STANDARD OPERATING PROCEDURES

For

Aedes Vector Surveillance in Sri Lanka

National Dengue Control Unit

Ministry of Health, Nutrition and Indigenous Medicine
Sri Lanka

STANDARD OPERATING PROCEDURES

FOR *AEDES* VECTOR SURVEILLANCE IN SRI LANKA

National Dengue Control Unit
Ministry of Health, Nutrition and Indigenous Medicine
Public Health Complex
555/5, Elvitigala Mawatha, Narahenpita, Colombo 05, Sri Lanka

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This document is the essential resource on Standard Operating Procedures for *Aedes* vector surveillance in Sri Lanka published by the National Dengue Control Unit in March 2019.

This SOP was developed based on the best available scientific evidences at the time of writing and the field experience of the expertise. The SOP will be reviewed periodically when new evidences become available. All the units in the content are SI units.

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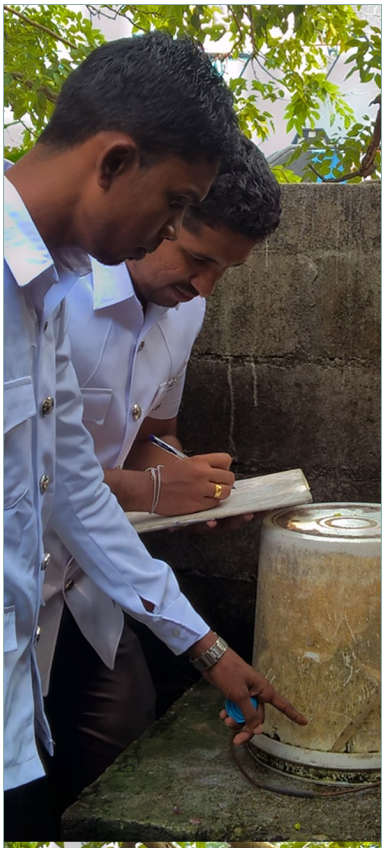

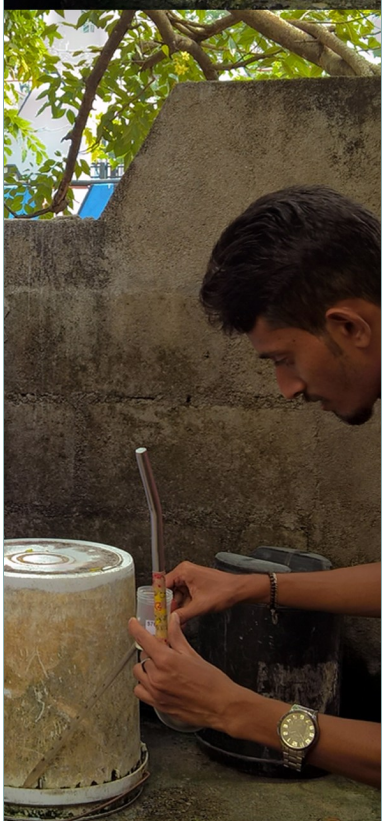

Aedes mosquito sampling from their typical breeding place

FOREWORD

Dengue continues to be a national and international challenge. In Sri Lanka, the burden of dengue fever is significantly effecting on the social and economic situations. Since the first serologically confirmed dengue case in 1962, Sri Lanka has experienced a fast distribution of dengue due to the rapid urbanization, increased population and enhanced transporting facilities.

"Standard Operational Procedures for Dengue Vector Surveillance in Sri Lanka" developed by National Dengue Control Unit, is expected to further improve existing knowledge and practice on *Aedes* vector surveillance in Sri Lanka to execute control activities more efficiently and effectively. Its guiding principle is to harmonize prevention via entomological surveillance within the existing health system ensuring this effort is coherent, sustainable and cost effective.

This document will be the key technical guide for both national and subnational vector surveillance teams for standard implementation of *Aedes* vector surveillance.

Dr. Hasitha Tissera

Director

National Dengue Control Unit

PREFACE

Entomology is one of the widest branch in biology and is the major area of science dealing with the disease transmitting insect vectors. *Aedes aegypti* and *Ae. albopictus* (Diptera: Culicidae) are responsible for the transmission of dengue, chikungunya, zika and yellow fever. Hence, scientifically collected entomological data are important for effective and efficient control of *Aedes* vector borne diseases specially the dengue.

This document comprehensively describes the entomological techniques on *Aedes* vector surveillance including *Aedes* immature (Larvae and pupae) survey, Ovitrap survey, Adult mosquito survey, insecticide susceptibility test for adult/larvae and cage bioassays. The content of this document is primarily based on the currently available guidelines, scientific knowledge and further supplemented by experiences of central and peripheral entomological staff on vector surveillance. The objective of this document is to plan and implement uniform and systematic *Aedes* vector surveillance system in order to achieve the best outcome from the ongoing *Aedes* vector surveillance and control programmes in Sri Lanka.

Hitherto, a complete and comprehensive document is not available for *Aedes* vector surveillance and this document would fill this gap and create a firm foundation to strengthen the current vector surveillance system throughout the country. Subsequently, this would aid to reinforce the prevailing vector control component of the dengue control programme. We hope that the detail provided in this SOP would be a scientific guide to help the national and subnational entomology teams and researchers for designing and running effective *Aedes* vector surveillance system in the country.

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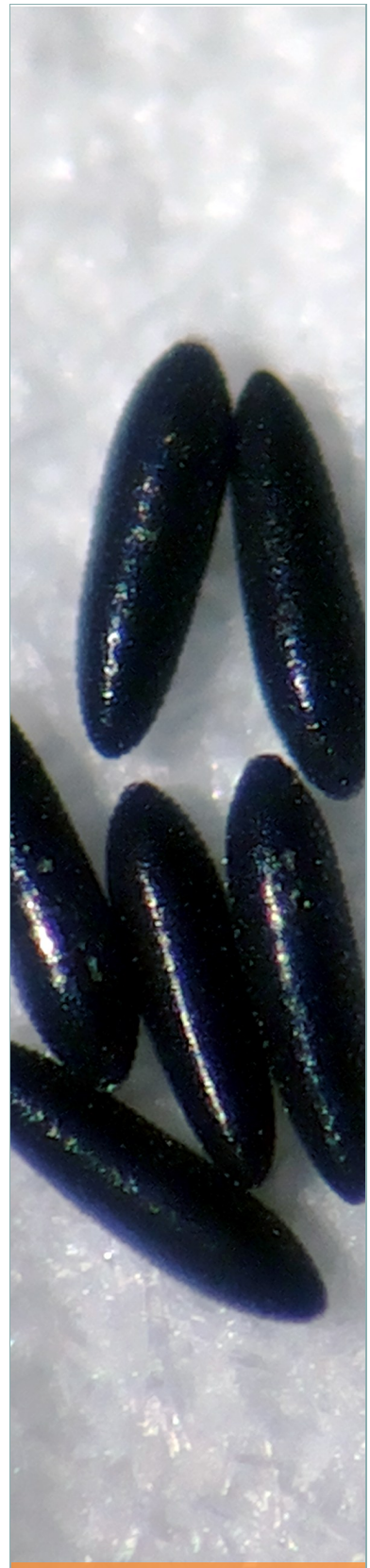

Eggs of Aedes mosquito

Larval stage of Aedes mosquito

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Pupal stage of Aedes mosquito

Dorsal view of a Aedes albopictus Adult mosquito

INTRODUCTION

Dengue is an arbo-viral disease complex which includes dengue fever (DF), dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS). The disease is caused by four serotypes of the dengue virus, namely, DENV-1, DENV-2, DENV-3 and DENV-4. Over the years of the past two decades, the number of dengue cases reported in Sri Lanka shows an increasing trend with epidemics at 2-3 year intervals and two seasonal peaks every year.

Dengue is transmitted by female mosquitoes of *Aedes aegypti* and *Aedes* albopictus. *Ae.aegypti* is considered as the primary dengue vector which shows primarily an antropophilic blood preference. *Ae.albopictus*; the secondary vector prefers blood meal from humans but also a variety of domestic and wild vertebrates inhabiting in the area. These vectors are container breeders and the most common breeding sites in Sri Lanka are discarded containers, tyres, water storage tanks and barrels. In the absence of a specific treatment or an effective vaccine for dengue virus so far, vector control is the mainstay of dengue prevention and control. Planning of vector control activities are heavily depend on the entomological data which are generated from entomological surveillance.

Entomological surveillance is the analysis and interpretation of systematically collected entomological data to determine changes in the temporal and spatial distribution of the vector, vector ecology, dynamics, bionomics and monitoring resistance for different insecticides.

This information facilitates appropriate decisions regarding vector control interventions, monitoring and evaluation of dengue control programmes. Therefore, collection of complete and uniform entomological data is of immensely important to generate sound entomological information.

This Standard Operating Procedures (SOP) was developed for use as the reference document for the entomological techniques carried out in sentinel, routine and spot sites. Including techniques are *Aedes* immature (Larvae and pupae) survey, Ovitrap survey, Adult mosquito survey, insecticide susceptibility test for adult/ larvae and cage bioassays. Furthermore it describes the objectives, frequency, equipment and materials, procedures, procedure notes, data collection, recording and reporting system of each technique.

Provincial Directors of Health Services, Regional Directors of Health Services and programme managers are requested to facilitate to implement this guideline by their entomological staff. Furthermore, researchers who are engaging the entomological works are requested to use as a guideline document for *Aedes* vector surveillance.

Dorsal view of a Aedes aegypti Adult mosquito

1. *Aedes* immature survey (Larvae and pupae)

1.1. Introduction

Aedes immature surveys provide information on the presence of *Aedes* species and their densities in an area. Whereas, the pupal surveys give the proxy of adult density in an area.

1.2. Objectives

Larval Survey

Breeding sites, Temporal and spatial distribution of Aedes aegypti and Aedes albopictus.

i. To identify the key breeding premises/habitats and to determine the density of *Aedes aegypti* and *Aedes albopictus.*

ii. To determine the temporal and spatial distribution of *Ae. aegypti* and *Ae. albopictus.*

Early warning of dengue outbreaks / epidemics

iii. To provide early warnings of dengue outbreaks/epidemics.

