

# **Guidelines for entomological surveillance of malaria vectors in Sri Lanka**

**Anti Malaria Campaign**

## 2009

### List of contributors

Dr.R.R.Abeyasingha, Consultant Community Physician, Anti Malaria Campaign

Dr. A.M. Yapabanadara, Regional Malaria Officer, Matale

Dr. P.H.D. Kusumawathie, Regional Malaria Officer, Kandy

Dr. D. Perera, Regional Malaria Officer, Kurunegala

Ms. B.S.L. Peiris, Regional Malaria Officer, Hambanthota

Ms. H.M.P. Hewavitharane, Entomologist, Anti Malaria Campaign

Ms. R.D.J. Harishchandra, Entomologist, Anti Malaria Campaign

## CONTENTS

1.0 Guidelines for Entomological surveillance	3
2.0 Entomological Field techniques	7
2.1 Hand collection of indoor resting mosquitoes	8
2.2 Pyrethrum Spray Sheet Collection	12
2.3 Outdoor Collection of mosquitoes	17
2.4 Cattle Baited Trap Collection	21
2.5 Cattle Baited Hut Collection	23
2.6 Window Trap Collection (Exit traps)	26
2.7 Human Landing Catches	29
2.7.1 Direct Landing Catches of Mosquitoes from Human Bait	29
2.7.2 Landing Catches of Mosquitoes from Human Baited Trap Nets - Double Trap Method	32
2.8 Larval Survey	37
2.9 Insecticide Susceptibility Test for adult mosquitoes	41
2.10 Insecticide Susceptibility Test for mosquito larvae	47
2.11 Bio Assay test for Insecticide deposits on wall surfaces	49
2.12 Bio Assay test Insecticide treated mosquito net surfaces	52
3.0 Entomological Laboratory techniques	58
3.1 Dissection of salivary glands for sporozoites	58
3.2 Ovary dissection for parity determination	60
4.0 References	63

## **1.0 Guidelines for Entomological surveillance**

Entomological investigations are important and essential aspect of malaria vector control, as these investigations provide information on vector species, their distribution, density, bionomics and susceptibility/ resistance to insecticides used for malaria control. In addition these investigations are useful for the monitoring of potential vectors and the role they could play in disease transmission. Information collected through entomological surveillance techniques assist in the understanding of the spatial and temporal changes in vector species, efficacy and effectiveness of vector control measures employed for malaria vector control.

Conducting of regular entomological surveillance on a wide scale is costly & labour intensive. Hence these investigations cannot be carried out in all locations where malaria transmission occurs but have to be limited to selected localities in each district/province. To increase the usefulness of data collected from surveillance and to maximize the use of available resources entomological investigations are carried out in

1. Sentinel sites
2. Selected localities where potential outbreaks are expected
3. During outbreaks or epidemics

### *Criteria for selection of sentinel sites*

Sentinel sites are identified at district level and are monitored at regular intervals to determine seasonal changes in vector densities and also more importantly to monitor changes in vector bionomics and characteristics.

A sentinel site should usually be an area where malaria transmission risk is present over a period of time or where increased potential for vector breeding is well established. High risk area may be a previously malaria risk area or an epidemic prone area.

Epidemic prone area could be an area;

- (I) subjected to frequent or seasonal out breaks/epidemics.

(11) where environmental changes have occurred due to human activity or as a result of changes in climate, resulting in increased vector breeding potential.

*Criteria for selection of areas for spot checks*

Spot checks need to be carried out in areas not covered by the sentinel surveys when

- (1) malaria cases are reported or an increase in malaria cases is reported.
- (11) environment changes in favour of vector breeding (e.g. climatic changes, disasters, development projects and human activities such as gem mining, timber felling, quarry pits etc.) is reported
- (111) migratory populations from malarious areas are present.

*Frequency of entomological surveys*

It is recommended that entomological surveys are carried out at least in two sentinel sites per month by the entomological teams attached to Regional Malaria Offices of the Anti Malaria Campaign. In addition depending on necessity a minimum of two spot checks in different localities within the district are recommended.

*Composition of an entomological team*

An entomological team consists of two Entomological Assistants, one Public Health Field Officer, five Spray Machine Operators or labourers and a driver and they are under direct supervision of Entomologist (AMC HQ)/ Regional Malaria Officer (AMC).

