Standard Operating Procedures for Entomological Surveillance

Anti Malaria Campaign

Sri Lanka

The standard operating procedures for entomological surveillance describe the activities performed in the field and in field laboratories detail. This manual has been developed by the Anti Malaria Campaign for use of all entomological teams involved in malaria entomological investigations. The SOPs were formulated through consultative meetings held at Anti Malaria Campaign with senior Regional Malaria Officers, Entomologists of AMC HQ, technical staff of AMC HQ and senior Entomological Assistants at regional and central level.
Standard Operating Procedures described in the manual are listed below.

Anti Malaria Campaign **SOP for Entomological Surveillance**					
SOP title:	**SOP for Hand collection of Indoor Resting Mosquitoes**				
SOP No.	**01**	**Revision No.**	**0.0**	**Effective Date**	**01.08.2016**
Replacement no.		**Dated**		**Page no.**	
Prepared by:	**Anti Malaria Campaign**			**Date**	**13.07.2016**
Approved by:	 **Director, Anti Malaria Campaign**			**Date**	**20.07.2016**

1.0 Purpose

This technique provides information on the resting surfaces of adult mosquitoes in indoors. The main objectives are

- To determine the indoor resting Anopheles mosquito species.
- To determine the resting surfaces of adult mosquitoes in indoors.
- To determine indoor resting density and assess the degree of endophilly.
- To monitor the indoor resting vector density and resting surfaces of vector mosquitoes over time.
- To assess the impact of insecticides used for IRS and LLIN by observing the tropic status and mortality.
- To collect mosquitoes for blood meal analysis.

2.0 Scope

This procedure applies to all entomological teams engaged in malaria entomological surveillance.

3.0 Responsibility

It is the responsibility of the Entomological Officer of Health as the team leader to ensure that the entomological team members carry out the SOP as written.

4.0 Equipment and Materials

4.1 Mouth aspirator

4.2 Three celled Torch (with spare bulb and 1.5V batteries)

4.3 Paper cups with net covers

4.4 Cotton wool

4.5 Pencil

4.6 Note book

4.7 Carrying bag

4.8 Hand lens

4.9 Stereo microscope

4.10 *Anopheles* Identification keys (adult)

4.11 GPS receiver

4.12 Container to transport paper cups/Plastic basin

4.13 Wet towel

4.14 Sugar solution (10%)

4.15 Papers to serve as packing materials

4.16 Ant Trap

4.17 H/AMC/E3 forms

5.0 Procedure

5.1 Start the survey by 08 00 and carry out up to 12 00 hours.

5.2 Houses should be selected randomly and they should be scattered throughout the village and it is often advantageous to select poorly constructed and less ventilated houses.

5.3 Visit the first house and get the consent of the household/ responsible occupant to perform indoor hand collection technique.

5.4 Inquire the household/ occupants which room was used by the highest number of

sleepers in the previous night.

5.5 If there is any person in the bedroom, ask him/ her to vacate the room at the time of collection.

5.6 Three team members (Entomological Officer of Health and two mosquito collectors) should enter to the bedroom, each person carrying a torch, a mouth aspirator and paper cups with net covers.

5.7 Collect mosquitoes as follows.

5.7.1 Start the collection from the door, moving one person clockwise and another person anti clockwise inside of the room. The other person should collect mosquitoes from furniture and from roof (if the roof can be reached) for collections. Time spent should be 10 minutes by each person per room.

5.7.2 Observe walls, roof, ceilings, underside of furniture and wall hangers, bed nets, curtains, empty containers if any.

5.8 Once an *Anopheles* mosquito is located, collect the mosquito using the aspirator into the paper cups. Make sure not to put more than 20-25 mosquitoes per cup (Separate cups should be used according to the different types of surfaces mosquitoes have been collected from).

5.9 Identify the species using hand lenses at the field and confirm the species with the use of Stereo microscope when returned to the field station.

5.10 Observe the abdominal condition, transfer the mosquito into the paper cup and make records in the field note book.

5.11 Label the paper cups with following information.

- Date and actual time started the collection
- House number or householders name
- Date
- Type of surface and location of the surface
- Mosquito species and abdominal condition

5.12 Record the following information in the field note book.

- GN area/Locality and GPS coordinates
- House number or house holders name

- Date and actual time started the collection
- Type of surface and location of the surface
- Time spent on collection
- Mosquito species and abdominal condition
- Whether sprayed or not with insecticide: if sprayed name of insecticide and date of spraying
- Whether LLIN is available in the room or not: If available, the type of net and date of receipt the net

5.13 After collection, keep the paper cups holding mosquitoes carefully in the plastic basin and cover with wet towels.

5.14 Repeat the above procedure till 10 houses are completed per day per locality.

5.15 Record the data in form H/AMC/E/3.

Procedure notes:

1. A minimum of 10 houses should be normally examined per day per site.
2. Abdominal conditions should be observed as soon as possible after the collection to avoid changes in the trophic status particularly in species such as *Anopheles subpictus.*
3. If the house is sprayed with an insecticide or provided with a LLIN keep the mosquitoes under suitable conditions (humidity and temperature controlled using wet towels with access to 10% sugar solution and away from ants) for 24 hours and observe the mortality after 24 hours.
4. All the collectors should be well trained to differentiate *Anopheles culicifacies* (which resembles Culicine mosquitoes by the resting posture) from Culicine mosquito species.

Anti Malaria Campaign **SOP for Entomological Surveillance**					
SOP title:	**SOP for Spray Sheet Collections**				
SOP No.	**02**	**Revision No.**		**Effective Date**	**01.08.2016**
Replacement no.		**Dated**		**Page no.**	
Prepared by:	**Anti Malaria Campaign**			**Date**	**13.07.2016**
Approved by:	**Director, Anti Malaria Campaign**			**Date**	**20.07.2016**

1.0 Purpose

Spray sheet collection provides information on indoor resting vector density and indoor resting vector species irrespective of the resting surface. The main objectives are

- To determine the indoor resting vector mosquito density and seasonal fluctuation
- To assess the degree of endophilly.
- To collect mosquitoes for blood meal analysis

2.0 Scope

This procedure applies to all entomological teams who are engaged in malaria entomological surveillance.

3.0 Responsibility

It is the responsibility of the Entomological Officer of Health as the team leader to ensure that the entomological team members carry out the SOP as written.

4.0 Equipment and Materials

4.1 White cotton sheets (size 2m x 1m-7 nos., 2m x 2m- 7 nos.,) – 14 sheets

4.2 Space spraying insecticidal (Pyrethrin) solution

4.3 Hand sprayer- the double action type with an air valve -02 sprayers

4.4 Haversack

4.5 Hand lens

4.6 Stereo microscope

4.7 *Anopheles* Identification keys (adult)

4.8 Petri dishes

4.9 Forceps

4.10 Container to transport mosquitoes in Petri dishes

4.11 Cotton wool

4.12 Three celled Torch (with spare bulb and 1.5V batteries)

4.10 Timer

4.11 Personal protective gear

4.12 GPS receiver

4.13 Pencil

4.14 Notebook

4.15 H/AMC/ E 2 forms

5. Procedure

5.1 Start the survey by 08 00 and complete by 12 00.

5.2 Houses should be selected randomly and they should be scattered throughout the village and it is often advantageous to select poorly constructed and less ventilated houses.

5.3 Visit the first house and get the consent of the household/responsible occupant to perform spray sheet collection technique.

5.4 Inquire the household/occupants which room was used by the highest number of sleepers in the previous night.

5.5 If there is any person in the bedroom, ask him/her to vacate the room at the time of collection.

5.6 Close the windows and other large spaces if any with cloth, papers or netting from outside.

5.7 Enter the bed room and remove food, drugs and pets (if any).

5.8 Spread white sheets to completely cover the floor of the room and all flat surfaces of the remaining furniture/ items ensuring covering under tables, beds and other places where mosquitoes may hide. When spreading sheets, start spreading from the distant side to the door and cover floor of the room as well as just outside the door (Figure 1).

5.9 Wear the Personal Protection Equipment.

5.10 Enter one team member into the room with the hand aerosol sprayer filled with right mixture of the insecticide and stay indoors. Another team member should stand outside and just opposite the team member inside carrying the sprayer with insecticide solution.

5.11 Close the door of the room (if the doors and windows are not covered cover them temporarily with cloths).

5.12 Start spraying the space between the roof and the wall, simultaneously form inside and outside while moving the team member inside the room in clockwise direction (start with shorter walls).

5.13 After completing spraying the space between the wall and the roof, the inner person/inside spray man should spray the roof and underside of furniture till the room is full of insecticide spray.

5.14 After spraying the inner person/inside spray should come outside room and close the door and start the timer.

5.15 After 10 minutes, open the door, remove the sheets from the room one by one holding 4 corners of the sheet. Remove the sheets starting from the sheets close to the door side and care must be taken to prevent escape of mosquitoes with the wind.

5.16 Examine each sheet carefully for *Anopheles* mosquitoes.

5.17 Collect them in Petri dish using forceps.

5.18 Use a separate Petri dish for mosquito collection in each room and label the container with all following data

- GN area / Locality
- Date and time
- House Number or householders name

5.19 Record the following information in the field note book.

- GPS coordinates
- Date and time of collection
- House number and householders name
- Whether the room has been sprayed previously and if so the date of spraying
- Availability of LLINs in the room, number & type.
- Name of the collector/supervisor

5.20 Identify to species and note the abdominal condition using hand lenses.

5.21 Fill the form H/AMC/E/ 2.

5.22 Visit the next house and repeat the same procedure for spray sheet collection till 10 houses are completed per day.

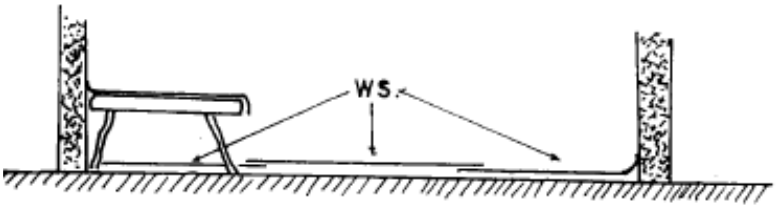


Figure 1. Preparation of a room for Spray Sheet Collections
WS: White Sheets

Procedure notes

1. Spray sheet collection should be performed in 10 houses per day per locality. In selection of houses, randomly selected poorly constructed and less ventilated houses with minimum spaces for escaping is preferred.
2. When weather is windy and rainy, a person can collect the mosquitoes on the sheets by moving through the room starting from the doorways using a torch and forceps.
3. Proper biosafety measures should be followed depending on the pyrethroid applied.
4. House selection: exclude houses with infants and sick persons.
5. Collect the blood fed mosquitoes for blood meal identification.

Anti Malaria Campaign **SOP for Entomological Surveillance**					
SOP title:	**SOP for Cattle Baited Net Trap Collection**				
SOP No.	**03**	**Revision No.**		**Effective Date**	**01.08.2016**
Replacement no.		**Dated**		**Page no.**	
Prepared by:	**Anti Malaria Campaign**			**Date**	**13.07.2016**
Approved by:	**Director, Anti Malaria Campaign**			**Date**	**20.07.2016**

1.0 Purpose

Cattle baited net trap collection provides information on outdoor feeding (exophagic) zoophillic *Anopheles* species density, abundance and seasonality.

2.0 Scope

This procedure applies to all entomological teams engaged in malaria entomological surveillance.

3.0 Responsibility

It is the responsibility of the Entomological Officer of Health as the team leader to ensure that the entomological team members carry out the SOP as written.