Evaluate the vector control activities

iv. To determine the appropriate vector control interventions.

v. To evaluate the impact of vector control interventions.

Pupal survey and pupal demographic survey

To identify most productive container habitats of Aedes aegypti and Aedes albopictus.

i. to identify the most productive container habitats of *Ae. aegypti and Ae. albopictus.*

To get information on proxy of adult density of Aedes aegypti and Aedes albopictus.

ii. to get information on proxy of adult density of *Ae. aegypti and Ae. albopictus.*

1.3. Frequency

Frequency of surveillance at sentinel, routine and spot survey sites.

Sentinel /routine survey sites – monthly surveys.

Spot survey sites — on requirement.

Note: See* Annexure I *for selection criteria of survey sites.

1.4. Equipment and materials

Field equipment

- Map of the study site
- Plastic dipper (capacity of ≈350 ml) with extendable handle
- Ladle (capacity of 120 - 150 ml)
- Plastic Pipettes (3 ml /10 ml/25ml/50ml)
- Larval collection vials with caps
- Portable ladder - optional
- Portable mirror with extendable handle
- Well net (fine mesh net – nylon gauze net, ring of iron wire 20-25 cm in diameter) - optional
- Torch
- Labels
- Marker pens, Pencil, Ball point pen
- Note book / Clipboard
- GPS device - optional
- Portable Multi parameter for Water quality assessment - optional
- Relevant forms (***NDCU/EN/01, NDCU/EN/02, NDCU/EN/03/I & II, NDCU/EN/04, NDCU/EN/05 and NDCU/EN/06***)
- Field bag

Laboratory equipment

- Binocular compound microscope
- Glass slides
- Cover slips
- 10 % Formalin solution
- Dissecting needles
- Hand lenses
- Enamel trays/bowls
- Larval (mosquito) identification keys

Figure 1.1: Ladle

Figure 1.2: Pipette

Figure 1.3: Vials

Figure 1.4: Compound microscope

Figure 1.5: Dissecting microscope

1.5. Procedure

A complete survey should cover 100 premises

- Start the survey at 7.30 a.m.
- The basic sampling unit is the house/ premises.
- Complete 33 – 35 households/premises per day.
- Cover 100 premises for a complete survey as follows,
  - ◊ by a team of 1 HEO and 2 SMO/SKS within 3 days
  - ◊ by a team of 3 HEO and 6 SMO/SKS within 1 day

Use systematic sampling method to select houses/premises. Select the first house/premises randomly.

- Select the first house/premises randomly. Then use the systematic sampling method to continue the survey in every n^{th} house.
- Definition of the n^{th} number is as follows,

$$n = \frac{\text{Number of premises in the area}}{\text{Number of premises required for the survey}}$$

- Visit each selected house/ premises, explain the purpose of visit and obtain the consent to examine the premises for mosquito breeding sites.

Examine both indoors and outdoors for mosquito breeding sites

- Examine indoor and outdoor areas (both ground and upper layers) for mosquito breeding sites ensuring that the whole area is examined.

Use one larval collection vial for one breeding site

- If a mosquito breeding site is found, collect 10 *Aedes* larvae and all pupae (if < 100 pupae) in to labeled larval collection vials separately. In instances where 10 larvae are not present, collect them all.

Collect 10 Aedes larvae and all pupae from each Aedes positive breeding site

- When there are more than 10 breeding containers of same type (eg. heap of tyres, coconut shells) in one place (record the approximate number), collect larvae from 10 randomly selected such containers.
- Use following collection methods depending on the type of breeding site (see Annexure II for more information).
  1. Dipping
  2. Pipetting
  3. Siphoning
  4. Netting

- Bring the immature stages to the laboratory and identify the 3rd and 4th stage larvae with the help of a compound microscope. Allow the 1st and 2nd stage larvae to develop to 3rd and 4th and the pupae into adults and identify the species.
- Calculate the larval indices, Container Index (CI), Premises Index (PI) and Breteau Index (BI).

CI : Percentage of water-holding containers infested with Ae. aegypti/Ae. albopictus larvae and/ or pupae.

PI : Percentage of premises infested with Ae. aegypti/ Ae. albopictus larvae and/ or pupae.

CI = Number of positive containers for *Ae. aegypti or Ae. albopictus* larvae and/or pupae X 100
Number of wet containers inspected

PI = Number of positive premises for *Ae. aegypti or Ae. albopictus* larvae and/or pupae X 100
Number of premises inspected

BI = Number of positive containers for *Ae. aegypti or Ae. albopictus* larvae and/or pupae X 100
Number of premises inspected

- Maintain the records in the forms ***NDCU/EN/01***, ***NDCU/EN/02***, ***NDCU/EN/03/I, NDCU/EN/03/II*** and ***NDCU/EN/04***.
- If there are pupae, calculate the following pupal indices. Pupal index and pupae per person

BI: Number of Ae. aegypti/ Ae. albopictus positive containers per 100 houses.

Pupal index = Number of *Ae. aegypti or Ae. albopictus* pupae in all containers X 100
Number of houses or premises inspected

Pupae per person = Number of *Ae. aegypti or Ae. albopictus* pupae in all containers
Human population in the surveyed houses

- Pupal index can be determined by pupal counts in different types of containers separately, giving the relative importance of different container types (e.g. tyres, water storage tanks etc.). This information is useful for targeted elimination of the most productive containers.
- Relative importance of different types of containers can be calculated as follows,

Pupal index, pupae per person and relative importance of a breeding site

Relative importance of particular breeding site = Total number of *Ae. aegypti /Ae. albopictus* pupae in particular container type
Total number of containers of that particular type

1.6. Procedural notes

Other premises such as construction sites, schools, factories, religious places etc. should be inspected

Avoid larval surveys while heavy rains.

When a construction site, school, factory, religious place or any other special premises is examined, obtain the permission of the head of the institution and examine all potential mosquito breeding sites and identify the dengue vector breeding sites. Give the potential/positive container types with number and container index.

1.7. Data recording and reporting

Data reporting formats

NDCU/EN/01

NDCU/EN/02

NDCU/EN/03/I

NDCU/EN/03/II

NDCU/EN/04

NDCU/EN/05

- To maintain the field records following formats should be used .
  - **Dengue Entomological surveillance field report – Details of positive/potential premises and containers** (***NDCU/EN/01***). This form is to be completed in the field.
  - **Dengue Entomological Daily Summary Report** – (***NDCU/EN/02***).
  - **Dengue Entomological Surveillance Report** (***NDCU/EN/03/I***).
  - **Dengue Entomological Surveillance containers and premises daily summary report (*NDCU/EN/03/II*)**.
  - **Entomological Survey Report on Institutions/ Construction sites/……. (*NDCU/EN/04*)**.
  - **Dengue Entomological Survey - Pupal Survey Report (*NDCU/EN/05*).**

- **Dengue Entomological Daily Summary Report** (***NDCU/EN/02***) is an interim report to be submitted to the respective Medical Officer of Health (MOH), just after the survey is completed in the area.
- After completion of the laboratory procedures, the completed forms of **Dengue Entomological Surveillance Report** (***NDCU/EN/03/I***) and **Dengue Entomological Surveillance containers and premises daily summary report (*NDCU/EN/03/II*)** have to be sent within 3 working days to the relevant MOH, Entomologist, Regional Malaria Officer (RMO), and Regional Epidemiologist (RE), Anti Filariasis Officer (AFO) of the District/Institution and to the National Dengue Control Unit.
- If the Entomological survey is conducted in **Institutions/ Construction sites/ Special premises** , the survey report (***NDCU/EN/04***) has to be sent to the relevant Entomologist/RMO and NDCU.
- The surveillance report of the **Pupal Survey** (***NDCU/EN/05***) has to be sent to relevant Entomologist/RMO and NDCU.

Send an daily summary report to MOH just after the survey is completed

Send the report of the completed larval survey within 3 working days to relevant MOH, Entomologist/RMO/AFO and RE at district/institution with a copy to NDCU

2. Ovitrap Survey

2.1. Introduction

An Oviposition trap (ovitrap) mimics the preferred breeding site for container breeding mosquitoes, including *Ae. aegypti* and *Ae. albopictus.*

2.2. Objectives

To determine the presence of adult Ae. aegypti and Ae. albopictus in an area

To evaluate the impact of vector control interventions

- To determine the presence of adult *Ae aegypti and Ae. albopictus* in an area.
- To evaluate the impact of vector control interventions.
- To collect *Ae. aegypti and Ae. albopictus* eggs to get larval and adult mosquitoes for susceptibility/bioassay tests.

2.3. Equipment and materials

Field equipment

- Ovitraps (Black plastic containers/cups - Capacity: 350 - 1000 ml, height: 80 - 150 mm; Diameter: 70 - 140 mm)
- Specified absorbent papers/fabric strips (prepared as per the size of the cup)
- Binder clips/ cloth pegs
- Paper towels
- Pair of scissors
- Hand lens (magnifying glass)
- Enamel trays/carrying boxes
- Field note book

Figure 2.1: Ovitrap

2.4. Procedure

- Select 50 premises in the study area and place the ovitrap as follows,
  - ◊ by a team of 1 HEO and 2 SMO/SKS within 2 days.
  - ◊ by a team of 2 HEO and 4 SMO/SKS within 1 day.
- Label the ovitraps with respective **ID numbers** (see Annexure III for labelling).

- Place the prepared absorbent paper/fabric strips to line the upper part of the trap and fix it using binder clips/ cloth peg (Figure 2.1).
- Fill the trap with water up to the level that ⅓ of the paper/ fabric is submerged.
- Place the prepared ovitraps in pairs in the selected premises; (1 outdoor and 1 indoor).
- Keep the traps 5 - 6 days in the field allowing mosquitoes to lay eggs.
- Collect the ovitraps on the 5th/6th day and transport to the laboratory.

Note: If the survey is continuing, retain the trap in the field and collect the paper/fabric strip. Clean the inner wall of the trap properly using a sponge and fix a new paper/fabric strip.