Work plans for sentinel site monitoring and spot checks are shown in table 1 and 2.

Table 1. Work plan for sentinel site monitoring

Time	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
4 -6		CBT/ CBH/WT Collection	CBT/ CBH/WT Collection	CBT/ CBH/W T Collecti on	Human Landing Catches (full night)	CBT/ CBH/WT Collection	CBT/ CBH/WT Collection
6-7	Identificati on of mosquitoes	Identificati on of mosquitoes	Identificatio n of mosquitoes		Identificati on of mosquitoes	Identificati on of mosquitoes	Identificati on of mosquitoes
7-8							
8-9		Susceptibili ty test	Bio assay for IRS and ITNS	Suscepti bility test		Susceptibili ty test	Outdoor Hand Collection
9-10	Departure	Larval Survey/ Indoor Hand Collection	Pyrethrum Spray sheet Collections	Larval Survey		Pyrethrum Spray sheet Collections	Identificati on of mosquitoes
10-11							Visit to RMO/MO H Office
11-12							
12-14							
14-15	Visit to RMO/MO H Office	Larval Identificati on	Identificatio n of mosquitoes, abdominal stages and Dissections	Larval Suscepti bility test		Identificati on of mosquitoes , abdominal stages and Dissections	Shift to next station/retu rn to office
15-16	Location survey						
16-17	Trap setting	Trap setting Preparation for HLC	Trap setting		Trap setting Preparation for HLC	Trap Setting** Preparation for HLC	
17-18				Human Landing Catches & Dissecti ons (full night)			
18-19		Human Landing Catches & Dissections (partial)				Human Landing Catches & Dissections (partial)	
19-20							
20-21							
21-22							

--	--	--	--	--	--	--	--

\*PSC - At least 20 houses should be checked during one day\*\*If the first 5 days were negative for CBTC trap setting on day 6 and collection on day 7 should be done

Table 2. Work Plan for spot checks

	Day 1	Day 2	Day 3	Day 4	Day 5
4-6		CBT/ CBH/ WT Collection	CBT/ CBH WT Collection	CBT/ CBH WT Collection	CBT/CBH WT Collection
6-7	Departure	Identification of mosquitoes	Identification of mosquitoes	Identification of mosquitoes	Identificatio n of mosquitoes
8-9		Susceptibility test	Susceptibility test	Bio assay for IRS and ITNS	Hand Collection
9-10		Larval Survey	Pyrethrum Spray sheet Collections	Pyrethrum Spray sheet Collections	Identificatio n of mosquitoes  Shift to next station/retur n to office
10-11					
11-12					
12-14					
14-15		Larval Identification	Identification of mosquitoes, abdominal stages and Dissections	Identification of mosquitoes, abdominal stages and Dissections	
15-16					
16-17	Location survey and trap setting	Trap setting Preparation for HLC	Trap setting	Trap setting Preparation for HLC	
17-18					
18-19		Human Landing Catches & Dissections (partial)		Human Landing Catches & Dissections (partial)	
19-20					
20-21					
21-22					

\*PSC - At least 20 houses should be checked during one day

## 2.0 Entomological Field techniques

The entomological field techniques that are generally used in malaria entomological investigations are listed below. During any survey the optimum combination of these techniques should be used based on local conditions prevalent to facilitate a proper collection of data necessary for appropriate decision making. The suggested minimum combination is shown in table 1 & 2 above, but may be changed depending on circumstances with approval of Entomologist/RMO.

1. Hand collection of indoor resting mosquitoes
2. Pyrethrum spray sheet collection
3. Outdoor collection of mosquitoes
4. Cattle baited net trap collection
5. Cattle baited hut collection
6. Window trap collection
7. Human landing catches
  - (1) partial night (18.00-22.00 hours)
  - (2) full night (18.00-06.00 hours)
8. Larval surveys
9. Insecticide susceptibility test  
(Adult & larvae)
10. Bio assay test for Insecticide deposits on wall surfaces
11. Bio Assay test for Insecticide treated mosquito net surfaces
12. CDC type Light trap collections

The methodologies of above techniques are described in the next part of this booklet. The data recording formats for all techniques are given at the end of the each method and the formats for summery of the survey are given from page 54 to 57.



