growth regulators (IGRs) and Bti products. It also outlines strategies to manage insecticide resistance in countries facing Zika virus and other viruses transmitted by this species of mosquito.

This document is for qualified entomologists at national and sub-national level who are responsible for evaluating the susceptibility status of local *Aedes* populations.

# Procedures to test adult mosquitoes

## Mosquito sampling and rearing

Representative sentinel sites need to be identified in Zika affected areas for the assessment of insecticide resistance status of *Aedes* populations. These should include neighbourhoods with the highest insecticide application in public health. Larval stages are easier to collect from the most productive breeding sites, and should be kept alive and taken to a local or centralized insectary facility for rearing. Usually the second or F2 generation is used, as enough number of larvae or adult mosquitoes are needed for the necessary tests.

Only female mosquitoes should be used in the tests. It is recommended that susceptibility tests be performed on non-blood fed females of 3–5 days old.

## Conditions

The optimum conditions for phenotypic susceptibility tests are 27 ± 2°C temperature, 75 ± 10 % relative humidity and low illumination that are usually maintained in an insectary. Where such infrastructure is not available, the tests should be done indoors in a building free from insecticidal contamination while maintaining optimum humidity and temperature using local procedures and avoiding extreme illumination and wind. Where possible, subsequent comparison test should be made under similar conditions of temperature and humidity.

## Materials

- 1 mosquito cage
- Live female mosquitoes (140 healthy specimens needed for testing)
- 14 plastic tubes (125mm L x 44mm D)
- 7 steel spring-wire clips
- 7 copper spring-wire clips
- Red marker, or 5 red dot stickers
- Yellow marker, or 2 yellow dot stickers
- 14 sheets clean white paper (12 x 15 cm)
- 5 sheets insecticide-impregnated paper
- 2 sheets oil-impregnated control paper
- 7 pads of cotton wool
- 10% sugar water solution
- 140 Eppendorf tubes

## Procedures for susceptibility testing[1]

1. Prepare seven **holding tubes** by rolling seven sheets of clean white paper (12 x 15 cm) into a cylinder shape. Individually insert each cylinder into a separate holding tube, and fasten into position with a steel spring-wire clip. Attached the tubes to slides.
2. In each of the holding tubes, aspirate 20 active female mosquitoes (in batches) from a mosquito cage through the filling hole in the slide. After the mosquitoes have been transferred, close the slide unit and set the holding tubes in an upright position for one hour at optimum test conditions. After one hour, replace any knocked-down, dead or damaged mosquitoes with healthy ones.
3. Prepare seven additional tubes in the same manner as the holding tubes. Mark five with a red dot (**exposure tubes**) and two with a yellow dot (**control tubes**). Line each exposure tube with a sheet of insecticide-impregnated paper, and the two control tubes with a sheet of oil-impregnated control paper. Fasten the papers to each tube with a copper spring-wire clip.
4. Attach the five exposure tubes to the vacant position on the slides, and open the slide unit. Blow the mosquitoes gently into the exposure tubes. Once all the mosquitoes are in the exposure tubes, close the slide unit, detach the exposure tubes and set them upright. Fill the two control tubes with mosquitoes in the same way.
5. Keep the mosquitoes in the exposure and control tubes for one hour. Make sure that the tubes are set in an upright vertical position with the mesh-screen on top.
6. After one hour, transfer the mosquitoes back to the holding tubes by reversing the procedure outlined in Step 4. Set all the holding tubes upright, with the mesh-screen on top. Soak a pad of cotton-wool in 10% sugar water solution and place on the mesh-screen.
7. Maintain the mosquitoes in the holding tubes for a 24 hour recovery period, under the optimum conditions previously described. It is important to keep the holding tubes in a place free from extreme temperature and humidity, ideally in an insectary facility. Temperature and humidity should be recorded during the recovery period.
8. At the end of the 24 hour recovery period, count and record the number of dead mosquitoes (refer to Annex 1 for definitions of knockdown and mortality).

   If mosquito mortality in the control tubes exceeds 10%, correct the mortalities of all treated groups using Abbott's formula (below). Discard the test and repeat if the corrected mortality in the control tubes exceeds 10%.

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

9. If supplementary tests (biochemical or molecular) are necessary after completing the susceptibility test, transfer each mosquito (dead or alive) to an individual, clearly labelled Eppendorf tube. Refrigerate and store the tubes until they can be processed for supplementary testing.