4.0 Equipment & Materials

4.1 Net trap **(**Size of the trap: **Length x width x height = 10' x 10' x 5'.**
when setting the trap the center of the trap should be 7' off the ground)

4.2 Mouth aspirators

4.3 Three celled torch (with spare bulb and 1.5V batteries)

4.4 Paper cups with net covers

4.5 Cotton wool

4.6 Container to transport paper cups/Plastic basin

4.7 Towels

4.8 Calf/cow

4.9 Four wooden sticks each of 6’ 6” long

4.10 One wooden stick of 7’ 6” long

4.11 Iron or wooden pole to tie the calf

4.12 Coir

4.12 Wooden Pegs

4.13 Hand lens

4.14 Stereo microscope

4.15 *Anopheles* Identification keys (adult)

4.16 Note book

4.17 Pencil

4.18 GPS receiver

4.19 H/AMC/E/4 forms

5.0 Procedure

5.1 Select a location for net trap preferably within 200-500 m distance from the vector mosquito breeding site (preferably with less abundance of cattle in the vicinity other than the selected bait).

5.2 The location should be of at least 10’ x 10’ open, flat, shady area to set the trap.

5.3 Be at the survey site one hour before sunset and set the net trap (at 17 00).

5.4 Fix the 6’ 6’’ wooden sticks at the 4 corners of the 10’ x 10’ square.

5.5 Fix the middle pole (7’ 6” wooden stick) at the center of the trap ensuring the center of the trap is 7’ high.

5.6 Tie the 4 upper corners of the net trap to the 4 sticks.

5.7 Tie the net to wooden pegs to keep the trap properly stretched at a height, keeping

4'' – 6" space between the lower edge of the net and the ground (Pegs should be fixed to the eight loops at the bottom edge of the net trap).

5.8 Fix and tie another strong wooden/iron pole to the middle pole to tie the calf.

5.9 Introduce the calf/ cow to the trap before sun set (17 30).

5.10 Leave the bait in the trap overnight.

5.11 On the following morning, go to the trap one hour before sunrise (at 5 00).

5.12 One team member should enter in to the trap carefully minimizing escape of mosquitoes and take the calf/cow out of the trap.

5.13 Enter 3 team members into the net trap and immediately after entering it, lower down the net to the ground by releasing the pegs. This prevents escaping mosquitoes that are trapped in the net.

5.14 Start collection of *Anopheles* mosquitoes trapped in the net using aspirators and torches, do not keep more than 5 mosquitoes in the aspirator at a time.

5.15 Transfer the collected mosquitoes into paper cups covered with nets. Do not put more than 20-25 mosquitoes in a single cup.

5.16 Place the cups of mosquitoes in a plastic basin and cover these with a wet towel to create a humid environment.

5.17 Transport the cups to the field laboratory as described in SOP no. 14.

5.18 Identify the species using standard mosquito identification keys with the use of hand lens and confirm using stereo microscope.

5.19 Keep different species of mosquitoes in separate paper cups with double nets and wet pads till the mosquitoes are used for susceptibility and bioassay tests or for mosquito rearing.

5.20 Record the data in form H/AMC/E/4.

Fig. 2 A Cattle baited net trap

Procedure notes:

1. Frequent washing may cause less attraction of mosquitoes and difficulties in handling the cow/calf.

2. Ensure the damaged parts of the net should be mended.

3. If the location is very much closer to the locations where other techniques are performed take a GPS coordinates from a representative location.

4. Before the mosquito samples are observed under the stereo microscope they should be killed using Ether/Chloroform.

Anti Malaria Campaign **SOP for Entomological Surveillance**					
SOP title:	**SOP for Cattle Baited Hut Collection**				
SOP No.	**04**	**Revision No.**		**Effective Date**	**01.08.2016**
Replacement no.		**Dated**		**Page no.**	
Prepared by:	**Anti Malaria Campaign**			**Date**	**13.07.2016**
Approved by:	**Director, Anti Malaria Campaign**			**Date**	**20.07.2016**

1. Purpose

Cattle baited hut collection provides information on zoophillic and endophillic *Anopheles* species particularly *An. culicifacies* and *An. subpictus* and their density.

Scope

This procedure applies to all entomological teams who are engaged in malaria entomological surveillance.

2. Responsibility

It is the responsibility of the Entomological Officer of Health as the team leader to ensure that the entomological team members carry out the SOP as written.

3. Equipment and materials

4.1 Cadjan Hut

(Size of the hut: **Length x width x height (center), corners = 6’ x 5’ x 6.5’, 5’)**

4.2 Mouth aspirators4.3 Three celled torch (with spare bulb and 1.5V batteries)

4.4 Paper cups with net covers

4.5 Cotton wool

4.6 Calf/ cow

4.7 Hand lens

4.8 Stereo microscope

4.9 *Anopheles* Identification keys (adult)

4.10 GPS receiver

4.11 Note book

4.12 Pencil

4.13 Container to transport paper cups/Plastic basin

4.14 Towels

4.15 Iron or wooden pole to tie the calf

4.16 H/AMC/E4 forms

5. Procedure

5.1 Select a location for the hut trap preferably within 200-500 m distance from the vector mosquito breeding site, and it should be in between the breeding site and human habitation (preferably a site with abundance of cattle in the vicinity).

5.2 The location should be a flat and a shady area to set the hut.

5.3 Use cadjan to construct the walls, roof and the door of the hut. Construct the hut with a length and width of 6' and5' respectively. The height of the hut at the sides is 5' and the middle (center) is 6.5'.

5.4 Set a door (2.5' x 5') with cadjan in one of the walls (5' x 6.5') facing any direction other than east.

5.5 Leave a space of 4-6" between the lower end of the hut wall and the ground.

5.6 Thatched walls and roof should be made of horizontally arranged cadjan as shown in figure 3.

5.7 Record the GPS coordinates of the hut.

5.8 Label the hut as "Experimental Hut, Do not Spray insecticides".

5.9 Be at the survey site preferably one hour before sunset (17 00).

5.10 Clean the wall and the floor of the hut to ensure free of spider webs, snakes etc.

5.11 Wet the inner walls and roof of the hut sprinkling some water.

5.12 Introduce the calf/cow before sunset (17 30). Fix a strong wooden pole inside close to the center of the wall opposite to the wall with the door and tie the calf to it.

5.13 Close the door and leave the hut overnight.

5.14 On the following day, go to the hut by 05 00 (preferably before sun rise).

5.15 One team member should enter the hut slowly opening the door taking care to prevent/ minimize the escape of mosquitoes and take the calf/cow out of the hut.

5.16 Then two team members should enter the hut and close the door immediately and start collecting all *Anopheles* mosquitoes resting on the walls and roof of the hut using mouth aspirators and torches one person moving in a clockwise direction and the other person in anti clock wise direction.

5.17 Do not keep more than 5 mosquitoes in the aspirator at a time. Transfer the mosquitoes into paper cups covered with netting. Do not keep more than 20 – 25 mosquitoes in a single cup.

5.18 Place the cups of mosquitoes in a plastic basin and cover these with a wet towel to create a humid environment.

5.19 Transport the cups to the field laboratory as described in SOP no. 14.

5.20 Identify the species using standard mosquito identification keys with the use of hand lens/stereomicroscope.

5.21 Keep different species of mosquitoes in separate paper cups with double nets and wet pads till the mosquitoes are used for susceptibility and bioassay tests or for mosquito rearing.

5.22 Record the data in form H/AMC/E/4.

Procedure Notes:

1. Number of cattle baited huts per sentinel site should be minimum of 01.
2. Before the mosquito samples are observed under the stereo microscope they should be killed using Ether/Chloroform.

Figure 3. Horizontal arrangement of cajdan

Figure 4. A cattle baited cadjan hut trap

Anti Malaria Campaign **SOP for Entomological Surveillance**					
SOP title:	**SOP for Outdoor Collection of mosquitoes**				
SOP No.	**05**	**Revision No.**		**Effective Date**	**01.08.2016**
Replacement no.		**Dated**		**Page no.**	
Prepared by:	**Anti Malaria Campaign**			**Date**	**13.07.2016**
Approved by:	**Director, Anti Malaria Campaign**			**Date**	**20.07.2016**

1.0 Background

Outdoor collection of mosquitoes gives information on outdoor resting surfaces of vector mosquito species.

Outdoor collection methods

For outdoor collection of *Anopheles* mosquito species following methods can be used.

- Hand collection
- Spray sheet collection (In the river beds *Anopheles* mosquitoes rest in the rock caves, brick kilns, animal sheds, culverts where collectors cannot reach for hand collections. In such places, spray sheet collection may be applied)
- Collections using Backpack aspirators

Choice of collection method depends on the behavior of the vector species and the types of resting sites and surfaces available in the study area.

2.0 Objectives

- To determine the *Anopheles* vector species that rest outdoors
- To determine the outdoor resting place and surfaces of vector mosquitoes
- To determine the seasonal changes in outdoor resting habitats.
- To determine the changes in relative abundance of outdoor resting vector species after vector control interventions.

3.0 Scope

This procedure applies to all entomological teams who are engaged in malaria entomological surveillance.

4.0 Responsibility

It is the responsibility of the Entomological Officer of Health as the team leader to ensure that the entomological team members carry out the SOP as written.

5.0 Outdoor Hand Collection

5.1 Equipment and Materials

5.1.1 Mouth aspirator / Mechanical aspirator

5.1.2 Three celled Torch (with spare bulb and 1.5V batteries)

5.1.3 Paper cups with net covers

5.1.4 Cotton wool

5.1.5 A pencil

5.1.6 Hand lens

5.1.7 Stereo microscope

5.1.8 *Anopheles* Identification keys (adult)

5.1.9 A note book

5.1.10 A bag to carry all the equipment

5.1.11 Container to transport paper cups/plastic basin

5.1.12 Wet towel

5.1.13 Sugar solution (10%)

5.1.14 Papers to serve as packing materials

5.1.15 H/AMC/E 7 form

5.2 Procedure

5.2.1 Be at the survey site by 0800 hours.

5.2.2 Search for potential resting sites of *Anopheles* species.

5.2.3 Select sites that are suitable for performing hand collection technique.

Such places include river banks, rock caves, cadjan and straw bundles etc.

5.2.4 Search for resting *Anopheles* mosquitoes in the resting site/ surface.

5.2.5 Collect the mosquitoes using torch and mouth/ mechanical aspirator.

5.2.6 Identify the species in live condition using identification keys using hand lens confirmed by stereo microscope.

5.2.7 Examine the trophic stage using the hand lens in live mosquitoes (unfed, bloodfed, semigravid, gravid)

5.2.8 Make records in the field note book.

5.2.9 Transfer the mosquitoes to a net covered paper cup (Do not put more than 20 – 25 mosquitoes per cup).

5.2.10 Use separate paper cups for different habitats and different localities and label

5.2.11 Transport the mosquitoes to the field laboratory (refer SOP 14).

5.2.12 Fill the H/AMC/E 7 form.

6.0 Outdoor collection of mosquitoes using spray sheet collection technique

6.1 Equipment and Materials

6.1.1 White cotton sheets (size 2mx1m, 2m x 2m)

6.1.2 Hand sprayer

6.1.3 Hand lenses

6.1.4 Stereo microscope

6.1.5 Space spraying Insecticidal solution

6.1.6 Small petri dishes

6.1.7 Paper cups with net covers

6.1.8 Forceps

6.1.9 Container to transport paper cups/Plastic basin

6.1.10 Cotton wool

6.1.11 Torch

6.1.12 H/AMC/E 7 form

6.2. Procedure

6.2.1 Be at the survey site by 08 00 hours.

6.2.2 Select the potential mosquito resting sites that are suitable for spray sheet collection.

6.2.3 Spread white sheets to completely cover the floor of the selected resting site.

6.2.4 Cover all the exit points (if any) except the mouth of the cave to prevent escaping of mosquitoes.

6.2.5 Spray inside the resting site with the hand aerosol sprayer filled with right mixture of the insecticide.

6.2.6 Close the opening of the resting site for 10 minutes.

6.2.7 After 10 minutes, take the sheets out pulling one by one. The sheets that are outer most of the resting site are pulled first. Ensure no mosquito is escaped with the wind.