- If larval/pupal stages are present in any trap, collect them in a labeled larval collection tube.
- In the laboratory, count the eggs on each paper/fabric strip using a hand lens / dissecting microscope.
- After completing the egg counting, submerge the paper/ fabric strip in a water filled cup (similar dimensions of ovitrap) to develop into 3rd and 4th larval stages.
- Identify the larvae in each cup and record species and their counts.
- If not rear eggs, store the paper/fabric strip with eggs.

Fill the trap with water up to the level that ⅓ of the lower part of the paper is dipped in water

Identify larvae at 3rd and 4th stages. Allow pupae to emerge to adults and identify at the adult stage

2.5. Data recording and reporting

- Record data in the form of **Ovitrap Surveillance Report** (***NDCU/EN/06***).
- Submit the completed form to the respective Entomologist and NDCU before the next survey.

Record data in the form of NDCU/EN/06 and submit to the respective Entomologist and NDCU before the next survey

3. Adult mosquito survey

3.1. Introduction

To determine the temporal and spatial distribution of indoor/ outdoor resting vector densities, their resting surfaces, and to assess the impact of space spraying and Indoor Residual Spraying (IRS)

Adult *Aedes* mosquitoes are collected from their resting places and flying locations to obtain necessary vector related information.

3.2. Objectives

- To determine the indoor/outdoor resting densities of *Aedes* species.
- To determine the resting surfaces of adult *Ae. aegypti* and *Ae. albopictus* (in indoors).
- To determine the temporal and spatial distribution of indoor /outdoor resting vector densities.
- To assess the impact of indoor space spraying and Indoor Residual Spraying (IRS).
- To collect mosquitoes for blood meal analysis and determination of viral infectivity.

3.3. Equipment and materials

Figure 3.1: Torch

Figure 3.2: Battery operated aspirator

- Aspirator (Mouth / battery operated/ backpack/Prokopack)
- Hand net
- Three celled Torch (with spare bulb and 1.5V batteries)
- Paper cups with net covers
- Cotton wool
- Hand lens
- Stereo microscope
- Dissecting instruments - optional
- Container to transport paper cups/Plastic basin
- Sugar solution (10%)
- Carrying bag
- *Aedes* Identification guides /keys (adult)
- GPS receiver - optional
- Wet towel
- Ant Trap - optional
- Field Note book
- Pencil /pen
- Relevant forms (***NDCU/EN/07***)

3.4. Procedure

- Start the survey by 7.30 a.m.
- Select **40 premises** in each site.
- Complete 10 households/premises per day.
- Cover 40 premises for a complete survey as follows,
  - ◊ by a team of 1 HEO and 2 SMO/SKS within 4 days.
  - ◊ by a team of 3 HEO and 6 SMO/SKS within 1-2 days.
- Visit the premises and get the consent from a responsible occupant to do the collection.
- Spend 20 - 30 minutes to complete the collection in one premises.

Note: spending time is depend on the size of the premises.

- Use a torch to examine indoor mosquito resting surfaces such as walls, roofs, ceilings, cloths, hangings, undersides of furniture, wall hangers, bed nets, curtains etc.
- In outdoors, examine for the adult mosquitoes in dark, humid places such as thick vegetation and other possible places where mosquitoes could be resting.
- Collect resting mosquitoes using a suitable aspirator or a hand net.
- At the field, identify the species and observe the abdominal conditions of the mosquitoes by using a hand lens.
- Transfer the collected mosquitoes in to labeled (see Annexure III for labelling) paper cups (Mouth of the paper cups should be covered with nets).
- Then carefully keep these cups in a plastic basin and cover with wet towels until transfer them to the laboratory for further investigations.

Note: confirm the species with the use of stereo microscope when returned to the field station/laboratory.

The basic sampling unit is a premises

Labelling:

Include date and time of collection, Sample ID, Type of surface and location of the surface, mosquito species and their abdominal condition

Confirm the species with the use of stereo microscope when returned to the field station/ laboratory

Make sure not to put more than 25 mosquitoes per cup

Separate cups should be used according to the different types of surfaces from where mosquitoes have been collected

3.5. Data recording and reporting

- Record the data in the form Dengue Entomological Surveys Indoor and Outdoor Adult Mosquito Resting Collection (***NDCU/EN/07***).
- Submit the completed form to the Entomologist on the following day after the completion the collection

Figure 3.3: Using of mouth aspirator for mosquito collection

Figure 3.4: Using of mechanical aspirator for mosquito collection

4. Insecticide susceptibility tests

4.1. Introduction

Insecticide susceptibility tests are used to detect and characterize the insecticide resistance in vector populations

Insecticide susceptibility tests are used to detect and characterize the insecticide resistance in vector populations. Standard guidelines and test kits are available with World Health Organization (WHO) and the Centers for Disease Control and Prevention (CDC). Further, the information provided by this tests also assist in determining mechanisms associated with resistance.

4.2. Objectives

- To determine the susceptibility level of a dengue vector population (in a defined area) for a particular insecticide using appropriate discriminative dosage.
- To monitor possible changes of insecticide susceptibility levels of vector populations at periodic intervals.

4.3. Frequency

Frequency is once in 6 months in the sentinel site

- Once in 06 months for each dengue vector species for each insecticide of interest in each sentinel site.
- If any indication of resistance is observed repeat the tests with insecticides of 5 times (x5) and 10 times (x10) diagnostic concentrations.

4.4. General Remarks

- These tests should be carried out in divisional, district or central entomology laboratories.
- The optimum conditions for susceptibility tests are 27 ± 2°C temperature, 75 ± 10 % relative humidity and low illumination that are usually maintained in an insectary.

Optimum temperature is 27 ± 2 °C and relative humidity is 75 ± 10 %

- 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 and avoiding extreme illumination and wind.

- Should carry out susceptibility tests for currently used insecticides when there is an continuing outbreak.

4.5. Susceptibility test for adult *Aedes* mosquitoes

4.5.1. Mosquito sampling and rearing

Collect larvae from breeding sites and collect eggs using ovitraps for the tests

- Collect larvae or eggs from each sentinel and routine survey sites separately (Larval stages are easier to collect from the most productive breeding sites. Egg collections from ovitraps could be substituted).
- Transport them to the local laboratory /insectary for rearing (with sugar feeds only).

Use adult female mosquitoes of F1 or F2 generation for the test

- Use adult female mosquitoes of F1 or F2 generation for the test.
- Perform tests on non-blood fed, 3–5 days old females.

4.5.2. Equipment and Materials

- 02 standard WHO diagnostic test kits.

Composition of a WHO standard diagnostic test kit

i. Fourteen (14) plastic tubes (125 mm in length and 44 mm in diameter). Each tube fitted at one end with 16-mesh screen. The 14 tubes are,

Use twelve plastic tubes for the test,

4-exposure tubes with red dots, 2-control tubes with yellow dots and 6-holding tubes with green dots

- Five (05) tubes, each marked with a *red dot* will be used as **exposure tubes**, (for exposing mosquitoes to the insecticide impregnated papers).
- Two (02) tubes, each marked with a *yellow* dot for use as **control tubes**, (for exposure of mosquitoes to the oil-treated control paper, (i.e. without insecticide).
- Seven (07) tubes, each marked with a *green* dot for use as **holding tubes** for pre-test sorting and post-exposure observation).

ii. Seven (07) slide units, each fitted with a screw-cap on both sides and a 15 mm filling hole.

iii. Fourteen (14) spring wire clips (07 steel and 07 copper) to hold the paper in position against the walls of the tubes; the 07 steel clips are to be used with the green-dotted holding tubes and 07 copper clips are to be used with the 05 red-dotted exposure and the two-yellow-dotted control tubes.

iv. Two (02) glass or plastic aspirator tubes of 12 mm internal diameter, together with 60 cm of tubing and mouthpieces.

v. Forty (40) sheets of clean paper (12 x 15 cm) for lining the holding tubes.

vi. One (01) roll of self-adhesive plastic tape.

vii. Instruction sheet, 20 copies of report forms.

- Live female mosquitoes (140 healthy specimens are required for one test
- 05 sheets insecticide impregnated papers
- 02 oil impregnated papers
- 07 sheets of clean white papers
- 07 pads of cotton wool
- 02 aspirators
- Mosquito cage
- Wet towel
- 10% sugar solution
- 01 Digital thermometer
- 01 Hygrometer
- 01 pair of gloves
- 01 pair of forceps

Use 06 steel and 06 copper spring wire clips to hold the paper in position

06 steel clips to green-dotted holding tubes. And the 06 copper clips for the red dotted exposure and yellow-dotted control tubes

Need 140 live healthy female mosquitoes for one test

4.5.3. Procedure

1. Roll 07 sheets of clean white papers (12 x 15 cm) separately to make them cylinder shape and insert each into seven holding tubes (one per tube) and secure them in position with two steel spring-wire clip. Then the tubes should be attached to slide units in the kit.

Carefully transfer 20 mosquitoes per tube without any damage to the mosquitoes

2. Transfer 20 mosquitoes per tube by using the aspirator provided (Figure 4.1). Mosquitoes should be transferred in groups of not more than 10 and gently transferred to the holding tubes through the filling-hole in each side (Figure 4.1). Damage resulting from careless handling of mosquitoes during collection may produce misleading high mortalities.

Let the mosquitoes in the holding tube for one hour before the test. After one hour, replace any knocked-down, dead or damaged mosquitoes with healthy ones

3. Once the mosquitoes have been transferred, the slide unit has to be closed and the holding tube set in an upright position for one hour (Figure 4.1). After one hour, replace any knocked-down, dead or damaged mosquitoes with healthy ones.

4. Five exposure tubes (tubes with red-dots) have to be prepared in much the same way, but lining each tube with a sheet of insecticide-impregnated paper. Use the copper wire clips to fix the paper.

5. Two yellow dotted control tubes should be lined with oil-impregnated papers; each has to be fastened into position with two copper spring-wire clip.

6. Attach the five exposure tubes to the vacant position on the slides, and with the slide unit open the mosquitoes need to be blown gently into the exposure tubes (Figure 4.1). Once all the mosquitoes are in the exposure tube, the slide unit has to be closed and detach the exposure tube and set them upright. Fill the two control tubes with mosquitoes in the same way and keep them upright.