## Interpretation of results

In light of new knowledge and the need for prompt action to counter the spread of resistance among vector populations, guidance on interpreting the results of the WHO bioassay test has been revised. The current recommendations are as follows[1]:

- Mortality between 98–100%: Susceptibility is indicated
- Mortality less than 98%: Resistance suggested. Further tests are needed to verify.
- Mortality between 90%–97% (corrected if necessary): Presence of resistant genes in the vector population must be confirmed. The confirmation of resistance may be obtained by performing additional bioassay tests with the same insecticide on the same population or on the progeny of any surviving mosquitoes (reared under insectary conditions) and/ or by conducting molecular assays for known resistance mechanisms. If at least two additional tests consistently show mortality below 98%, then resistance is confirmed.
- Mortality less than 90%: Confirmation of existence of resistant genes in the test population with additional bioassays may not be necessary, as long as a minimum of 100 mosquitoes were tested. However, further investigation of the mechanisms and distribution of resistance should be undertaken.

## Sourcing test kits and papers

Insecticide impregnated papers are currently prepared at University Sains Malaysia, Penang, Malaysia[1] (on behalf of WHO). The information on discriminating concentrations of insecticides is currently limited[2], therefore local tests may be carried out to establish baseline doses using test papers with serial doses that can be ordered with the facility in Penang, Malaysia.

As a routine, the papers are prepared only with the discriminating concentrations of the relevant insecticides and those are packed in plastic boxes; each box contains 8 papers. The equipment and/or insecticide impregnated papers can be ordered separately. Table 1 shows the discriminating doses for *Aedes*.

Given the logistical challenges of securing these supplies timely and the fact that these are needed to support the current Zika epidemic, exceptionally, other reputable sources – especially of test papers are encouraged to supply them.

**Table 1. Discriminating concentrations and exposure time of insecticides used for *Aedes* mosquitoes[2]:**

| Insecticide class | Insecticide | Discriminating concentrations | Exposure period (hours) |
|---|---|---|---|
| Pyrethroids | Cyfluthrin | 0.15%[b] | 1 |
| | Deltamethrin | 0.03%[a] | 1 |
| | Lambdcyhalothrin | 0.03% | 1 |
| | Permethrin | 0.25% | 1 |
| | Etofenprox | 0.5%[b] | 1 |
| | Alpha-cypermethrin | 0.03%[a] | 1 |
| Organophosphate | Fenitrothion | 1% | 1 |
| | Malathion | 0.8% | 1 |
| | Pirimiphos methyl | 0.21%[b] | 1 |

[a] Tentative

[b] Determined for *Anopheles* mosquitoes[1], tentative for *Aedes*.

[1] Ordering instructions can be found at http://www.who.int/whopes/resistance/en/WHO_CDS_CPE_PVC_2001.2.pdf.

[2] For more information, see http://who.int/whopes/resistance/en/Diagnostic_concentrations_May_2014.pdf.

# Procedures to test larvae

## Larvae collection

*Aedes* larvae can be collected from the most productive breeding containers by using dippers or by the use of ovitraps. The larvae must be sorted into different instars in the insectary.

## Conditions

The optimum conditions for larvae susceptibility tests are similar as for adult mosquitoes: a proper insectary facility in which enough larvae are reared for the tests is required. This should include a bench in which the testing cups can be maintained without disturbance during the tests. Note that for some insecticides such as insect growth regulators, the tests could last around 2 weeks. 27 ± 2°C temperature, 75 ± 10 % relative humidity and low illumination that are usually maintained in an insectary. Where such infrastructure is not available, the tests should be done indoors in a building free from insecticidal contamination while maintaining optimum humidity and temperature using local procedures and avoiding extreme illumination and wind. Where possible, subsequent comparison test should be made under similar conditions of temperature and humidity.

## Materials

- 140 x 3th –4th instar larve
- 1 pipette capable of delivering 100–1000 µl
- 5 x 1 ml pipettes (insecticides)
- 1 x 1 ml pipette (control)
- 100 x 100 µl disposable tips
- 100 x 500 µl disposable tips
- 3 droppers with rubber suction bulbs
- 1 small strainer or a loop of plastic screen
- 7 disposable cups (if not available, use 120ml and 250ml glass bowls/beakers)
- 1 graduated measuring cylinder
- Data recording forms
- Log–probit software or paper
- Alcohol (or organic solvent)