6.2.8 Examine each sheet carefully for *Anopheles* mosquitoes, if there are *Anopheles* mosquitoes, collect them into a petri dish lined with cotton wool with the aid of forceps.

6.2.9 Identify the species and note the abdominal condition.

6.2.10 Fill the (H/AMC/E7) form.

7.0 Outdoor collection of mosquitoes using backpack aspirator

For the collection of mosquitoes that rests in the heaps of coconut husks, bushes, river banks, termite mounds, tree trunks, rock caves, animal burrows etc. backpack aspirators are used.

7.1 Equipment and Materials

7.1.1. Backpack aspirator

7.1.2. Chloroform

7.1.3. Cotton wool

7.1.4. Petri dishes (glass)

7.1.5. Forceps

7.1.6. Hand lens

7.1.7. Rubber bands

7.1.8. Net covers

7.1.9. Collection vials

7.1.10 Container to transport paper cups/Plastic basin

7.2 Procedure

7.2.1. Be at the survey site by 0800 hours.

7.2.2. Select the potential mosquito breeding sites that are suitable for collections by back pack aspirators.

7.2.3. Start collection using the back pack aspirator ensuring all resting surfaces are reached by the aspirator.

7.2.4. Cover the collection cup with a net using a rubber band while keeping the back pack aspirator switched on.

7.2.5. Switch off the back pack aspirator and remove the collection cup.

7.2.6. Place a cotton wool soaked in chloroform on the net cover of the collection cup and cover with a glass Petri dish (take care not to damage the plastic collection cup by chloroform).

7.2.7. Transfer the anaesthetized mosquitoes in to the Petri dish.

7.2.8. Identify the mosquitoes, record the abdominal condition and transfer the specimens to collection vials.

7.2.9. Make records and transport the mosquitoes to the field laboratory.

8.0 Labeling containers, keeping the records (common for all three methods)

8.1 Use a separate container for mosquito collection in each type of outdoor habitat.

8.2 Label the all the paper cups with all relevant data, including

- Location
- Date and time of collection
- Method of collection

- Type of resting site and surface
- Name of the collector/supervisor
- Whether the village was treated with insecticides or with insecticides treated bed net

8.3 Write this information with a pencil directly on paper cups or on piece of paper, which are placed inside the container.

8.4 Keep a separate record in the field note book.

8.5 Record the data in form H/AMC/E/7.

Procedural notes

1. S**election of collection sites and surfaces**

   Suspected outdoor resting sites and surfaces of *Anopheles* mosquitoes are selected for outdoor collection of mosquitoes. Some important sites are: open huts, bundles of cadjan, straw etc., heaps of coconut husks, firewood etc., abandoned brick kilns, river banks, culverts, rock caves, tree holes, among tree roots, shrubs, bushes etc.

2. **Time of collection**

   From 0800 – 1200 hours

3. **Personnel required**

   01 Entomological Assistant and 02 mosquito collectors per team is required (For the back pack aspirator in rotation, 2 mosquito collectors for one machine are required).

4. Define trophic stages of collected mosquitoes as unfed, freshly fed, semi gravid and gravid. Blood fed mosquito samples should be taken for blood meal identification.

Anti Malaria Campaign **SOP for Entomological Surveillance**					
SOP title:	**SOP for Window Trap Collection (Exit Traps)**				
SOP No.	**06**	**Revision No.**		**Effective Date**	**01.08.2016**
Replacement no.		**Dated**		**Page no.**	
Prepared by:	**Anti Malaria Campaign**			**Date**	**13.07.2016**
Approved by:	**Director, Anti Malaria Campaign**			**Date**	**20.07.2016**

1.0 Background

Window trap (exit) technique provides information on indoor biting and outdoor resting behavior of *Anopheles* mosquito species. In addition, this technique can be used to determine the effect of indoor residual spraying and LLINs on the movement (degree of excito-repellency) and feeding habits of mosquitoes and to determine the residual efficacy of insecticides as indicated by the 24 hour mortality rate of mosquitoes found alive in the traps.

2.0 Scope

This procedure applies to all Entomological teams who are engaged in malaria entomological surveillance.

3.0 Responsibility

It is the responsibility of the Entomological Officer of Health as the team leader to ensure that the entomological team members carry out the SOP as written.

4.0 Materials and Equipment

4.1 Window trap with frame and 16- mesh netting (Figure 5 & 6).

4.2 Dark coloured covering materials such as cloth, polythene or paper

4.3 Mouth aspirators

4.4 Paper cups with net covers

4.5 A towel

4.6 Container to transport paper cups/Plastic basin

4.7 Cotton wool

4.8 Petri dishes

4.9 Forceps

4.10 Hand lenses

4.11 Stereo microscope

4.12 *Anopheles* Identification keys

4.13 Note book

4.14 Pencil

4.15 H/AMC/E/5 forms

5.0 Procedure

5.1 Select a house located preferably within 200-500 m distance from the vector mosquito breeding site.

5.2 Select a bed room (less ventilated, without large spaces under the eaves or having minimum exit points for mosquitoes with higher number of sleepers in the previous night) with a small window preferably facing east for setting the exit window trap.

5.3 Record the data regarding house hold data, house type and vector control interventions used in the house in the field note book.

5.4 Set the window trap by 5.30 p.m.

5.5 Fix the trap to a window preferably at least at a height of 4’ above the ground level with the collecting sleeve facing outward.

5.6 Cover the openings of the room with dark covering materials to prevent escape of mosquitoes other than the window to which the trap is fixed.

5.7 Leave the trap overnight.

5.8 On the following day, visit the trap by 6.30 a.m.

5.9 Collect the trapped mosquitoes through the sleeve of the trap into paper cups with net covers using an aspirator.

5.10 Collect dead mosquitoes first to the petri dishes and identify to species and separate them according to trophic stage.

5.11 Record the data in the field note book.

5.12 Then collect the live mosquitoes one by one using an aspirator and identify species and separate them according to trophic stage into labeled paper cups with nets.

5.13 Label the cup clearly using a pencil and include the following information.

- Location of the window trap (house number or householder's name)
- Date of collection
- No. of died and live mosquitoes in the trap at the time of collection
- Date of last insecticide spraying
- Availability of LLINs in the house/room and date of issue
- Type of wall and roof

5.14 Transport the mosquitoes to the field laboratory.

5.15 When the traps are fitted in houses that have been sprayed with insecticides or in houses using LLINs, keep the mosquitoes caught live for 24 hours.

5.16 After 24 hours record the number survived.

5.17 Record the data in form H/AMC/E/5.

Figure 5. A window trap fixed to a window

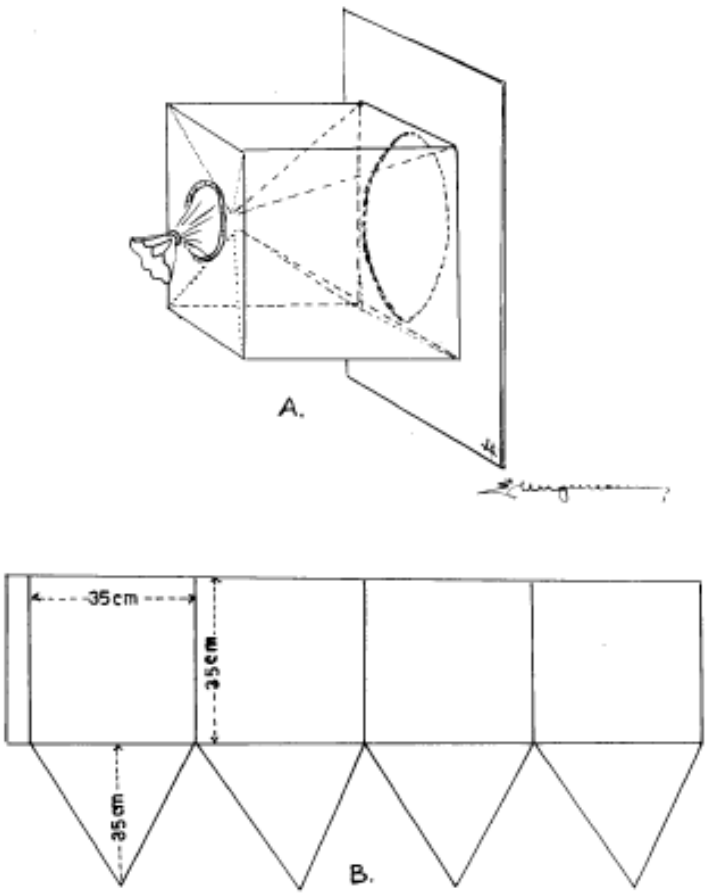


Figure 6. Construction of an exit trap

A. Completed trap B. Pattern for cutting mosquito netting

Anti Malaria Campaign **SOP for Entomological Surveillance**					
SOP title:	**SOP for Human Landing Night Collection of Anopheline vectors**				
SOP No.	**07**	**Revision No.**		**Effective Date**	**01.08.2016**
Replacement no.		**Dated**		**Page no.**	
Prepared by:	**Anti Malaria Campaign**			**Date**	**13.07.2016**
Approved by:	**Director, Anti Malaria Campaign**			**Date**	**20.07.2016**

1.0 Purpose

This technique provides information on Female *Anopheles* mosquitos' attraction to human to obtain blood meals both indoor and outdoor. The main objectives are

- To determine human biting Anopheline mosquito species
- To determine the biting time of a particular vector mosquito species
- To determine the peak biting period of a vector mosquito species
- To determine whether the vector mosquito species is biting indoor or outdoor
- To collect mosquitoes for parity determination and for salivary gland dissections
- To determine the seasonal variation of vector mosquito biting

2.0 Scope

This procedure applies to all entomological teams who are engaged in malaria entomological surveillance.

3.0 Responsibility

It is the responsibility of the Entomological Officer of Health as the team leader to ensure that the entomological team members carry out the SOP as written.

4.0 Equipment and Materials

4.1 Mouth aspirators

4.2 Test tubes

4.3 Three celled Torch (with spare bulb and 1.5V batteries)

4.4 Paper cups with net cover

4.5 Cotton wool

4.6 Rubber bands

4.7 Thermo meter and hygro meter

4.8 A plastic basin

4.9 A towel

4.10 Stereo microscope

4.11Compound microscope

4.12 Hand lens

4.13 Glass slides & cover slips

4.14 Dissecting set

4.15 Chloroform

4.16 Table lamp

4.17 Normal saline

4.18 Distilled water

4.19 *Anopheles* identification keys (adult)

4.20 Note book

4.21 Pencil

4.22 H/AMC/E6 forms

Criteria for selection of a house for HLNC.