Keep mosquitoes in the exposure and control tubes for one hour in an upright vertical position with the mesh-screen on top

7. Keep 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 (Figure 4.1).

Note: When testing for pyrethroids, timed observation of the rate of knock down (kd) should be made after 10, 15, 20, 30, 40, 50 and 60 minutes of exposure

8. Transfer back the mosquitoes to the holding tubes at the end of the 1-hour exposure period, by reversing the procedure outlined in step 6. Set all the holding tubes upright, with the mesh-screen on top. Soak a pad of cotton-wool in 10% sugar solution and place on mesh-screen.

When testing with pyrethroids, note the knock down (kd) after 10, 15, 20, 30, 40, 50 and 60 minutes of exposure.

9. Maintain mosquitoes in the holding tubes for 24 hours (the recovery period). During this time, it is important to keep the holding tubes in a shady, sheltered place free from extremes of temperature (an insectary is ideal). If conditions are very hot and dry, a moist chamber may be prepared by suspending damp toweling in a container. If necessary, the tubes should be protected from ants by placing them on a platform standing in a pan of water. Temperature and humidity should be recorded during the recovery period.

The recovery period is 24 hours.

10. At the end of recovery period (i.e. 24 hours post-exposure), count and record the number of dead mosquitoes, defined as in Table 4.1.

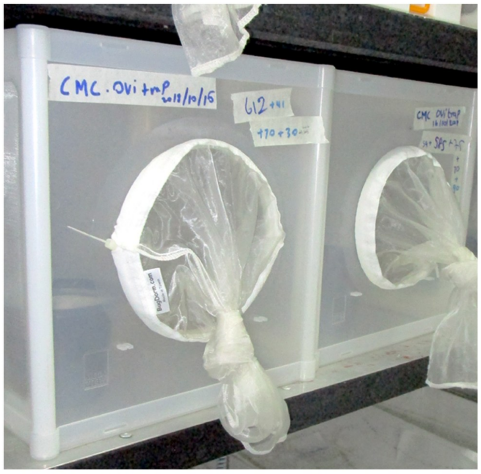
11. If supplementary tests (biochemical or molecular) are necessary after completing the susceptibility test, transfer each mosquito (dead or alive) to an individual, clearly labeled Eppendorf tube.

12. Refrigerate and store the tubes until they can be processed for supplementary testing.

Table 4.1: Classification of adult mosquitoes in bioassays

Alive	Moribund*	Dead*
Can stand on and fly in a coordinated manner	Cannot stand (e.g. has 1 or 2 legs) Cannot fly in a coordinated manner Lies on its back, moving legs and wings but unable to take off Can stand and take off briefly, but falls down immediately	No sign of life; immobile; cannot stand

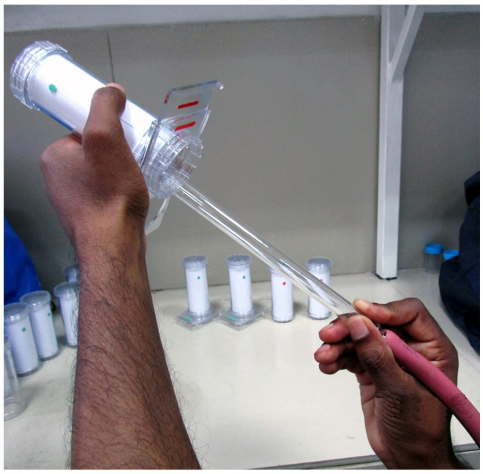
* ***Knocked down after 60 minutes or dead after 24 hours of exposure***



A

B

C



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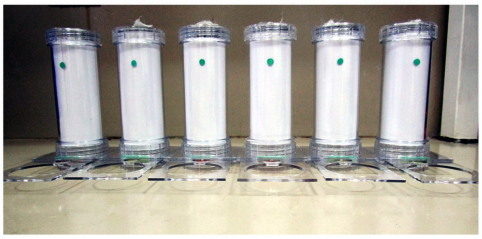
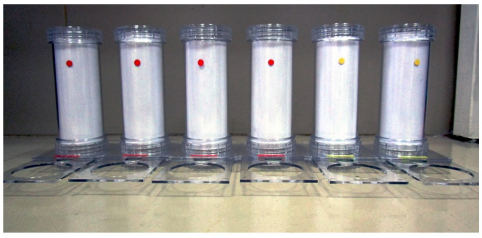


Holding tubes with *green dots*



Exposure tubes (*red dots*) and Control tubes (*yellow dots*)

Figure 4.1: Methodology of the adult mosquito susceptibility test

4.5.4 Insecticide impregnated papers and discriminating concentrations

Table 2. Discriminating concentrations and exposure periods of insecticides used for *Aedes* mosquitoes

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

[a] Tentative [b] Determined for Anopheles mosquitoes1, tentative for Aedes. Source :(WHO/ZIKV/VC/16.1)

- Manufactured date and expiry date of insecticide papers are mentioned in the box. All papers have to be used before the expiry date (expiry date is valid only if the packages are kept sealed all the time).
- After the impregnated paper has been removed, papers should be kept in their original plastic box, sealed with tape and stored in a container or refrigerator at 4°C or, if this is not possible, in a darkened cupboard at room temperature. If papers have been stored at 4°C, they should be brought to room temperature before being used in an exposure test. Test papers should never be exposed to direct sunlight. Date of expiry of each batch is given on the box and should be strictly adhered to.
- The efficacy of impregnated papers declines with the number of uses and the number of mosquitoes tested. This is especially true of the pyrethroid-impregnated papers.

No insecticide-impregnated paper should be used more than 06 times within 2 weeks. Pyrethroid papers should not be used more than 05 times

4.5.5. Calculation of mortality and knock-down rates

$$\text{Observed mortality} = \frac{\text{Total number of dead mosquitoes}}{\text{Total sample size}} \times 100$$

- If mosquito mortality in the control tubes exceed 10%, correct the mortalities of all groups exposed to insecticides using Abbott's formula (below). If the corrected mortality in the control tubes exceed 10% discard the test and repeat .

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

If the mortality between 98-100 %, susceptible.

Less than 98 %, resistance suggested and repeat the test to verify

4.5.6. Interpretation of susceptibility test results

- 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.

4.5.7. Data recording and reporting

Use the form ***NDCU/EN/08*** to report data to respective Entomologist and NDCU.

4.6. Susceptibility test for *Aedes* larvae

4.6.1 Obtaining mosquito larvae

- Collect *Aedes* larvae from the study area. It is easier to collect more larvae from the most productive breeding sites.
- Transport the larvae to the local laboratory /insectary.
- Identify the 3rd and 4th instar larvae and place different species (*Ae. aegypti* and *Ae. albopictus*) in separate containers.
- As an alternative method for larval collection, *Aedes* mosquito eggs can be collected by placing ovitraps in the study area.
- When using ovitraps for obtaining larvae, estimate the number of ovitraps to be placed for obtaining the required number of larvae.
- Place the ovitraps both indoors and outdoors in order to collect both vector species; *Ae. aegypti* and *Ae. albopictus*.
- Collect the ovitraps after 5 – 6 days of placement and bring them to the laboratory.
- Keep the egg strips for 2 days in the laboratory.
- Hatch the eggs and allow them to develop to 3rd and 4th instar larvae.
- Identify the 3rd and 4th instar larvae and place different species (*Ae. aegypti* and *Ae. albopictus*) in separate containers.

Note: Use 3rd and preferably early 4th instar larvae for the test.

Collect larvae from the breeding sites.

Collect eggs using ovitraps

4.6.2. Equipment and materials

A. Larval test kit. It contains

- two (02) 1 ml pipettes (1 for insecticides and 1 for ethanol) with rubber suction bulbs.
- two (02) droppers with rubber suction bulbs (eye droppers)

How to make a strainer

i. Cut and fix two pieces of netting to opposite side of the large end of the wire loop.

ii. Trim the netting with scissors, after drying

WHO diagnostic dosage for Aedes

Insecticide	*Diagnostic dosage (mg/litre)*
Temephos	*0.0125*

- following materials for making a strainer:
  - 02 wire loops
  - 01 piece of nylon netting (30 cm^2)
  - 01 tube of glue (UHU)
  - one (01) polyethylene bottle, 50 ml

B. 140 healthy specimens of 3rd and/or early 4th instar larvae of *Aedes aegypti* and/or *Aedes albopictus*

C. Seven disposable plastic cups or beakers (250 ml)

D. Larval trays

E. Measuring cylinder

F. Thermometer

G. Twenty (20) data recording forms

H. Alcohol or organic solvent

I. Mosquito larval foods

J. Labels

K. 1 pipette capable of delivering 100–1000 µl

L. 5 x 1 ml pipettes (insecticides)

M. 100 x 100 µl disposable tips

N. 100 x 500 µl disposable tips

O. Log–probit software or paper

4.6.3. Insecticide solutions (in 50 ml bottle)

- Temephos 3.125 mg/L,

4.6.4. Procedure

A. Retain the selected larvae in trays/bowls containing de-chlorinated water until they are used for testing.

B. Label 5 disposable plastic cups/beakers with test concentration and remaining 2 as controls.

C. Prepare the test solution.

Instructions to prepare the test solutions.

i. Add 225 ml of distilled water to all test and control cups/ beakers.

ii. Add 1 ml of the insecticide solution to each test cup/ beaker.

iii. Stir the solution vigorously for 30 seconds with the pipette.

iv. Add another 24 ml of water to make the test solution of 250 ml (the final concentration of this solution is 0.0125 mg/L).

v. For the control cups/ beakers, Add 1 ml of alcohol solution to each control cup/beaker.

vi. Stir the solution vigorously for 30 seconds with the pipette.

vii. Add another 24 ml of water to make the solution of 250 ml.

D. Within 15-30 minutes of preparation of the solutions, transfer batches of 20 larvae by means of a strainer to the test and control cups/ beakers.

E. Record larval mortality after 24 hours of exposure period and calculate the percentage mortality (dead and moribund larvae).

- Dead larvae: the larvae that cannot be induced to move when they are probed with a needle on the siphon or the cervical region.
- Moribund larvae: The larvae that are incapable of rising to the surface or not showing the characteristic of diving reaction when the water is disturbed.