## 2.1 Hand collection of indoor resting mosquitoes

### Objectives:

1. To determine the common resting places of adult mosquitoes
2. To determine distribution of mosquitoes on different types of surfaces
3. To determine indoor resting density
4. To determine seasonal changes in the density

Hand collecting can also provide live specimens for:

- Susceptibility and bioassay tests
- The production of eggs
- Observations on mortality among insects from insecticide treated houses and nets
- Taxonomic studies
- Laboratory studies on rates of infection and survival

### Essential equipment:

Mouth aspirator / Mechanical aspirator

A torch (with spare bulb and batteries)

Paper cups with net covers

Cotton wool

A pencil

A note book

A bag to put all equipment

For transport mosquitoes

Insulated picnic box or other suitable container

Sugar solution (5-8%)

Paper cups with net covers

Cotton wool

Towels

News papers to serve as packing material

### **Selection of houses**

- In any village, a minimum of 20 houses should normally be examined per day in order to provide a representative sample. The houses should be randomly selected and scattered throughout the village.
- It is often advantageous to select the poorly constructed and inadequately ventilated houses because they usually contain the largest numbers of mosquitoes. Houses on the fringe of a village or near known breeding sites will often yield more day-resting mosquitoes.

### **Collection of mosquitoes**

- Normally take place early in the morning at about 8: 00 hrs and should be done after obtaining consent of the occupants.
- Examine the bed room in which people slept the previous night. Try to select rooms with few external openings.

### **Use of mouth aspirators**

- With the aid of the torch, look for mosquitoes on walls, ceiling, the roof and under furniture.
- Search systematically starting from the door and move clockwise around the inside of the house or room.
- Look for mosquitoes on wall hangings and curtains, behind and under furniture and inside large pots and jars.
- While collecting observe the resting places normally chosen by mosquitoes. If you record in the note book what you see including the numbers of the mosquitoes on the upper, middle or lower parts of walls, you can determine the proportion resting in each location
- Collection can be carried out 15 minutes in each house per person

### **Labeling paper cups**

- During hand collecting transfer mosquitoes to the paper cups. Separate paper cups should be used for each house.
- The cup must be clearly labeled in pencil with at least the following essential information.
  - Location
  - Date and time of collection
  - Time spent on collection
  - House number or house holders name
  - Type of structure (house, animal shelter, stores etc.)
  - Parts of the surface (Upper, medium or lower part of wall)
  - Whether sprayed or with insecticide treated bed nets and if so when
  - Collector's name



## 2.2 Pyrethrum Spray Sheet Collection

### Objectives:

1. To determine indoor resting density
2. To determine their blood digestion/egg development stages
3. To determine seasonal changes in the density
4. To determine the proportion having sporozoites in their salivary glands
5. To determine the proportion that has fed on human blood

### Essential equipment:

White floor sheets (size 2m x 2m) can be made of cotton about 4 numbers

Hand lenses

Pyrethrum solution

Small Petri dishes

Paper cups with net covers

Forceps

A container preferably a picnic box

Cotton wool

Filter papers

A torch

Hand sprayer- the double action type with an air valve

Pyrethrum solution at a concentration of 0.2-0.3% in kerosene

### Selection of rooms for spray sheet collection

- It is often advantageous to select the poorly constructed and inadequately ventilated houses because they usually contain the largest numbers of mosquitoes. Houses on the fringe of a village or near known breeding sites will often yield more day-resting mosquitoes.
- Rooms selected should be those in which one or more persons slept the previous night
- Collections normally carried out in the morning after the occupants of houses have arisen. Permission is required from householders to make collection in their houses.
- It is normal for the work to be performed by a team of three or four people so that collection can be made in twenty rooms in each locality.

### Preparation of the room

- Remove all people, animals and birds in the room
- Remove or cover all food
- Remove all small items of furniture
- Cover all openings and eaves with cloth or netting
- Spread the white sheets to completely cover the floor and all flat surfaces of the remaining furniture. Sheets also should be spread under tables, beds and other places where mosquitoes may hide.
- Close all windows and doors.