- It should be closer (preferably 200-500 m) to the vector breeding sites in the area.
- If there is a reported malaria case, the residence of malaria patient should be selected.
- If there are no malaria cases in the location, it is advantageous to a select most poorly constructed and less ventilated house.
- Smokes should not be in the area.
- There should not be any cattle sheds in the vicinity (not applicable for malaria case based entomological investigations)

5.0 Procedure

5.1 Get permission from the house holder of the house selected for HLNC.

5.2 Start the survey at 6.00 p.m.

5.3 Select a room for indoor collection which should be a bed room. Collections should not be made in an open verandah, as it is considered neither inside nor outside.

5.4 Select the ideal place for positioning for outdoor collection at the outside of the house. It is better if the space normally used by people to sit outdoors during the evening can be selected.

5.5 Mosquito collectors should be seated indoor near to each other. Same number of members should be seated outdoor about 2-3 meters apart from each other.

5.6 Collectors should be seated quietly with their legs exposed up to the knee. Lower arms should also be exposed.

5.7 Each collector must carry a test tube, torch and labeled paper cup with net cover and cotton wool.

5.8 Collectors must catch mosquitoes landing on their exposed body surface by the test tube by themselves.

5.9 Different paper cups should be used for each hour of collection and labeled accordingly before collection starts.

5.10 Mosquitoes should be transferred to the relevant paper cup with a net cover immediately.

5.11 Label the paper cup with following information using a pencil

- Whether indoor or outdoor/site of collection
- Time interval or hour of collection
- Date

5.12 Record the following information in the field note book.

- Locality
- Type of breeding site in the vicinity
- When the location was last sprayed
- When the house was given LLIN
- Reading of Temperature in thermo meter
- Reading of Relative Humidity in hygro meter

5.13 After collection, keep the paper cups holding mosquitoes carefully in the plastic basin and cover with wet towels and transport to the field laboratory (refer SOP 14).

5.14 Record the temperature and Relative humidity data hourly (It should be started 30 minutes after initiation of the collection and then continue hourly). In case of outdoor collections, weather conditions (presence of rain, moon light and wind) should be recorded.

5.15 Record the data in for H/AMC/E6

5.16 If vector mosquitoes are collected dissections should be carried out (ovary and salivary gland refer the SOP No. 16 & 17)

Procedure Notes

1. No of baits must be equal in both indoor and outdoor collections.

2. Its preferred that the collectors avoid taking bath or washing just before the survey.

3. Mosquito repellent substances, should not be used during the work. Collectors shouldn't use any perfumes or fragrances. Also they should not use alcohol or smoke while collecting.

4. Baits indoors and outdoors should be changed hourly, to minimize possible bias in their attractiveness to mosquitoes (one indoor collector should go out and one outdoor collector should come in after every hour of collection).

5. If it is a partial night collection, collections should be made during 6.00pm to 9.00pm.

6. In a full night program, hourly collections should be made during entire period from 6.00pm – 6.00 am hours (Dusk to Dawn).

7. Full night collection is a laborious activity, therefore minimum eight members are required as two teams of collectors and each team should be working half of the night.

8. Both indoor and outdoor collections should be done to accommodate the normal resting and sleeping habits of the local people.

Figure 7. Collection of mosquitoes from human bait

Anti Malaria Campaign **SOP for Entomological Surveillance**					
SOP title:	**SOP for Survey of *Anopheles* mosquito larvae**				
SOP No.	**08**	**Revision No.**		**Effective Date**	**01.08.2016**
Replacement no.		**Dated**		**Page no.**	
Prepared by:	**Anti Malaria Campaign**			**Date**	**13.07.2016**
Approved by:	**Director, Anti Malaria Campaign**			**Date**	**20.07.2016**

1.0 Purpose

Larval survey provides information on the presence and density of immature (larvae and pupae) stages of *Anopheles* species in a variety of habitats in a locality. This technique is performed with the objectives of

- Establishing the breeding habitats of different *Anopheles* species and evaluate the active breeding sites.
- Determining the temporal and geographical distribution of *Anopheles* species.
- Evaluating the impact of anti larval measures and other vector control interventions determining optimal times for the application of larval control measures.
- Forecasting the need and timing for adult mosquito control.
- Collecting larvae for larval and adult susceptibility test.

2.0 **Scope**This procedure applies to all entomological teams who are engaged in malaria entomological surveillance.

3.0 Responsibility

It is the responsibility of the Entomological Officer of Health as the team leader to ensure that the entomological team members carry out the SOP as written.

4.0 Equipment and Materials

4.1 Plastic dipper (capacity of 350 ml) with extensible handle

4.2 Ladle (capacity of 120 ml)

4.3 Larval vials (3 sizes: Large, medium and small)

4.4 Enamel trays (small)

4.5 Pipettes

4.6 Compound microscope

4.7 Glass slides/Cover slips

4.8 10 % Formalin solution

4.9 Pairs of boots

4.10 Pairs of gloves

4.11 Fine mesh net-nylon gauze net

4.12 Ring of iron wire (20-25 cm in diameter)

4.13 Rubber tubes

4.14 Collection bottles

4.15 Labels

4.16 Marker pens

4.17 Carrying bag

4.18 *Anopheles* Identification keys (Larvae)

4.19 GPS receiver

4.20 Note book

4.21 H/AMC/E8 forms

5.0 Procedure

5.1 Start the survey as early as possible in the morning when there is sufficient sunlight. Duration of the survey would depend on the number and extent of breeding sites.

5.2 Explore the focal area in order to identify all potential *Anopheles* breeding sites.

5.3 Note down the identified breeding sites in the area.

5.4 Record the types of vector control interventions in place in focal area, if any.

5.5 Use appropriate larval collection method/s according to the type of breeding site.

5.6 Methods of larval collections

- Dipping
- Netting
- Pipetting

5.6 Dipping

This method applies for sampling from relatively large water bodies where the water level is high enough for dipping. Such water bodies include stagnant, shallow and slow flowing river and stream margins, sand pools, connected pools and rock pools of river beds, burrow pits, quarry pits, gem pits, clay pits, brick fields, natural and manmade irrigation water canals and channels, drainages, tank and reservoir beds and margins, lagoon margins, ponds, paddy fields, tire prints ditches, culverts, wells, rain water pools/ ground pools and any type of similar breeding places.

5.6.1 Estimate the active breeding surface area visually in square meters.

5.6.2 Determine the number of dips to be taken from the breeding sites at the rate of 6 dips per squaremeter water surface from small pools (<10m^2 surface area). In the large water bodies such as pools, tanks, seepages, flowing streams and irrigation channels, dip at the same rate in 0.5mx10m quadrates along the margins. Select the spots for the survey randomly.

5.6.3 Proceed slowly and carefully to avoid disturbing the water surfaces as much as possible (waves and vibrations from footsteps will stimulate immature to dive from the water surface).

5.6.4 The collector should be in a position avoiding casting his shadow in the water during dipping.

5.6.5 Submerge the leading edge of the dipper gently with an angle of 45^0 and about 2.5 cm below the water surface.

5.6.6 If the breeding site is shallow, long and has no obstructions, draw the dipper along the water surface till ¾ of the dipper is filled and lift it.

5.6.7 If the breeding site deep or with obstructions, lower the dipper gently to allow water and larvae to flow into the dipper and then lift the dipper.

5.6.8 Let the dipper to fill ¾ of water in both steps 5.6.5 and 5.6.6.

5.6.9 Use the ladle for dipping in small water collections where the dipper cannot be used as described in 5.6.3.

5.6.10 Keep 2-3 minute intervals between two dips to allow larvae to come to the water surface again.

5.6.11 Record the number of dips taken, number of dips positive for *Anopheles* larvae according to the type of breeding site.

5.6.12 In each dip, count the number of 1^{st} and 2^{nd} stage larvae, 3^{rd} and 4^{th} stages larvae and pupae separately (identify the larval instars stage by observing the relative size of the collar).

5.6.13 Transfer larvae and pupae from the dipper to labeled vials according to different breeding sites using a pipette. The label should contain the following details.

- Date
- Locality/GN area
- Type of breeding place
- Collection method

5.6.14 Transfer the collected larvae to the field station or to the laboratory.

5.6.15 If 3rd and 4th stage larvae found, identify all 3rd and 4th larvae to the species level using standard mosquito identification guides for *Anopheles* larvae.

5.6.16 In the absence of vector species among the 3rd and 4th larvae and if 1st and 2nd stage larvae are found, allow them to develop to 3rd and 4th instar larval stages in separate net covered plastic/ enamel trays for different breeding sites each tray filled with 1 liter of water from the respective breeding place.

5.6.17 Identify these larvae as in step 5.6.15.

5.6.18 If pupae are found in the survey, allow them to emerge into adult in an enamel tray covered with a net and identify the adult *Anopheles* using adult *Anopheles* mosquito identification keys up to the species level.

5.6.19 Calculate the larval density per 100 dips for each species for each type of larval breeding habitat separately.

5.6.20 Record the following environmental parameters at the time of survey.

- Water quality parameters–clear, turbid, polluted
- Presence or absence of larvivorous fish and other known predators
- Presence of vegetation-floating, emergent, submerged
- Exposure to sunlight-sunlit, shaded
- Weather condition at the time of collection-Rainy / Dry

5.6.21 Record the GPS coordinates of each breeding site sampled in focal area.

5.6.22 Enter data on H/AMC/E8.

5.7 Netting

5.7.1 This method is normally used to collect larvae and pupae in deep water bodies such as wells and ponds.

5.7.2 The fine mesh net mounted on a circular frame should be dipped slowly in the water of the well keeping half the border of the net above the water.

5.7.3 Sweep the surface water by moving the net along the margin of the water body. Hold the net with about 45^0 angle to the water surface and drag across the surface.

5.7.4 Take the net out and invert the net and wash out in an enamel tray with water.

5.7.5 Collect and transfer the larvae with a pipette in to a labeled vial.

5.7.6 After waiting 2-3 minutes to allow the disturbed larvae to return to the water surface, repeat the steps 5.7.3- 5.7.5.

5.7.7 Identify the larvae following the procedure as in dipping 5.6.11 to 5.6.18.

5.7.8 Calculate the larval density per well, pond, etc.

5.7.9 Follow the steps in 5.6.20 & 5.6.21.

5.7.10 Record the data in the H/AMC/E8 form.

5.8. Pipetting

5.8.1. This method is used for collection of larvae from small breeding sites such as mini rock pools, tree holes, hoof prints, containers, small puddles and plant axils.

5.8.2. Explore the presence of breeding sites in the focal area which are appropriate for pipetting.

5.8.3. Pipette the whole water content and pour in to the enamel tray separately according to the type of breeding sites

5.8.4. Use separate vials according to the type of breeding sites and label properly.

5.8.5. Transport the larvae to the field station or to the laboratory.

5.8.6. Follow the counting and identification procedure as mentioned in the dipping method (5.6.11 to 5.6.21).

5.8.7. Enter data in the H/AMC/E8 form.

Procedural notes

1. Do not carry out a larval survey immediately after heavy rains.
2. If the number of 3rd and 4th stage larvae is unusually high, identify a sample of about 50% and estimate the density accordingly.
3. The breeding sites of *Anopheles* mosquitoes in Sri Lanka are classified in Annexure I.
4. Prepare temporary mounts of *Anopheles* mosquito larvae for identification.
5. If there is a reported malaria case, the larval survey should cover all mosquito breeding sites of an area of 1 km radius area.
6. Do not throw the residual water back into the breeding place when dipping, as this may further disturb the larvae and pupae.