Use de-chlorinated water for the test. If tap water is used, allow to de-chlorinate by keeping the water for 24 hours

Record larval mortality after 24 hour exposure period

If more than 10% of the larvae in the controls pupated during the experiment, discard the test and repeat it

F. Discard the larvae that have pupated during the test. If more than 10% of larvae in the controls are pupated in the course of the experiment, discard the test and repeat it.

If the control mortality is between 5%- 20%, use Abbott's formula to calculate corrected mortality

G. Calculate the corrected mortality using Abbott's formula if the control mortality is between 5%- 20%

Note: For the test, optimum average temperature of the water is 25°C (20°C - 30°C).

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

4.6.5. General remarks

Tightly close the solution bottles after using

- Keep the insecticide bottle closed tightly after use in order to prevent evaporation of alcohol in the insecticide solution since it affects the concentration of the insecticide solution.
- Do not use the insecticide when it decreased below 5 ml.

Clean carefully the beakers/ plastic cups after use to remove traces of insecticides

- Clean the beakers/plastic cups after use by rinsing and scrubbing them thoroughly with detergent and water ensuring no traces of insecticides in those.
- Clean the pipettes thoroughly with acetone or alcohol.
- Use distilled water or well water for washing and cleaning (tap water can be used after keeping 24 hours to de-chlorinate).

4.6.6. Data recording and reporting

Use the given form to report data (***NDCU/EN/09***) to respective Entomologist and NDCU.

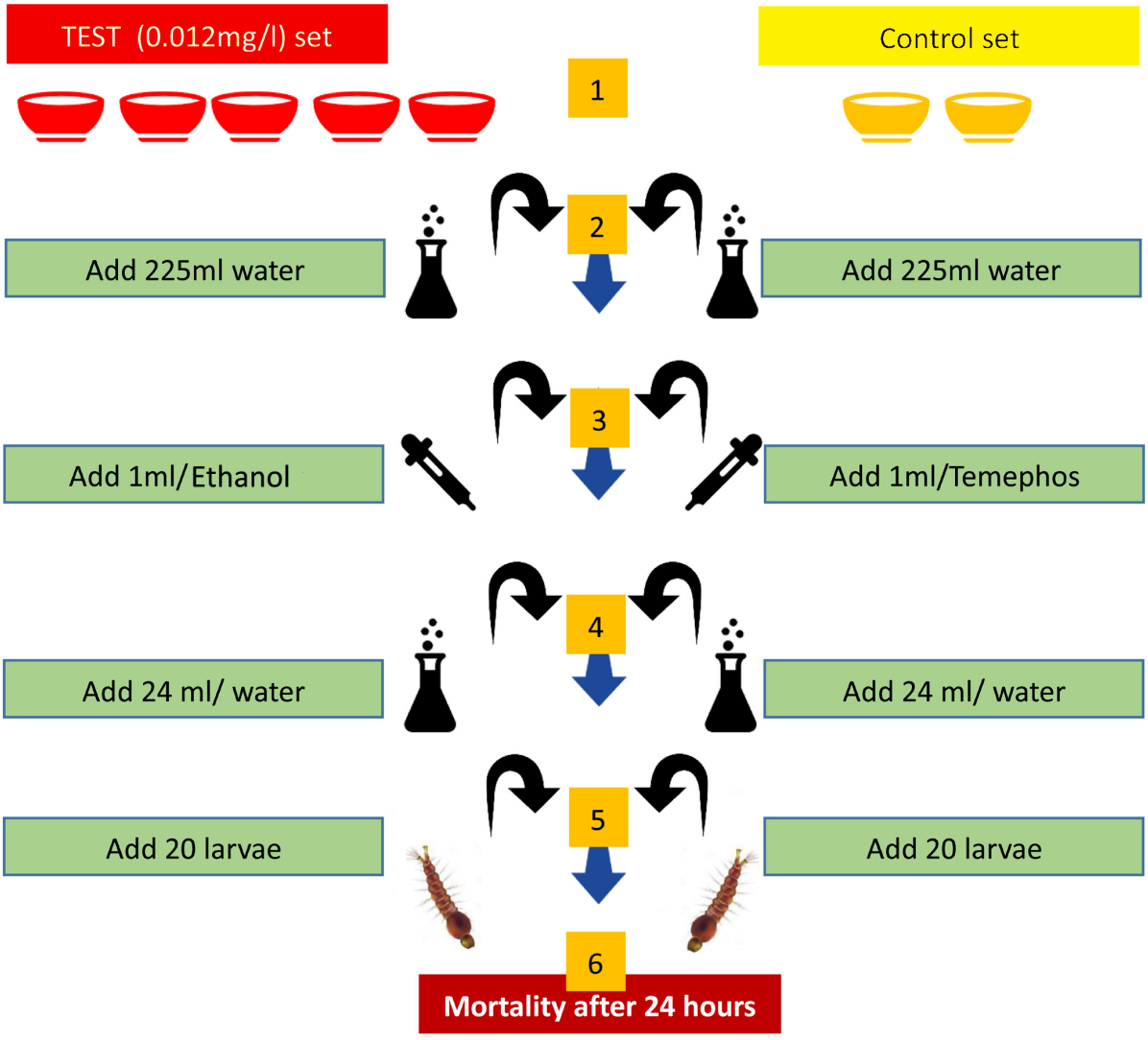


Figure 4.2: Methodology of the larval mosquito susceptibility test

5. Cage Bioassays

5.1. Introduction

Evaluate the effectiveness of space spraying

Cage bioassays are used to evaluate the effectiveness of space spraying operations.

5.2. Objectives

- To evaluate the effectiveness of space spraying (fogging) operations.
- To regularize the on-going space spraying activities.

5.3. Frequency

Cage bioassay tests should be perform at least once in three months

- Test could be repeated at least once in 6 months (or accordingly MOH request, for clarification of usage of space spraying).

5.4. Equipment and materials

- F_0 or F_1 progeny of field collected *Aedes* mosquitoes (Larval/ovitrap collections)
- Standard bio-assay cages
- Mosquito rearing cages
- Aspirator tubes
- Paper cups
- 50 m tape

5.5. Procedure

- Time – before 10 a.m. or after 3 p.m.
- Collect eggs or larvae from field and rear them in the lab/ insectary to obtain adult mosquitoes for bioassays.
- Take 8 mosquito bioassay cages.
- Transfer 2 – 3 days old 20 female mosquitoes fed on a 10% sugar to each bioassay cage, shortly before the treatment to be assessed.

- The cages should be transported to and from the field in mosquito cage protected from extreme heat.
- At least five houses in the treatment area should be used.
- As a minimum for evaluation of space sprays, cages should be located at each house at the following sites,

i. For indoors at an exposed site and in a sheltered site.

ii. For outdoor in front and at the rear of the houses (within 5 m - 30 m different distances).

- The same number of cages should be exposed at similar sites in the untreated area.
- Thirty minutes (30 min) after exposure, the cages should be removed and returned to the laboratory in their transport cages.
- Then the mosquitoes are transferred to clearly mark clean holding cages, and are provided with sugar solution and maintained at ambient temperature.
- Mortality in all cages should be determined 24 hours after the spray application.
- Temperature and relative humidity should be recorded during the test (both the exposure and the holding periods). Ideal temperature for testing is 25± 2°C, and should not higher than 30°C. Relative humidity should be 70-80%.
- Chambers should be hung vertically.
- Cages should be washed thoroughly before use in another experiment.

The mosquito cages should be protected from extreme heat

Consider the wind direction when placing the cages during a space spraying

The cages should be removed and transported to the laboratory after 30 minutes of the exposure

24 hour mortality should be determined after exposure

Ideal temperature for the testing is 25± 2°C and relative humidity of 70-80%

Wash the cages thoroughly before the nest experiment

5.5.1. Interpretation of susceptibility test results

$$\text{Observed mortality} = \frac{\text{Number of dead mosquitoes}}{\text{Number of test mosquitoes}} \times 100$$

If control mortality is between 5-20%, the average observed mortality should be corrected by Abbott's formula.

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

5.6. Data recording and reporting

Use the given form to report data (***NDCU/EN/10***) to respective Entomologist and NDCU.

ANNEXURE I

Sentinel site surveillance

Sentinel site dengue vector surveillance is a surveillance system in which regular entomological surveys are carried out in pre-arranged and designated areas to collect entomological data that are useful to make trend observations on vector density, dynamics of vector breeding sites, changes in vector behaviour and monitoring vector susceptibility/ resistance status to insecticides that are used in dengue vector control. Sentinel site vector surveillance facilitates early warning and forecasting of dengue outbreaks.

Criteria for selection of sentinel sites

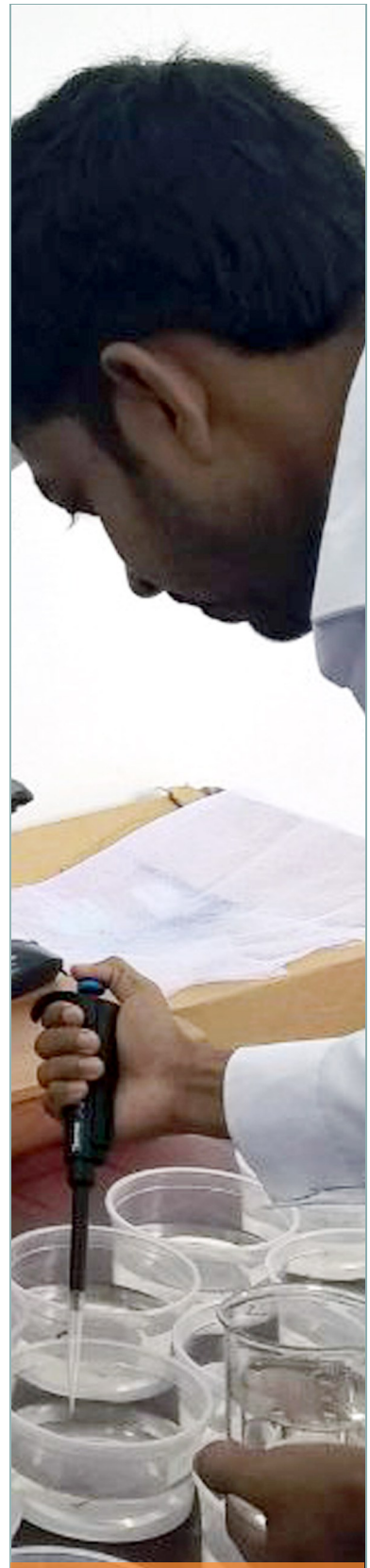
a. Sentinel sites are identified at district level and monitored monthly. It is recommended that a minimum of 02 (one urban, one semi urban or rural) sentinel sites per district to be established and monitored.

b. Sentinel site should be (i) an area where dengue transmission/ high-risk of transmission is present over a period of time or (ii) an epidemic prone area (areas experiencing/potential for periodic or seasonal outbreaks (it may be a cluster of Municipal wards/ Grama Niladari areas or a Public Health Inspector area).

c. In a sentinel site adjacent Municipal wards/ Grama Niladari areas having more or less homogeneous prevalence of *Ae. aegypti* and reported dengue cases for the past 3-5 years should be selected for entomological surveillance.