Annexure I

Running water systems	**Permanent or semi - permanent standing water**	**Temporary water habitats**	**Container habitats**
River margins	Marshes	River/stream bed pools	Tree holes
Stream margins	Swamps	Rock pools	Rock holes
	Seepages	Sand pools	Leaf axils
	Lagoon margins	Connected pools	Cement tanks
	Ponds	Rain water collections	Barrels
	Tank margins	Ground pools	Ornamentals
	Wells (domestic)	Quarry pits	
	Agricultural wells	Gravel pits	
		Burrow pits	
		Brick pits	
		Clay pits	
		Gem pits	
		Irrigation canals	
		Paddy fields	
		Tire prints	
		Canal bed pools	
		Animal foot prints/hoof prints	
		Drainage canals	
		Ditches	
		Culverts	

Anti Malaria Campaign SOP for Entomological Surveillance					
SOP title:	**SOP to Determine the Susceptibility of Adult Mosquitoes to Insecticides**				
SOP No.	**09**	**Revision No.**		**Effective Date**	**01.08.2016**
Replacement no.		**Dated**		**Page no.**	
Prepared by:	**Anti Malaria Campaign**			**Date**	**13.07.2016**
Approved by:	**Director, Anti Malaria Campaign**			**Date**	**20.07.2016**

1.0 Background

Insecticides play an important role in malaria vector control programmes. Continuous and long-term application of insecticides will select resistance gene(s) in the population of malaria vectors. Monitoring of susceptibility levels of vector/s population at different time intervals is important for selecting appropriate insecticide and dosage for vector control.

2.0 Objectives

1. To detect susceptibility levels of the vector population to different insecticides.
2. To monitor changes of susceptibility levels of vector population to discriminative dosages of different insecticides at periodic intervals.

3.0 Scope

This procedure applies to all entomological teams who are engaged in susceptibility/resistance monitoring of malaria vectors against different insecticides.

4.0 Responsibility

It is the responsibility of the Entomological Officer of Health to carry out the SOP as written.

5.0 Materials

5.1 WHO standard susceptibility test kit for adult mosquitoes (WHO, 2013)

Composition of the test kit

- 12 plastic tubes (125 mm in length and 44 mm in diameter), with each tube fitted at one end with 16-mesh screen or net. The 12 tubes include:Four marked with a **red dot** for use as exposure tubes,
- Two marked with a **yellow dot** for use as control tubes, Six marked with a **green dot** for use as holding tubes, Six slide units, each with a screw-cap on either side, and provided with a 15 mm filling hole;
- 40 sheets of clean paper (12x15 cm) 12 spring wire clips, (6 steel and 6 copper).
- Two glass or plastic aspirator tubes of 12 mm internal diameter, together with 60 cm of tubing, and mouthpieces;
- One role of self-adhesive plastic tape or one sheet of label tag;
- Instruction sheet, and 20 copies of the report form;

5.2 Insecticide impregnated papers

For routine monitoring of insecticide susceptibility, papers impregnated at the discriminating concentrations are used. The impregnated papers for each insecticide at the discriminating concentration and control papers are packed in plastic boxes. Each box contains 8 papers. Impregnated papers should be stored at 4 °C. After using the papers they should be immediately returned to the boxes, resealed carefully with the plastic tape and stored at 4 °C for further use. Papers should not be used after the expiry dates shown on the box.

Discriminating concentrations of insecticides for adult Anopheline mosquitoes (WHO, 2013)

Insecticide	Discriminating Concentration	Exporsure period	Control paper
DDT	4%	1hr.	Risella oil
Malathion	5%	1hr.	Olive oil
Fenitrothion	1%	2hr	Olive oil
Bendiocarb	0.1%	1hr.	Olive oil
Propoxur	0.1%	1hr.	Olive oil
Permethrin	0.75%	1hr.	Silicone oil
Lambda-cyhalothrin	0.05%	1hr.	Silicone oil
Deltamethrin	0.05%	1hr.	Silicone oil
Cyfluthrin	0.15%	1hr.	Silicone oil
Etofenprox	0.5%	1hr.	Silicone oil
Bifenthrin	0.2%	1hr.	Silicone oil

5.3 Forceps

5.4 Rubber bands

5.5 5-8% Sugar solution

5.6 Hygrometer (to record relative humidity , maximum and minimum temperature)

5.7 Wet towels

5.8 Ant traps

5.9 Extra 12 spring wire clips,(6 steel and 6 copper)

5.10 Paper cups with netting.

5.11 Cotton wools

5.12 Adult mosquito cage

5.12 Note book

5.14 Pencil

5.15 H/AMC/E9 Form

6.0 Sampling Protocols

6.1 Selection of test specimens

6.1.1 Ideally non-blood fed adult females, aged no more than 3-5 days post emergence.

- Through larval collections. Type of breeding site should be specified. Samples from the same place and the same type of breeding sites can be pooled before testing.
- From the F1 progeny from wild caught females which should ideally be collected from number of different locations.

For species or places where larval collections are not possible, tests can be performed on wild caught females. In this case, their physiological status (unfed/blood fed, semi-gravid, gravid) should be recorded.

6.2 Sample size

6.2.1 Hundred (100) adult female mosquitoes should be tested for any insecticide at the discriminative concentration, with 4–replicates each of around 25 mosquitoes per test tube.

6.2.2 A minimum of two controls of 50 mosquitoes (two replicates each of around 25 mosquitoes) should be carried out.

6.3 Conditions for test

6.3.1 The tests should be done indoors, free from insecticidal contamination and extremes of temperature, humidity, illumination and wind.

6.3.2 Tests should be carried out ideally at a temperature of 25 ± 2 °C and at 80 ± 10% relative humidity (RH). Tests should never be done at a temperature higher than
30 °C.

6.3.3 The number of times the impregnated papers can be re-used depends on the insecticide. The maximum number of times for use of impregnated papers is 15 for organochlorine papers, 10 for organophosphate and carbamate papers, and 5 for pyrethroid papers.

6.3.4 To avoid multiple manipulations, impregnated papers can remain in the tubes, provided that they are wrapped in aluminium foil and kept at 4^0C between successive tests.

7.0 Test procedure

7.1 Roll and insert a piece of clean white paper (12x15 cm). Into each holding tube to line the wall and fasten it in position with two spring-wire clips (silver). Attach the slides to the tubes.

7.2 Aspirate 150 active adult females gently from the mosquito cage in batches and release them into the 6 holding tubes through the filling-holes giving 25 mosquitoes per a tube. In each aspiration do not collect more than five (5) mosquitoes. Insert a cotton pad into the filling-hole.

7.3 Set the holding tubes upright for one hour. If any damaged mosquito is found replace it with a healthy mosquito.

7.4 Roll and insert relevant control paper to the insecticide to be tested into control tubes to line the wall and fasten it in position with two steel clips using a forcep. Similarly introduce the insecticide impregnated paper into each exposure tube rolled into a cylinder of the exposure tube facing insecticide impregnated side inward as indicated in the box and fasten it with two copper spring-wire clips.

7.5 Introduce the mosquitoes into the exposure tube by attaching it to the vacant screw-top in the slide. The slide should be pulled out to a point beyond the filling hole so that no part of it occludes the tube openings; the mosquitoes are then blown gently into the exposure tube ensuring all the mosquitoes are transferred. Close the slide. Detach the holding tube and set it aside. Similarly transfer the mosquitoes in to the control tubes.

7.6 Keep all exposure tubes and control tubes in a vertical position with the mesh screen end uppermost, for the recommended time period.

7.7 At the end of the exposure period, transfer the mosquitoes to the holding tube by reversing the procedure step 7.5. Open the slide and gently blow the

mosquitoes into the holding tube; close the slide and remove the exposure tube. Then set the holding tube so that it stands on the slide and place a pad of a moist cotton-wool on the screen.

7.8 Keep the holding tubes for 24 hours in a secluded, shaded place, the tubes should be protected from ants by placing them on an ant trap (platform standing in a pan of water). If conditions are very hot and dry the tubes can be stored in a container and covered by a damp towel.

7.9 Temperature and relative humidity should be recorded during both the exposure and the recovery periods.

7.11 Make mortality counts at 24-hour post-exposure. Adult mosquito should be considered as live when they are able to fly regardless of the number of legs remaining. Any knocked-down mosquitoes and those with lost legs or wings are considered moribund and they should be counted as dead.

7.11 When testing pyrethroids, recording of the knock down (kd) rate of mosquitoes during exposure should be made at 10, 15, 20, 30, 40, 50 and 60 minutes of exposure. If after 60 minutes the observed KD rate is less than 80%, another count at 80 minutes should be made of the mosquitoes in the observation tube.

7.12 The results should be recorded on the form H/AMC/E9.

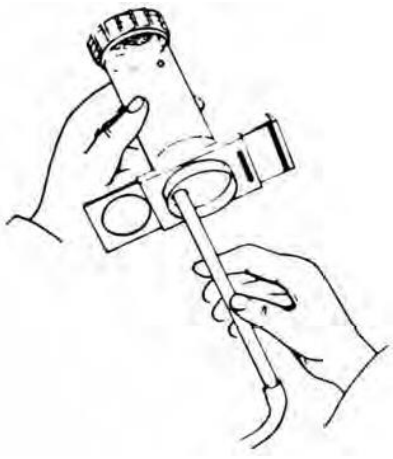


Figure 9. Releasing mosquitoes to the holding tube

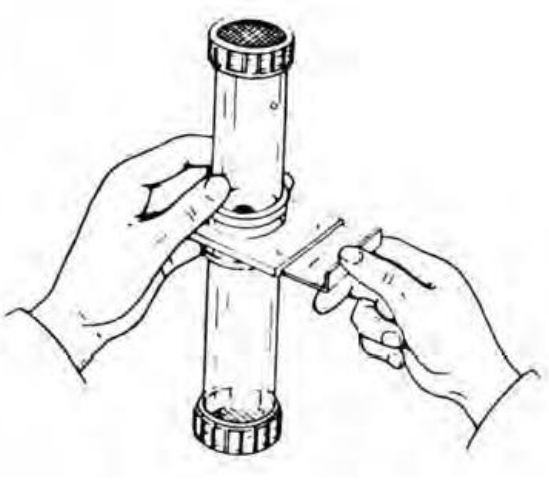


Figure 10. Introducing mosquitoes to the exposure tube

8.0 Analysis of the susceptibility test results

Percentage mortality after the 24-hour recovery period should be recorded.

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

A similar calculation should be made in order to obtain a value for the control mortality.

If the control mortality is above 20%, the tests must be discarded. If the control mortality is greater than 5% and less than 20%, the mortality observed should be corrected by applying Abbott's formula as follows:

$$\frac{\%\text{ test mortality} - \%\text{ control mortality}}{100 - \%\text{ control mortality}} \times 100$$

If the control mortality is below 5%, it can be ignored and no correction is necessary.

9.0 Interpretation of susceptibility test results (WHO, 2013)

- 98-100% mortality indicates susceptibility

- <98% mortality suggests possibility of resistance that needs further investigations for confirmation. Perform additional tests using the same insecticide on the same species and if at least two additional tests showed mortality <98% then resistance is confirmed.

- <90% mortality suggests resistance

Procedure notes

1. If it's not possible to collect enough mosquitos on a single occasion (if working with wild caught females for instance) then it is possible to store live mosquitoes until sufficient

numbers have been collected. When relying on pooled samples, mosquitoes should be provided with access to a sugar meal until the bioassay can be carried out.

2. Number of tests subjects

   At least 100 mosquitoes should be tested for any insecticide at the diagnostic concentration, with at least 4 replicates of 20-25 mosquitoes per test. When it is not possible to test this number of mosquitoes on a single day, tests can be conducted over a few days until this number is reached.

3. Knockdown rate

   Observations on knocked down mosquitoes should be after 10,15,20,30,40,50 and 60 minutes of the exposure period with last observation just before transfer to the observation tube. If, after 60 minutes, the observed knockdown rate is less than 80% another count should be made at 80 minutes in the observation tube.

Anti Malaria Campaign **SOP for Entomological Surveillance**					
SOP title:	**SOP to determine the susceptibility of mosquito larvae to discriminative dosage of insecticide.**				
SOP No.	**10**	**Revision No.**		**Effective Date**	**01.08.2016**
Replacement no.		**Dated**		**Page no.**	
Prepared by:	**Anti Malaria Campaign**			**Date**	**13.07.2016**
Approved by:	 **Director, Anti Malaria Campaign**			**Date**	**20.07.2016**

1.0 Purpose:

The purpose of the susceptibility test on *Anopheles* larvae is to detect the presence of resistance in a larval population as early as possible so that alternative control measures can be made in time to manage the insecticide resistance.

2.0 Scope

This procedure applies to all entomological teams engaged in malaria entomological surveillance.

3.0 Responsibility

It is the responsibility of the Entomological Officer of Health to carry out the SOP as written.

4.0 Equipment and Materials

4.1. World Health Organization (WHO) standard larval susceptibility test kit could be used (WHO, 2013)

Composition of the test kit

- Droppers with rubber suction tubes for each insecticide
- Strainers (2 wire loops, 1 piece of nylon netting (30 cm2) and 1 tube of glue) for each insecticide different solutions of larvicide (in 50 ml bottles)
- Pesticide solvent (normally ethanol)
- Instruction sheet
- Log-probit papers

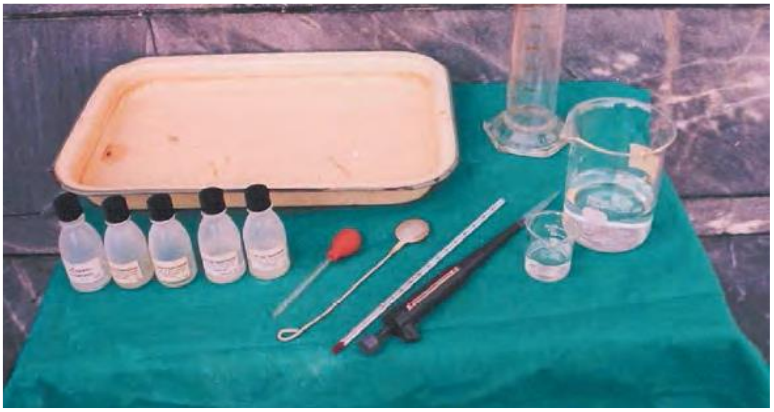


Figure 11. Composition of the test kit for larval susceptibility testing

4.2 Additional equipment

- 24 beakers of 400ml
- 24 beakers of 30ml
- Compound microscope
- Standard Anopheles mosquito larvae identification keys
- Tally counter
- Water thermometer
- Measuring cylinder
- Larval tray

- Data recording format

Discriminating dosages of insecticides for Anopheline mosquito larvae (mg/l=ppm) (WHO, 2013 b)

Insecticide	Discriminating Concentration
Temephos	0.25
Malathion	3.125
Fenitrothion	0.125

5.0 Procedure

5.1 Collect *Anopheles* larvae from same type of breeding sites in sufficient numbers.

5.2 Check a 10 % of sample of the collected larvae to ensure that they are of the same species.

5.3 Select robust (healthy) third instar or early fourth instar larvae for the test.

5.4 Take 25 ml of water to a small beaker (30 ml) and introduce 20–25 larvae by means of a strainer and keep the larvae for 30 minutes.

5.5 Any larvae that look abnormal, such as those that appear unhealthy, should be discarded.

5.6 Pour 224 ml of water into a 400 ml beaker (distilled water, rain water or tap water; even water obtained from a well or stream may be used, but hard or chlorinated tap water should not be used).

5.7 Add 1 ml of insecticide test solution (1000 ppm) using a dropper to the large beaker that makes the total volume 225 ml. The final test solution of insecticide to which larvae will be exposed is 4 ppm (1000/250).

5.8 Stir the solution thoroughly and leave the solution for 10 minutes.

5.9 Measure the temperature of the water (the optimum temperature for the test is 25 °C). The water temperature must be between 20 °C and 30 °C.

5.10 Add 25 ml of water containing 25 larvae into a large beaker containing 225 ml that makes the total volume 250 ml.

5.11 Record the time.

5.12 Leave the larvae for 24 hours.

5.13 Discard the larvae that have pupated during the test.

5.14 For each discriminative concentration of an insecticide at least 4 replicates representing 100 mosquito larvae should be tested.

5.15 Simultaneously two replicates of controls are set up with water in which 1ml of ethanol is added.

5.16 If more than 10% of the control larvae pupate during the experiment, the test should be discarded.

5.17 Tests with control mortality of 20% or more are unsatisfactory and should be repeated.

5.18 Record the number of dead and moribund larvae after the 24-hour exposure period for each replicate. (Dead larvae cannot be induced to move when they are probed with a needle in the siphon or the cervical region. Moribund larvae are those incapable of rising to the surface within a reasonable period of time. They may also show discoloration, unnatural position, tremors, and lack of coordination or rigour).

5.19 Identify all larvae used for the test and control using standard *Anopheles* larvae identification keys.

5.20 Repeat the test until 100 larvae of same species is tested for a particular insecticide.

6.0 Analysis of the susceptibility test results

6.1. Percentage mortality after the 24-hour recovery period should be recorded.

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

6.2. A similar calculation should be made in order to obtain a value for the control mortality.

6.3. If the control mortality is above 20%, the tests must be discarded. If the control mortality is greater than 5% and less than 20%, the mortality observed should be corrected by applying

Abbott's formula as follows:

$$\frac{\text{\% test mortality - \% control mortality}}{\text{100 - \% control mortality}} \times 100$$

7.0 Interpretation of susceptibility test results (WHO, 2013)

Under optimum conditions with a sample size of >100 mosquito larvae, test results can be interpreted as follows:

- 98–100% mortality indicates susceptibility.
- <98% mortality suggests the possibility of resistance that needs further investigation for confirmation. Between 90% and 97% mortality the presence of resistant genes in the vector population must be confirmed.
- If mortality is less than 90%, suggests resistance.

Procedural Notes:

1. Susceptibility testing should be done twice a year in selected sentinel sites.
2. Susceptibility testing should be carried out ideally using larvae collected from F1 of a known species.

SOP for Entomological Surveillance					
SOP title:	**SOP for Susceptibility testing of *Anopheles* mosquito larvae to determine Discriminative Dosages of Insecticides**				
SOP No.	**11**	**Revision No.**		**Effective Date**	**01.08.2016**
Replacement no.		**Dated**		**Page no.**	
Prepared by:				**Date**	**13.07.2016**
Approved:	**Director, Anti Malaria Campaign**			**Date**	**20.07.2016**

1. **Purpose**:

   The purpose of this test is to establish the diagnostic or discriminative concentration to detect the presence of insecticide resistance in a larval population.

2. Scope

This procedure applies to all entomological teams engaged in malaria entomological surveillance.

3. Responsibility

It is the responsibility of the Entomological Officer of Health to carry out the SOP as written.

4. Equipment and Materials

4.1 World Health Organization (WHO) standard larval susceptibility test kit (WHO/VBC/81.807 Composition of the test kit)

- Droppers with rubber suction tubes for each insecticide
- Strainers (2 wire loops, 1 piece of nylon netting (30 cm^2) and 1 tube of glue)
- Series of different standard solutions of larvicide (in 50ml bottles)

Larvicide	Concentration 1	Concentration 2	Concentration 3	Concentration 4
Temephos	156.25 mg/l	31.25 mg/l	6.25 mg/l	1.25 mg/l
Malathion	781.25 mg/l	156.25 mg/l	31.25 mg/l	6.25 mg/l
Fenitrothion	31.25 mg/l	6.25 mg/l	1.25 mg/l	0.25 mg/l

- Insecticide solvent (Ethanol)

4.2 24no.s of beakers 400ml

4.3 24 nos. of beakers 30ml

4.4 Compound microscope

4.5 Standard *Anopheles* mosquito larvae identification keys

4.6 Tally counter

4.7 Water thermometer

4.8 Measuring cylinder

4.9 Larval tray

4.10 Data recording format

4.11 Micropipette (10-100 and 100-1000 microlitres)

4.12 Instruction sheet

4.13 Log-probit mortality sheets

5.0 Procedure

5.1 Collect about 100-400 *Anopheles* larvae of 3rd instar or early 4th instar from laboratory reared population, or larvae of F1 generation obtained from field collected adult mosquitoes with known susceptibility.

5.2 Take 12 beakers of 30 ml and add 25 ml of water to each (distilled water, rain water or tap water; even water obtained from a well or stream may be used, but hard or chlorinated tap water should not be used).

5.3 Distribute lots of 20- 25 larvae to each beaker by means of a strainer.

5.4 Larvae that look abnormal or unhealthy should be discarded.

5.5 Take 12 beakers of 400 ml and Pour 224 ml of water to each

5.6 Prepare the test concentration by pipetting 01 ml of appropriate standard insecticide solution. Care should be taken the pipette should not intact with the surface of water in each beaker (to avoid contamination).

5.7 Stir vigorously for 30 seconds with a glass rod.

5.8 Lowest concentration should be prepared first.

5.9 Prepare at least2 replicates at each concentration,

5.10 At least two replicates of controls are set up simultaneously with water in which 1ml of ethanol is added.

5.11 Leave the solution for 15-30 minutes.

5.12 Measure the temperature of the water (the optimum temperature for the test is 25 °C). The water temperature must be between 20 °C and 30 °C.

5.13 Add 25 ml of water containing 25 larvae into a large beaker that makes the total volume 250 ml. Record the time.

5.14 Leave the larvae for 24 hours.

5.15 Discard the larvae that have pupated during the test.

5.16 If more than 10% of the control larvae pupate during the experiment, the test should be discarded.

5.17 Tests with control mortality of 20% or more are unsatisfactory and should be repeated.

5.18 Record the number of dead and moribund larvae after the 24-hour exposure period for each replicate. (Dead larvae cannot be induced to move when they are probed with a needle in the siphon or the cervical region. Moribund larvae are those incapable of rising to the surface within a reasonable period of time. They may also show discoloration, unnatural position, tremors, lack of coordination or rigors).

5.19 Identify all larvae used for the test and control using standard *Anopheles* Larvae Identification keys.

5.20 Repeat the test until 100 larvae of same species is tested for a particular insecticide.

5.21 Calculate the corrected mortality. Percentage mortality after the 24-hour recovery period should be recorded.

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

A similar calculation should be made in order to obtain a value for the control mortality.

- If the control mortality is above 20%, the tests must be discarded. If the control mortality is greater than 5% and less than 20%, the mortality observed should be corrected by applying Abbott's formula as follows:

$$\frac{\text{\% test mortality - \% control mortality}}{\text{100 - \% control mortality}} \times 100$$

5.22 Draw the mortality –regression line in the log - probit sheet using corrected percentage mortalities with the respective concentrations. The concentration that gives 50% mortality is known LC50: that gives 95% mortality is LC95. The curve can be extended to estimate the LC99.9.