Entomological techniques to be carried out in sentinel site surveillance

- Larval surveys.
- Pupal surveys.
- Indoor and outdoor adult mosquito resting collections.
- Human bait collections (using the double net method).
- Insecticide susceptibility/ resistance tests.
- Bio-efficacy tests for larvae and adult *Ae. aegypti* and *Ae. albopictus*.



Conducting a susceptibility test for mosquito larvae

Mosquito breeding sites in construction sites should be eliminated

Routine site surveillance

Routine vector surveillance is a surveillance method in which regular entomological surveys are carried out in high dengue transmission/ transmission risk areas (localities) to collect entomological data that are useful to guide dengue vector control activities and for epidemic prevention. Immature surveys (larvae and pupae) are the commonly used entomological surveillance method in routine vector surveillance. Monitoring of larval density helps to identify (i) potential dengue transmission areas and seasons well ahead of the outbreak period and (ii) area and time specific vector breeding sites that facilitate application of most appropriate and cost-effective vector control interventions.

Criteria for selection of routine surveillance sites

a. Routine surveillance sites are identified at the district or sub district (MOH) level and monitored regularly (ideal if monitored fortnightly; otherwise at least monthly).

b. Routine surveillance site is (i) an area where dengue transmission/ high risk of transmission is present over a period of 3 years or (ii) an epidemic prone area. Epidemic prone areas include:

- the areas that are subjected to frequent or seasonal outbreaks/ epidemics
- areas with increased vector breeding sites due to development activities, urbanization, interruptions of regular water supply etc.

Entomological techniques to be carried out in routine site surveillance

- Larval surveys –fortnightly/monthly.
- Insecticide susceptibility/ resistance tests (once in 6 months).
- Bio-efficacy tests for larvae and adult *Ae. aegypti* and *Ae. albopictus* (once in 6 months).

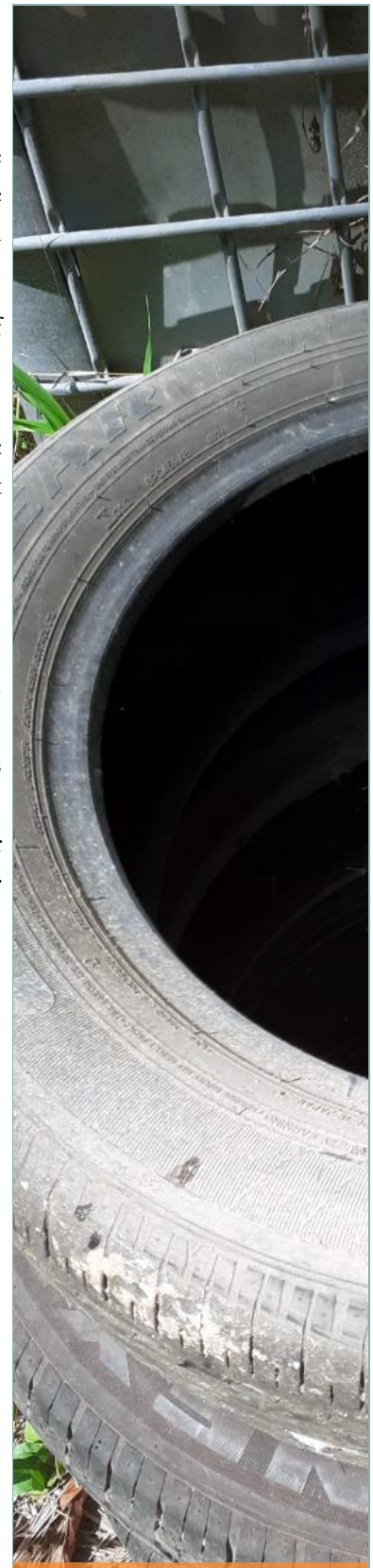
Spot checks

Spot check is a surveillance method that is carried out to generate entomological information for a particular locality for guiding the vector control activities in that locality/site. Spot checks are carried out:

- in an areas where there are outbreaks of dengue in spite of regular vector control interventions.
- in contact places of reported dengue cases.
- in high-risk institutions such as schools, bus depots, public places, hospitals, religious places, areas where development projects are carried out and construction sites etc.
- in new areas where dengue cases are reported.
- in an area where there is an increase in the reporting of fever / suspected dengue cases.
- When environment changes occur favouring vector breeding (eg. flooding, development projects etc).
- When there is a need to evaluate the impact of control measures (e.g. cleaning programmes, fogging etc.).
- to identify the new establishment of *Aedes aegypti* or *Aedes albopictus* in areas where there were no reports of the vector previously.

Entomological techniques to be performed during spot checks

- Larval surveys.
- Indoor and outdoor adult mosquito resting collection.
- Bio-efficacy tests for larvae and adult *Ae. aegypti* and *Ae. albopictus.*



Discarded tyres, a critical place of Aedes mosquito breeding

ANNEXURE II

Dipping

Figure i

Let the dipper fill ¾ of water

- This method applies for sampling from relatively large water bodies where the water level is high enough for dipping. Such water bodies include water storage tanks/ barrels, cement lined drains, shallow wells and any type of similar breeding place.
- The collector should be in a position avoiding casting his shadow in the water during dipping.
- Let the dipper fill ¾ of water.
- If larvae/pupae are visible, collect the larvae using the dipper (Figure i).
- If larvae/pupae are not visible, dip from 4 corners (in a rectangular structures) or along the edge (in circular containers) at the rate of 6 dips per m^2, allow sufficient time (2-3 minutes) in between dips to larvae to come up as they sink when water is disturbed.
- Check the dipping content for Mosquito larvae.
- Transfer the collected larvae/pupae to the collection vial using a pipette.
- Label the vial accordingly.
- Identify the larvae at the laboratory using standard identification keys.
- Allow the pupae to develop into adults and identify the species using standard identification keys.

Netting

Netting is applicable to wells and large water storage containers.

- This method is normally used to collect larvae and pupae in wells, large water storage containers, etc.
- The fine mesh net mounted on a circular frame (Figure ii) should be dipped slowly in the water of the well keeping half the border of the net above the water.

- Sweep the surface and the column water by moving the net along the margin and middle of the water body. Hold the net with about 45^0 angles to the water surface and drag across the surface.
- Take the net out and invert the net and wash out in an enamel tray with water.
- Collect and transfer the larvae with a pipette in to a labeled vial.
- After waiting for 2-3 minutes to allow the disturbed larvae/ pupae to return to the water surface, repeat the steps.
- Identify the larvae at the laboratory using standard identification keys.
- Allow the pupae to develop into adults and identify the species using standard identification keys.

Figure ii

Hold the net with about 450 angles to the water surface and drag across the surface.

Siphoning

- This method is used to collect larvae/pupae from small water collections such as tree holes etc.
- The top of the siphon should be fitted with two pieces of rubber tubes inserted through it (Figure iii)
- Place one length of tubing in the tree hole while sucking the other tube to start water siphoning out and water will continue to flow out.
- Siphon out all the water into a transparent bottle.
- Examine for the presence of larvae or pupae.
- If larvae and pupae are present, collect and transfer them from the bottle to a vial using a pipette.
- Label the vial accordingly.
- Identify the larvae at the laboratory using standard identification keys.
- Allow the pupae to develop into adults and identify the species using standard identification keys.

Figure iii

Siphon out the water into a transparent bottle

Figure iv

Pipetting

- This method is used to collect larvae/pupae from small breeding sites such as tree holes, small receptacles, plant axils, tyres and roof gutters etc.
- Examine the container and pipette (Figure iv) the whole water content and pour in to the ladle.
- If larvae/pupae are present, collect and transfer them from the ladle to a vial using a pipette.
- Label the vial accordingly.
- Identify the larvae at the laboratory using standard identification keys.
- Allow the pupae to develop into adults and identify the species using standard identification keys.

ANNEXURE III

Labelling of field samples of mosquito larvae, adults and ovitraps

Labelling of field samples of mosquito larvae, adult and ovitraps is of critical importance in entomological surveillance process. The label should include information on the locality, place, surface, date of sampling, sample ID and other key information for proper recording of data. Following are model sample labels that can be used for labeling samples of mosquito larvae, adult and ovitraps.

Locality/GN: .....

Breeding Place:.....

Sample ID:.....

Date:.....

Name of the HEO:.....

Figure v: Model label for mosquito larval samples

Locality/GN: .....

Collected surface:.....

Sample ID:.....

Date:.....

Name of the HEO:.....

Figure vi: Model label for mosquito adult samples

Name of the institution

DO NOT REMOVE

Institutional telephone No.

MOH: .....

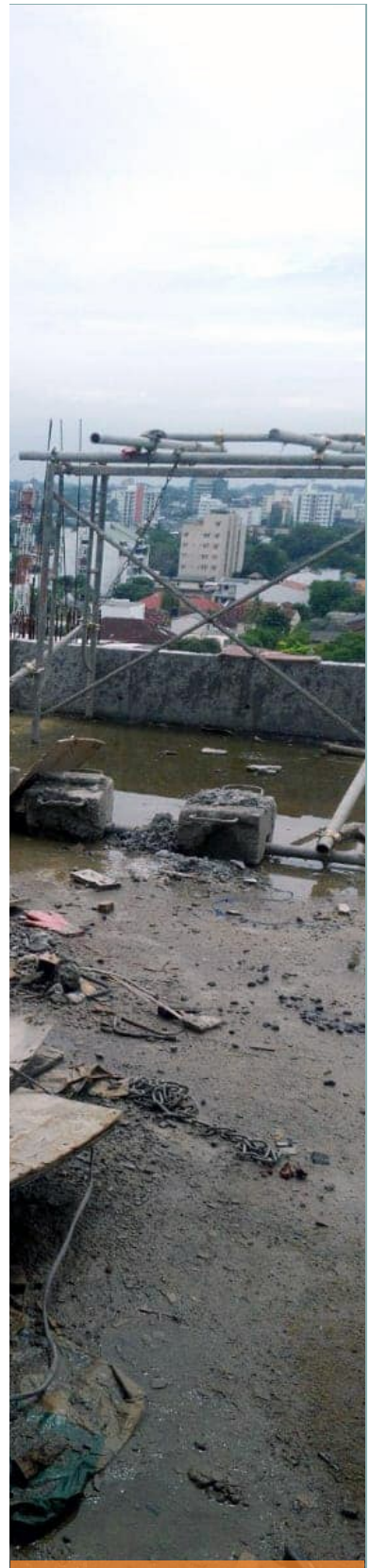
House No: .....

Setup Place: .....

Setup Date: .....

Date of Recollection:.....

Figure vii: Model label for oviposition trap

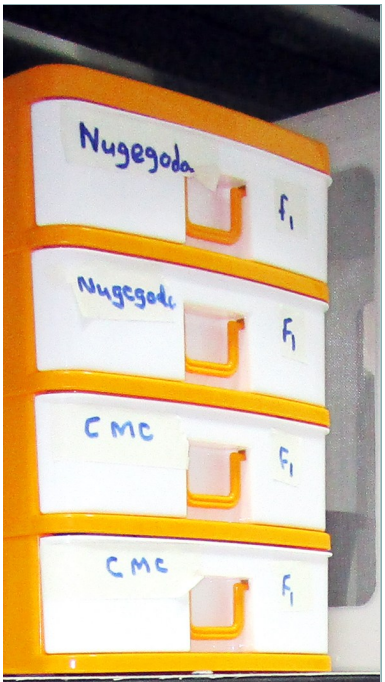


A mosquito breeding place in a construction site

Comb scales of Aedes aegypti larvae

INDEX

Discarded items - a breeding habitat of Aedes mosquitoes



Mosquito rearing facility

GLOSSARY

The definitions given below apply to the terms as used in this SOP. They may have different meanings in other contexts.

Anthropophagic	Descriptive of vectors that show a preference for feeding on humans, even when non-human hosts are
Anthropophilic	Descriptive of vectors that are attracted to humans
Bioassay	In applied entomology, experimental testing of the biological effectiveness of a treatment (e.g. infection, insecticide, pathogen, predator, repellent) by deliber-
Breteau index	Number of containers with larvae and/or pupae per 100 houses inspected
Container index	Percentage of water holding containers with larvae and/or pupae
Endemic area	An area in which there is an ongoing, measurable incidence of mosquito-borne transmission over a
Epidemic	Occurrence of a number of dengue cases highly in excess of that expected in a given place and time
Insecticide	Chemical product (natural or synthetic) that kills insects: Ovicides kill eggs; larvicides (larvacides) kill larvae; pupacides kill pupae; adulticides kill adult mosquitoes. Residual insecticides remain active for an extended period.
Insecticide, dose	Amount of active ingredient of insecticide applied per unit area of treatment (mg/m2) for indoor residual spraying and treated mosquito nets, or per unit of space (mg/m3) for space spraying and per unit area of application (g/ha or mg/m2) or per volume of water (mg/L) for larvicides
Insecticide resistance	Property of mosquitoes that can survive exposure to a standard dose of insecticide that may be the result of physiological or behavioural adaptation
Integrated vector	Rational decision-making for optimal use of resources

Mosquitoes in a rearing cage

Larvicide	Substance used to kill mosquito larvae
Mosquito trap	Device designed for capturing mosquitoes with or without attractant components (light, CO_2, living
Premise index	Percentage of premises with larvae and/or pupae
Susceptibility tests	Vectors are given a controlled dose of insecticide and observed to see whether they die or survive; a measure of phenotypic resistance.
Vector control	Measures of any kind against dengue-transmitting mosquitoes, intended to limit their ability to transmit the disease
Vector surveillance	A systemic monitoring of the seasonality and abundance of vector populations

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NDCU/EN/01

National Dengue Control Unit
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Telephone: (0094) 011 2368416, 2368417 Fax: (0094) 011 2369893 Email: ndcu2010@yahoo.com

Dengue Entomological Surveillance Field Report

RDHS area: PHI area: Type of survey: Date:
MOH area: GN division: Locality:

SN	Name and Address	Type of the premise**	Type of breeding place*	No. inspected containers	No. potential		No of (+) containers		Sample ID	Larval range#	Pupal range§	Remarks
					Wet	Dry	I	O				

* **WSB**-Water sorage barrels, **WSCT**-water sorage cement tanks, **WSO**-water storage other, **Con.sl**-concrete slab, **Gut**-Gutter, **Ornam**-Ornamental, **Natu**-Natural, **T.wells**-Tube wells, **A/C,Ref**-A/c,refrigerator, **T.R.I**-Temporary remove items, **Cov**-Covering items, **Dis(deg)** -discared degradable , **Dis(nd)**-discarded non degradable, **Dis(r)**-Discarded reuse, **PFC**-Pet feeding cups, **Nuc/c**-non use cisterns/commode

** **G**-Houses, **H**-Commercial sites, **I1**-Government institutions, **I2**-Private Institutions, **J**-Construction sites, **K**-Open areas, **L1**-Schools, **L2**-Other Education Centers, **M**-Religious places, **N**-Other

#Larval range : <10, 10-50, 50-100, 100< §Pupal range : <10, 10-20, 20< I : Indoor O : Outdoor

Type of container	WSB		WSCT		WSO		Con. Sl		Gut		Tires		Ornam.		Natu		Ponds		Wells		T. wells		A/C ref.		TRI		Cov		Disc		PFD		NUC/C		Other		Total	
	I	O	I	O	I	O	I	O	I	O	I	O	I	O	I	O	I	O	I	O	I	O	I	O	I	O	I	O	I	O	I	O	I	O	I	O
Dry																															.					
Wet																																				
With Larvae																																				

Type of the inspected site	G		H		I1		I2		J		K		L1		L2		M		N		Total	
	I	O	I	O	I	O	I	O	I	O	I	O	I	O	I	O	I	O	I	O	I	O
Number of larvae positive																						

WSB-Water sorage barrels, **WSCT**-water sorage cement tanks, **WSO**-water storage other, **Con.sl**-concrete slab, **Gut**-Gutter, **Ornam**-Ornamental, **Natu**-Natural, **T.wells**-Tube wells, **A/C,Ref**-A/c,refrigerator, **TRI**-Temporary remove items, **Cov**-Covering items, **Dis(deg)** -discared degradable , **Dis(nd)**-discarded non degradable, **Dis(r)**-Discarded reuse, **PFC**-Pet feeding cups, **Nuc/c**-non use cisterns/commode

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Larval ranges : <10, 10-50, 50-100, 100< Pupal range : <10, 10-20, 20< I : Indoor O : Outdoor

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Dengue Entomological Daily Summary Report

NDCU/RMO/AFU/MOH …….. Entomology team

RDHS area: MOH area:

PHI area: GN area:

Locality: Date:

	Type of the premises*																				Total	
	G		H		I 1		I 2		J		K		L 1		L 2		M		N		Ot			
	I	O	I	O	I	O	I	O	I	O	I	O	I	O	I	O	I	O	I	O	I	O	I	O
Total number of premises inspected:																								
Number of positive premises:																								
** percentage(%) of premises positive																								
Number of wet containers:																								
Number of dry containers :																								
Number of positive containers:																								

Comments and suggestions:

Date | Name of the reporting Officer | Designation | Signature

* **G**-Houses **H**-Commercial Sites **I1**-Government Institutions **I2**-Private Institutions **J**-Construction Sites **K**-Open Areas **N**-Factories **Ot**-Other places
L1-Schools **L2**-Other Education centres **M**-Religious places **I**- Indoor **O**-Outdoor
Target: should expect to keep this <1%

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Dengue Entomological Surveillance Report

RDHS area: PHI area: Period: from to Type of survey:
MOH area: GN division: Locality:

No. of premises inspected												No. of containers inspected			No. of premises positive for			No. of containers positive for			Premise Index (%)			Container index (%)			Breteau Index		
G	H	I1	I2	J	K	L1	L2	M	N	O	Total	Wet	Dry	Total	A	B	AB	A	B	AB	A	B	C	A	B	C	A	B	C
												A << No of premises positive for the breeding places of A									Identified other species with breeding places								
												B << No of premises positive for the breeding places of B																	
												AB << No of premises positive for the breeding places of AB									Total no.of containers positive for *An.stephensi*								

Summary of breeding places

	No. of containers positive							No. of containers positive							No. of containers positive							No. of containers positive						
	Wet	Dry	A	B	AB	S		Wet	Dry	A	B	AB	S		Wet	Dry	A	B	AB	S		Wet	Dry	A	B	AB	S	
WSB							Ornam							TRI							Nuc/c							
WSCT							Natu							COV							.....							
WSO							Ponds							Dis(deg)							.....							
Con.sl							Wells							Dis(nd)							.....							
Gut							T.wells							Dis(r)							.....							
Tyres							A/C,ref							PFD							.....							