5.23 When 4 replicates at each concentration have been performed with the same population of mosquito larvae, adequate data should be available for constructingbase- line susceptibility.

5.24 Take the LC 99.9 and double the dosage that gives LC 99.9 and use that concentration for routine insecticide susceptibility monitoring for mosquito larvae.

Procedure Notes:-

1. Initially the mosquito larvae should be exposed to a wide range of test concentration bydiluting the standard solutions to find out the active range of insecticide. After determining mortality of larvae in wide range of concentrations, yielding between 10%-95% mortality can be used to determine LC50 and LC90 values.
2. It should be noted that distilled water obtained commercially may contain traces of poisonous heavy metals and this will give high mortalities in the controls. Certain species

such as salt marsh or tree hole mosquitoes may suffer upon transfer to relatively pure water, an effect that will also be reflected in high control mortalities; in this case water from the breeding site should be used provided that it is free from insecticides and filtered to exclude most of the organic matter.

Ani Malaria Campaign **SOP for Entomological Surveillance**					
SOP title:	**SOP for Bioassay Test to Determine the Residual Effect of Insecticides on different types of resting Surfaces**				
SOP No.	**12**	**Revision No.**		**Effective Date**	**01.08.2017**
Replacement no.		**Dated**		**Page no.**	
Prepared by:	**Anti Malaria Campaign**			**Date**	**13.07.2016**
Approved:	Director, Anti Malaria Campaign			**Date**	**20.07.2016**

1.0 Purpose:

The main objective of the bioassay is to assess the potency of an insecticide deposit against adult mosquitoes with proven susceptibility, at various times after application on different surfaces. The test provides information on the decline of toxic effect of the insecticide on the surface and thereby to decide the appropriate insecticide dosage and spacing of spraying cycles for effective vector control. Results of bioassays can be used to assess the quality of spraying operations.

2.0 Scope

This procedure applies to all entomological teams engaged in malaria entomological surveillance.

3.0 Responsibility

It is the responsibility of the Entomological Officer of Health to carry out the SOP as written.

4.0 Equipment and Materials

4.1 Twenty four (24) conical chambers of transparent plastic, 8.5cm in diameter at the base and 5.5cm high.

4.2 Ten (10) hard glass aspirator tubes, 1cm in outside diameter with one end bent to facilitate reaching all parts of the exposure chamber, together with 60cm of flexible rubber or plastic tubing.

4.3 Ten (10) straight glass aspirators.

4.4 Rolls of thick and thin plastic adhesive sponge tape

4.5 One box of pins

Selection criteria for test mosquitoes

- Preferably endophillic vector species (*Anopheles culicifacies* and *Anopheles subpictus*) should be used. Known susceptible species should be used (SOP no. 09) in the absence of above species any other susceptible *Anopheles* species should be used.

Selection of test specimens

- Blood fed adult females

Selection of houses for the bio assay tests on different indoor resting surfaces

- It is necessary to test and to evaluate separately the potency of the insecticide deposit on each main type of resting surface. Therefore select houses which represent different types of indoor resting surfaces in the locality.
- In selection of resting surfaces priority should be given to the major types of indoor resting surfaces available in the selected locality.
- At least 10 points, variously situated should be chosen for testing on a given day.
- They should be distributed in several houses, with not more than 3 points in any one house.
- Points for testing on a selected surface should be on three locations such as upper, middle and lower and this should be replicated in at least four houses in a locality.

  At least 100 for test and 02 controls with 20 for the control.

5.0 Test Procedure

5.1 Select the spot of wall surface to which the exposure chamber is fastened. Four exposure chambers should be fastened at the top (70 cm below the roof), 4 exposure chambers in the middle, and 4 chambers in the lower part of wall (60–70 cm over the floor surface).

5.2 The exposure chamber could be fastened with an appropriate device to hold it tight against the surface.

5.3 Female mosquitoes of known age and susceptibility should be taken for the bioassay tests. Release 10 mosquitoes with a straight aspirator into each chamber by blowing gently. Care should be taken to ensure that the end of the tube does not touch the test surface.

5.4 The cage of stock mosquitoes to be used in bioassays should never be taken inside a house that has been sprayed with insecticide. It should be kept on an insecticide-free surface outside the house. The cages should be handled with care to avoid contaminating them with insecticide from the hands of operators.

5.5 Leave the chambers undisturbed for 30 minutes.

5.6 At the end of exposure period, the mosquitoes are collected carefully by means of a bent aspirator.

5.7 Transfer the mosquitoes immediately into the holding container. Paper or plastic cups can be used as holding containers.

5.8 Record the number of knocked down mosquitoes if a pyrethroid insecticide is used for IRS.

5.9 Room temperature and relative humidity (RH) are recorded at the beginning and end of tests.

5.10 Keep the holding containers for 24 hours in a secluded and shaded place, where the temperature does not exceed 30 °C. Maximum and minimum temperatures during the recovery period should be recorded. The humidity should be kept high by the use of damp toweling where necessary.

5.11 The same procedures should be carried out for the controls. Chambers are fastened in a room similar to that used for the test cones, but with no insecticidal application. Controls must be run at a rate of at least 2 controls for every 10 bioassay tests.

Results and Interpretation

- After 24 hours, the dead and live mosquitoes are counted and recorded.
- It is essential that observed mortality is recorded for each individual test. If the control mortality is above 10%, it is recommended that the number of controls in the subsequent series of the tests should be increased to 4 for each series of 10 tests. Where control mortalities exceed 20%, the series of tests should be considered unsatisfactory and repeated.
- The mortality at different points (on one type of surface only) is averaged.
- Where the control mortality is between 5-20% the average observed mortality is corrected by Abbott's formula:

$$\frac{\%\ \text{test mortality} - \%\ \text{control mortality}}{100 - \%\ \text{control mortality}} \times 100$$

- Average mortality should be calculated for each part of the wall.

<u>Procedure notes</u>

1. The bio assay tests should be carried out before 10.00 a.m.
2. After the end of each bio assay test bio assay cones should be washed properly with water. Do not use any soap or detergents.

Anti Malaria Campaign **SOP for Entomological Surveillance**					
SOP title:	**SOP for Bioassay Test to Determine the Residual Efficacy of Insecticides on Long Lasting Insecticidal Nets**				
SOP No.	**13**	**Revision No.**		**Effective Date**	**01.08.2016**
Replacement no.		**Dated**		**Page no.**	
Prepared by:	**Anti Malaria Campaign**			**Date**	**13.07.2016**
Approved by:	**Director, Anti Malaria Campaign**			**Date**	**20.07.2016**

1.0 Purpose

The objective of bioassay is to assess the potency of an insecticide deposit on LLINs to adult mosquitoes with proven susceptibility after number of washes of insecticide treated net/various time intervals. There are three different methods for the biological efficacy of pyrethroids on LLINs and the test done using WHO cones is described below.

2.0 Scope

This procedure applies to all entomological teams engaged in malaria entomological surveillance.

3.0 Responsibility

It is the responsibility of the Entomological Officer of Health to carry out the SOP as written.

4.0 Equipment and Materials

4.1 Standard WHO bio assay cones of transparent plastic, 8.5 cm in diameter at the base and 5.5 cm high.

4.2 glass (or plastic) aspirator tubes of 12 mm internal diameter, together with 60 cm of tubing, and mouthpiece

4.3 ole of self-adhesive plastic tape or one sheet of label tag

4.4 Rubber bands

4.5 Paper cups

4.6. Stop watch

4.7. Digital hygrometer

4.8. Netting

4.9. Cotton wool

4.10. Normal mosquito net without insecticide treatment

4.11. Hand lenses

4.12 . AMC /11 form

5.0 Procedure

5.1 Randomly select five LLIN nets in a locality.

5.2 Wash the net as per the provided guidelines provided for the particular net and keep for 24 hours.

5.3 Start the testing procedure early in the morning preferably before 10.00 a.m.

5.4 Four (4) cones should be gently fitted on to the LLIN. Cones should be fitted at top, upper, middle and lower part of the net.

5.5 Use susceptible 1–3 day old, non-blood fed female *Anopheles* mosquitoes per assay.

5.6 Introduce five (5) mosquitoes in to each cone the time interval between each "4 cone" set should be as brief as possible.

5.7 Expose the mosquitoes to the LLIN for 3 minutes.

5.8 After exposure, transfer mosquitoes into paper cups. Provide a sucrose solution added on a cotton plug and maintain in a humid condition with the use of damp towels for 24hrs.

5.9 Mosquitoes from the first 4 cones tested are grouped in separate four paper cups. The same procedure should be followed for the second net and similar procedure should be carried out for 5 nets per locality.

5.10 The knock-down (KD) is recorded at 60 minutes following exposure, starting once the 4th cone (in each set) has been transferred to the cup, and ending when about

80% of mosquitoes are KD. Observation is stopped after 60 minutes even if 80% KD has not occurred.

5.11 Knock down (KD) rate at 60 minutes post-exposure and mortality rate after 24 hours should be recorded.

5.12 Simultaneously carry out a two control tests per one LLIN using plain nets.

Interpretation of the test results

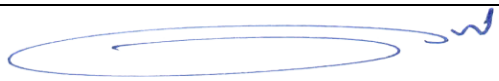
Percentage mortality should be recorded after the 24-hour recovery period. If control mortalities exceed 20%, the results should be recorded and test repeated. If the control mortality is in the range 5–20%, the average observed mortality should be corrected by Abbott's formula as follows:

$$\frac{\%\ \text{test mortality} - \%\ \text{control mortality}}{100 - \%\ \text{control mortality}} \times 100$$

Procedure notes:

1. Test conditions

   Tests should be carried out ideally at 25 ± 2 °C and 70–80% relative humidity (RH); tests should never be done at temperatures greater than 30 °C.
2. The LLINs used for testing should be washed at every three month interval according to the guidelines provided for the usage of particular LLIN type (washing should be done in front of the Entomological team and the date of washing should be recorded).
3. Bio assay should be repeated in three month intervals.
4. Testing should be carried out at least for five nets at a location.

Anti Malaria Campaign **SOP for Entomological Surveillance**					
SOP title:	**SOP for Transportation of Live Adult Mosquitoes**				
SOP No.	**14**	**Revision No.**		**Effective Date**	**01.08.2016**
Replacement no.		**Dated**		**Page no.**	
Prepared by:	**Anti Malaria Campaign**			**Date**	**13.07.2016**
Approved by:	 **Director, Anti Malaria Campaign**			**Date**	**20.07.2016**

1.0 Purpose

Samples of live mosquitoes are to be transported to and from field to the testing site.

2.0 Scope

This procedure applies to all entomological teams who are engaged in malaria entomological surveillance

3. 0 Responsibility

It is the responsibility of the Entomological Officer of Health as the team leader to ensure that the entomological team members carry out the SOP as written.

4.0 Equipments and Materials

4.1 A Plastic basket to be used as a carrier with a sufficient size/capacity to hold the required number of paper cups with mosquitoes.

4.2 Paper cups with net cover

4.3 A rack made of light weight material

4.4 Clean towel

4.5 Rubber bands

4.6 Distilled water

4.7 Thermometer and hygrometer

4.8 Labels

4.9 Packing materials

5.0 Procedure

5.1 The Plastic basket must be new or clean and free from any sources of contamination at all times. It is good practice to use different boxes for transporting mosquitoes to the field and from the field to the testing site.

5.2 Place the rack in the plastic basket.

5.3 Introduce the paper cups containing live mosquitoes into the racks or in the packing materials in an upright position.