Comments:

Date	Name of the reporting officer	Designation	Signature

Date	Name of the head of the institution /Designation	Signature

A- *Aedes aegypti* , B - *Aedes albopictus* , S - *Anopheles stephensi* , C - common

G-Houses, H-Commercial sites, I1-Government institutions, I2-Private Institutions, J-Construction sites, K-Open areas, L1-Schools, L2-Other Education Centers, M-Religious places, N-Other

WSB-Water sorage barrels, **WSCT**-water sorage cement tanks, **WSO**-water storage other, **Con.sl**-concrete slab, **Gut**-Gutter, **Ornam**-Ornamental, **Natu**-Natural, **T.wells**-Tube wells, **A/C,Ref**-A/c,refrigerator, **TRI**-Temporary remove items, **Cov**-Covering items, **Dis(deg)** -discared degradable , **Dis(nd)**-discarded non degradable, **Dis(r)**-Discarded reuse, **PFC**-Pet feeding cups, **Nuc/c**-non use cisterns/commode

Report to be forwarded by e-mail: ndcu2010@yahoo.com

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Dengue Entomological Surveillance Containers and Premises Detail Summary Report

RDHS area: PHI area: Type of survey: Date:
MOH area: GN division: Locality:

Type of container		WSB		WSCT		WSO		Con. Sl		Gut		Tires		Ornam.		Natu		Ponds		wells		T. wells		A/C ref.		TRI		Cov		Disc		PFD		NUC/C		Other		Total	
		I	O	I	O	I	O	I	O	I	O	I	O	I	O	I	O	I	O	I	O	I	O	I	O	I	O	I	O	I	O	I	O	I	O	I	O
Dry																														.							
Wet																																					
With Larvae	A																																				
	B																																				
	AB																																				
	Os																																				
Total																																					

Type of the inspected site		G		H		I1		I2		J		K		L1		L2		M		N		Total	
		I	O	I	O	I	O	I	O	I	O	I	O	I	O	I	O	I	O	I	O	I	O
Number of larvae positive	A																						
	B																						
	AB																						
	Os																						

Date Name of the reporting officer Designation Signature

WSB-Water sorage barrels, **W.S.C.T**-water sorage cement tanks, **WSO**-water storage other, **Con.sl**-concrete slab, **Gut**-Gutter, **Ornam**-Ornamental,**Natu**-Natural, **T.wells**-Tube wells, **A/C,ref**-Air conditioner and refrigerators , **TRI**-Temporary remove items, **Cov**-Covering items, **Dis(deg)** -discared degradable , **Dis(nd)**-discarded non degradable, **Dis(r)**-Discarded reuse, **PFC**-Pet feeding cups, **Nuc/c**-Non use cisterns/commode

G-Houses, **H**-Commercial sites, **I1**-Government institutions, **I2**-Private institutions, **J**-Construction sites, **K**-Open areas, **L1**-Schools, **L2**-Other Education Centers, **M**-Religious places, **N**-Other

A-*Aedes aegypti* **B**-*Aedes albopictus* **Os** - Other species **I**-Indoor **O**-Outdoor

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Entomological Survey Report on Institutions/construction sites/…….

Entomological survey format* - Institutions / construction sites /…….

NDCU/RMO/AFU/MOH…… Entomology Team

RDHS area: PHI area: Survey type: Date:

MOH area: GN area: Name of the premises /Site surveyed:

SN	Specify the locality surveyed within the premises/site	Type of breeding	Number of containers						Presence of *Aedes* spp.				Presence of other mosquito sp.		Type of identified *Aedes* sp.	Remarks	Action taken
			With water		Without water		Positive for mosquito larvae		No. of *Aedes* larvae		No. of *Aedes* pupae						
			I	O	I	O	I	O	I	O	I	O	I	O	A/B/AB		
Total																	

Date Name of the reporting officer Designation Signature

Date Name of the head of the institution / Designation Signature

A-*Aedes aegypti* **B**-*Aedes albopictus* **AB** - A & B mix breeding **I**- In door **O** - Out door *Please use one format per premise/site inspected.

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Dengue Entomological Surveys - Pupal Survey Report

RDHS area:		GN division:	
MOH area:		Locality:	
PHI area:		Date:	

Breeding place	No. of Wet Containers	No. of Containers Positive For Pupa	No. of Pupae	
			Ae. aegypti	*Ae.albopictus*
Water Storage Barrel				
Water Storage Cement Tank				
Water Storage Other				
Concrete Slab				
Roof Gutters				
Tyres				
Ornamentals				
Natural				
Ponds				
Wells				
Tube Wells				
A.C./ Refrigerator				
Temporary Removed Items				
Covering Items				
Discarded (degradable)				
Discarded (non degradable)				
Discarded (reusable)				
Pet Feeding Cups				
Non use commode/ cistern				
Other-				
Other-				
Other-				
Other-				
Other-				
Total				

No. of Premises Examined: No. of Pupae (*Ae. aegypti, Ae. albopictus*)

Pupal Index**

**[Pupal Index=(No. of Pupae/No. of Premises Inspected)*100]

Date	Name of the reporting officer	Designation	Signature

Date	Name of the head of the institution / Designation	Signature

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Dengue Entomological Surveys - Ovitrap Surveillance Report

RDHS area:
MOH area:
PHI area:
GN Division:
Locality:

Ovitrp set date
Ovitrap collection date

Climatic Conditions: Bright sun shine Gloomy Raining heavily Drizzling

Nature of Locality: Residential Public site Town Other

	Field works								Laboratory works								
									Indoor				Outdoor				
SN	Type of premises *	Name & address of the house holder/public site	Ovitrap placement area[1]		Ovitrap placement place[2]		Positive /Negative (On the collection day)		No. of eggs	No. of A** larvae	No. of B ** larvae	Mix	No. of eggs	No. of A** larvae	No. of B** larvae	Mix	Total
			I	O	I	O	I	O									

Date Name of the reporting officer Designation Signature

Date Name of the head of the institution / Designation Signature

A: *Aedes aegypti* **B: *Aedes albopictus* **I**: Indoor **O**: Outdoor * **G**: Houses **H**: Commercial sites **I1**: Government institutions **I2**: Private institutions **J**: Construction sites **K**: Open areas **L1**: Schools **L2**: Other education centres **M**: Religious places **N**: Factories **Ot**: Other places **Area[1]**: living room,bed room,bathroom,kitchen, garden,other (specify) **Place[2]**: under/on the furniture,under the bed,among vegetation,other (specify)

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Dengue Entomological Surveys - Indoor and Outdoor Adult Mosquito Resting Collection

RDHS area: GN Division: Date:
MOH area: Locality: Start at:
PHI area: Weather Condition: Finished at:

SN	Address (positive for adult dengue vector mosquitoes)	Start time	End time	Type of the roof*	Type of the wall**	I	O	Resting			No. of Mosquitoes Collected				Condition of the abdomen	Other Mosquito sp
											Ae.aegypti		*Ae.albopictus*			
								Area[1]	Place[2]	Height	Male	Female	Male	Female		

No. of Premises Examined:

No. of Premises Positive for Adult Dengue Vector Mosquitoes — Female: Male:

Total No. of Dengue Vector Mosquitoes Collected — Female: Male:

Date	Name of the reporting officer	Designation	Signature

Date	Name of the head of the institution/Designation	Signature

***Roof type**: Cadjan, roof tiles, roof sheets, other ****Wall types**: Clay wall, Brick wall, cement wall, other **I**: Indoor **O**: Outdoor **Area[1]**: living room, bed room, bathroom, kitchen, garden, other (specify) **Place[2]**: wall, under the furniture, under the bed, among vegetation, other (specify)

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Report Form of Insecticide Susceptibility Test on Adult Mosquitoes

Collection information

RDHS area: PHI area: Date:
MOH area: GN Division: Collection stage: Larvae/egg
Locality:

Species information

Species tested: Generation: Sex:
Conditions: Unfed /Sugar fed Age (days):

Test information

Test date: Time started:
Insecticide tested: Concentration:
Exposure period Exposure Temperature (℃) Exposure Relative Humidity %

Holding time: Temperature range (℃) Relative Humidity %
Min: Min:
Max: Max:

Results

Knockdown rate

	10 min	15 min	20 min	30 min	40 min	50 min	60 min	80 min
Number exposed								
Number knockdown								

	Exposure Mortality (24 hr)						Control Mortality		
	E1	E2	E3	E4	E5	Total	C1	C1	Total
No. of exposed									
No. of dead									
Mortality (%)									

Corrected Mortality

Date | Name of the reporting officer | Designation | Signature

Date | Name of the head of the institution / Designation | Signature

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Report Form of Insecticide Susceptibility Test on Mosquito larvae

Collection information

RDHS area: PHI area: Date:
MOH area: GN Division: Collection stage: Larvae/egg
Locality:

Species information

Species tested: Generation:

Test information

Test date: Time started:
Insecticide tested: Concentration:
Exposure period Exposure Temperature (℃) Exposure Relative Humidity %

Expiry date: Temperature range (℃) Relative Humidity %
Min: Min:
Max: Max:

Results

	Exposure Mortality (24 hr)						Control Mortality		
	E1	E2	E3	E4	E5	Total	C1	C1	Total
No. of exposed									
No. of dead									
Mortality (%)									

Corrected Mortality

Date	Name of the reporting officer	Designation	Signature

Date	Name of the head of the institution / Designation	Signature

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Report Form of Cage bio assay test

Collection information

RDHS area: | PHI area: | Date:
MOH area: | GN Division: | Collection stage: Larvae/egg
Locality:

Species information

Species tested: | Generation: | Sex:
Conditions: Unfed /Sugar fed | Age (days):

Test information

Test date: | Time started:
Type of insecticide | Expiry date | Batch no:
Type of machine use: | Concentration:
Exposure period | Exposure Temperature (℃) | Exposure Relative Humidity %
Holding time: | Temperature range (℃) Min: Max: | Relative Humidity % Min: Max:

Results

Test area

Cage no.	Distance to the fogging route (m)	Height (cm)	Wind direction*	Environmental conditions	No. of female mosquitoes	In/Out	Knockdown in 30 minutes	Mortality after 24 hours	Corrected mortality

Control area

Cage no.	Distance to the fogging route (m)	Height (cm)	Wind direction*	Environmental conditions	No. of female mosquitoes	In/Out	Knockdown in 30 minutes	Mortality after 24 hours	Corrected mortality

Corrected Mortality

Date	Name of the reporting officer	Designation	Signature
Date	Name of the head of the institution / Designation		Signature

Wind direction*- Towards, Away