5.4 Provide mosquitoes with 10% sugar solution or 10% glucose for unfed mosquitoes.

5.5 Once all cups of mosquitoes have been placed into the plastic basket, cover the basket with its lid.

5.6 Once all cups of mosquitoes have been placed in the racks, packing material should be placed between the cups to minimize movement.

5.7 Cover the cups with the damp towel and keep the towel damp until the mosquitoes reach the field station/laboratory.

5.8 Avoid shaking and keep the plastic basket upright during transportation to/from the field.

Procedure notes

1. Prepare 10% sugar solution taking 5g of sugar into a small vial and mixing with 45ml of distilled water.

Anti Malaria Campaign
SOP for Entomological Surveillance

SOP title:	**SOP for Transportation of Live mosquito larvae**				
SOP No.	**15**	**Revision No.**		**Effective Date**	**01.08.2017**
Replacement no.		**Dated**		**Page no.**	
Prepared by:	**Anti Malaria Campaign**			**Date**	**13.07.2016**
Approved by:	**Director, Anti Malaria Campaign**			**Date**	**20.07.2016**

1. Purpose

Samples of live mosquitoes are to be transported to and from field to the testing site.

2. Scope

This procedure applies to all entomological teams who are engaged in malaria entomological surveillance

3. Responsibility

It is the responsibility of the Entomological Officer of Health as the team leader to ensure that entomological team members carry out the SOP as written.

4. Equipment and materials

4.1 Larval vials

4.2 Plastic basket or cool box

4.3 Large vacuum flask

5. Procedure

5.1 Place all the larvae captured from a particular breeding site in one vial and label it.

5.2 Cap each vial very tightly preventing spill of water from it.

5.3 Make sure that there is air space in the top 1-2cm so that the larvae and pupae can breathe for few hours.

5.4 If the journey to the laboratory takes more than 2-3 hours, remove the stoppers every 2 hours to provide the specimens with fresh air.

5.5 Pack the bottles in the basket/cool box very carefully.

5.6 If the larvae are to be used in insecticide susceptibility tests they should be transported in water in a large vacuum flask or other large container.

Anti Malaria Campaign **SOP for Entomological Surveillance**					
SOP title:	**SOP for Ovary dissection for parity determination**				
SOP No.	**16**	**Revision No.**		**Effective Date**	**01.08.2016**
Replacement no.		**Dated**		**Page no.**	
Prepared by:	**Anti Malaria Campaign**			**Date**	**13.07.2016**
Approved by:	**Director, Anti Malaria Campaign**			**Date**	**20.07.2016**

1.0 Purpose

To determine the proportion of parous mosquitoes in a collected sample to assess the average daily survival rates of the man biting mosquitoes. The assessment of average daily survival rate is essential to assess vectorial capacity (receptivity) of the area of concern and assessment of the impact of vector control interventions.

2.0 Scope

This procedure applies to all entomological teams who are engaged in malaria entomological surveillance.

3.0 Responsibility

It is the responsibility of the Entomological Assistant as the team leader to ensure to carry out this technique as per stated in the SOP.

4.0 Equipment and materials

4.1 Dissecting microscope

4.2 Compound microscope

4.3 Dissecting needles

4.4 Fine forceps

4.5 Glass slides

4.6 Pipettes

4.7 Distilled Water

4.8 Ether/Chloroform

4.9 Normal Saline

4.10 Sample mosquitoes to be dissected

4.11 Gloves

4.12 Mask

4.13 H/AMC/E6 form

5.0 Procedure

5.1 Collect female *Anopheles* vector mosquitoes during Human Landing Night Collection technique (SOP No.7) to perform this technique.

5.2 Transport collected mosquitoes according to the SOP No. 14 to the field station/laboratory within three hours (ideally perform the dissection at the premises used for Human Landing Night Collection).

5.3 Kill the female mosquito with Ether/Chloroform.

5.4 Remove legs and wings using fine forcep and a needle.

5.5 Place the mosquito on a glass slide, add a drop of distilled water and examine under a dissecting microscope (magnification).

5.6 Cut the abdomen on each side of the body between the sixth and the seventh segment.

5.7 Holding the one needle on the thorax pull the tip of the abdomen away from the rest of the body with another needle held in the right hand.

5.8 Cut through the common oviduct and separate the ovaries from the rest of the specimen.

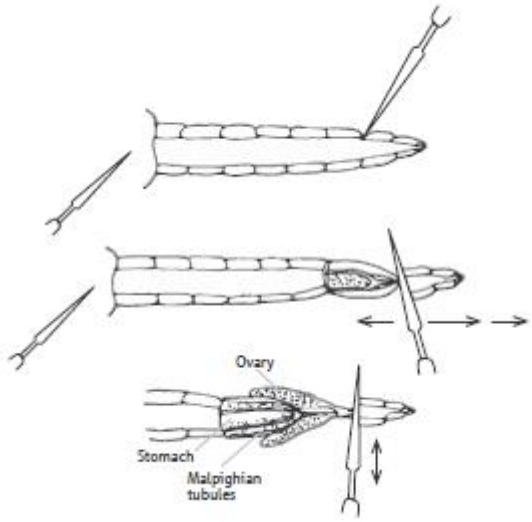


Figure 9. Dissection of ovaries

5.9 Remove all the other particles except the ovaries and allow the ovaries to dry. Examine the dried ovaries under a compound microscope using 10Xobjective and if necessary confirm using 40Xobjective.

5.10 Interpretation

- Females in which the ovaries have coiled tracheoles skeins are nulliparous (Figure a).
- Ovaries in which the tracheoles have become stretched out are parous (Figure b).

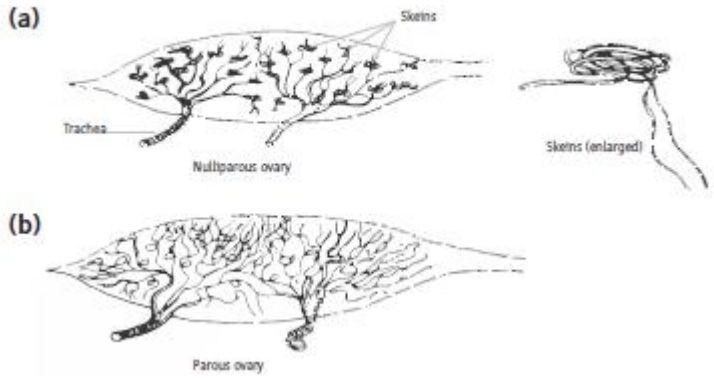


Figure 10. Appearance of a) nulliparous ovaries b) parous ovaries

(c)

Figure 11. Appearance of freshly dissected ovaries

- The proportion of parous females and parous rate can be calculated as follows
- The proportion of parous females = $\frac{\text{No. of parous females}}{\text{Total No. of females examined}}$
- Parous rate = $\frac{\text{No. of parous females x 100}}{\text{Total No. of females examined}}$

Procedure notes:

1 The body parts of the dissected mosquito can be used for other studies (identification of sibling species and salivary gland dissections).

Anti Malaria Campaign **SOP for Entomological Surveillance**					
SOP title:	**SOP for Salivary gland dissection for sporozoites**				
SOP No.	**17**	**Revision No.**		**Effective Date**	**01.08.2016**
Replacement no.		**Dated**		**Page no.**	
Prepared by:	**Anti Malaria Campaign**			**Date**	**13.07.2016**
Approved by:	**Director, Anti Malaria Campaign**			**Date**	**20.07.2016**

1.0 Purpose

To determine

- mosquito species carrying the malaria parasite
- sporozoite rates

2.0 Scope

This procedure applies to all entomological teams engaged in malaria entomological surveillance.

3.0 Responsibility

It is the responsibility of the Entomological Officer of Health to carry out the SOP as written.

4.0 Equipment and materials

4.1 Stereo microscope

4.2 Compound microscope

4.3 Dissecting needles

4.4 Fine forceps

4.5 Glass slides

4.6 Pipettes

4.7 Distilled Water

4.8 Ether/Chloroform

4.9 Sample mosquitoes to be dissected

4.10 Normal Saline

4.11 Gloves

4.12 Masks

4.13 H/AMC/ E6 forms

5.0 Procedure

5.1 Collect female *Anopheles* vector mosquitoes during HLNC technique (SOP No. 7).

5.2 Transport collected mosquitoes according to the SOP No. 14 to the field station/laboratory within three hours (ideally perform the dissection at the premises used for Human Landing Catches).

5.3 Wearing gloves and mask kill the female mosquito with Ether/Chloroform. Keep the mosquitoes in a paper cup with netting and put a piece of cotton wool soaked with Ether/Chloroform on the netting and cover with a glass lid.

5.4 Keep for 2-3 minutes (allowing to die).

5.5 Remove a mosquito using a forcep onto a glass slide under a dissecting microscope.

5.6 Remove legs and wings using a dissecting needle and place the mosquito to another slide. Add a drop of saline solution to the slide and place the mosquito on the saline drop, mosquito on its side on the slide with the head pointing to the right.

5.7 Take 2 dissecting needles, hold the thorax firmly with the blunt end of dissecting needle in left hand (if the performer is a left handed person this should be done with the right hand).

5.8 Place the other needle (held in right hand) in between the neck and the thorax of the mosquito but do not cut the neck.

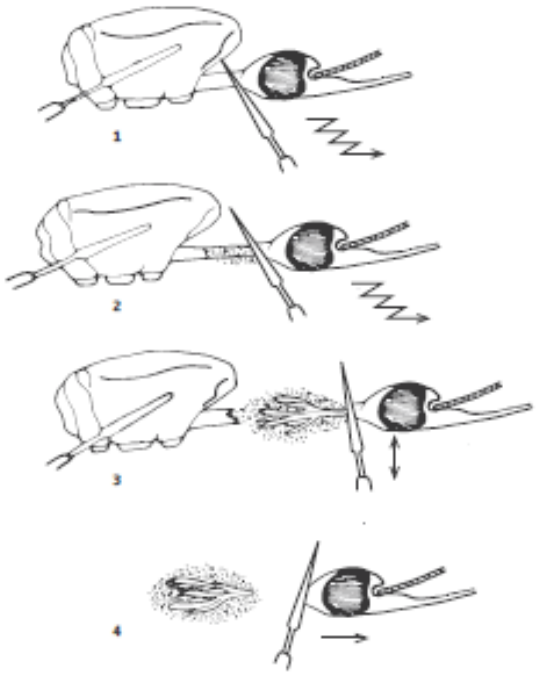


Figure 11. Dissection of the female mosquito for sporozoites

5.9 Gently pull the head away from the thorax, so as to get the salivary glands out of the thorax, attached to the head.

5.10 If the glands do not come out with the head, they should be obtained by gently squeezing the thorax.

5.11 Separate the glands with the other needle, and place into the drop of saline solution.

5.12 Remove the body of the mosquito.

5.13 Cover the salivary gland with a cover slip.

5.14 If the glands have not been crushed by the cover slip, gently press the cover slip with a dissecting needle so that the glands break and sporozoites are released.

5.15 Examine the glands under a high power x40 objective so that the unstained sporozoites can be seen moving. Reduce the illumination either by lowering the condenser or by partially closing the iris diaphragm to get better contrast for an easier detection of sporozoites.

5.16 Calculate the sporozoite rate as follows.

Sporozoite rate:

$$\frac{\text{Number of mosquitoes with sporozoites}}{\text{Number of mosquitoes dissected}} \times 100$$

References

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