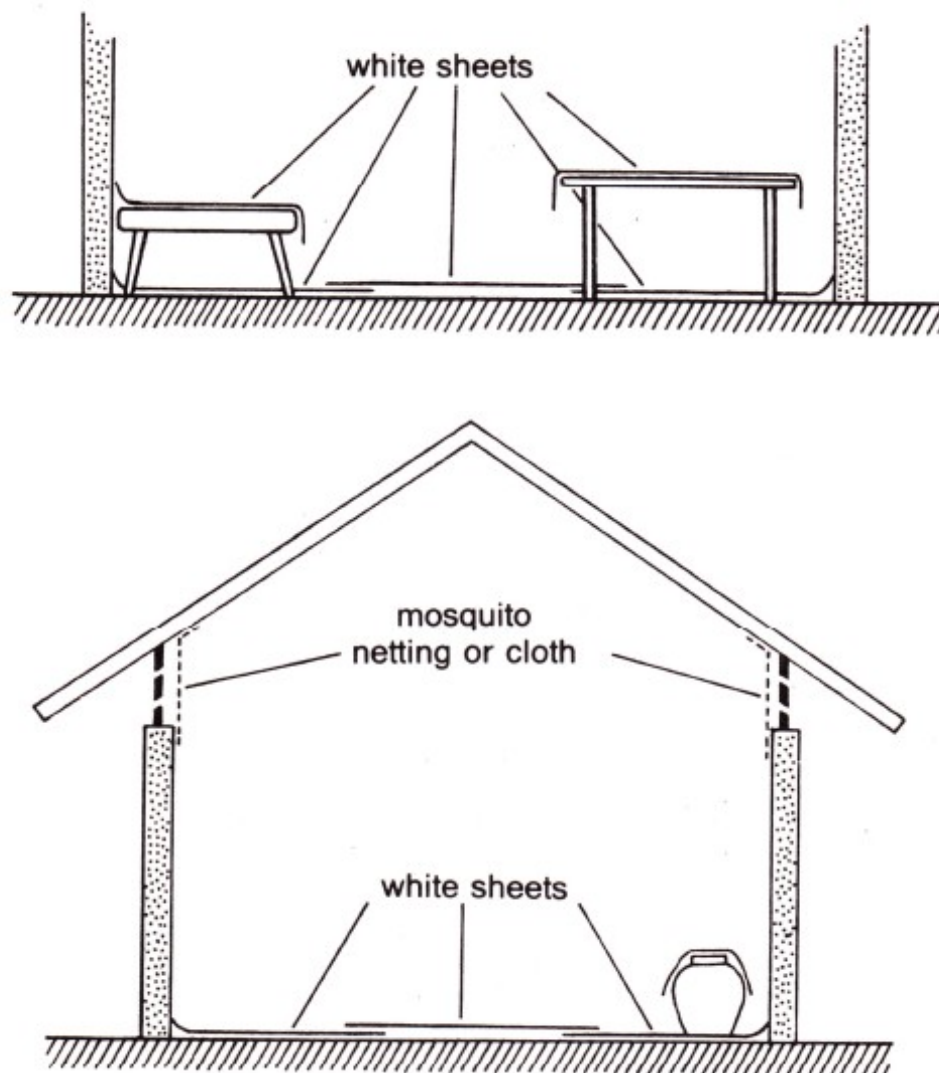


Fig. 1 Preparation of a room for PSC

### **Carrying out space spraying**

- One of the team members should walk around outside of the room and spray in open spaces or holes in the walls and eaves.
- Another member of the team should then enter the room close the door and move in a clockwise direction apply spray towards the ceiling until the room is filled with a fine mist.
- The operator should leave the room quickly and make sure that the door remains closed for at least 10 minutes.

### **Collection of mosquitoes from sheets**

- Open the room. Starting from the doorway, pick up the sheets one at a time by their corners. Carry the sheet outside. Collect and examine the mosquitoes outside in daylight.
- *If it is windy and rainy;* Open the room. Move gradually through the room starting from the doorways. With the aid of the torch, collect the mosquitoes from the sheets leaving the sheet in position.

### **Labeling containers, keeping the records and transporting them to the laboratory**

- A separate container should be used for mosquito collection in each room.
- The container should be labeled with all relevant data, including
  - Location
  - Date and time of collection
  - House number or householders name
  - Number of people and /or animals in the room during the previous night
  - Whether the room has been sprayed previously and if so the date of spraying
  - Availability of LLINs in the room, number & type.
  - Availability of plain nets or ITNs in the room
  - Use of mosquito repellants the previous night, specify what coil, mat, vapourizer etc.
  - Name of the collector/supervisor
  - This information must be written in pencil directly on paper cups or on piece of paper, which are placed inside the container.
- Keep a separate record in your note book or in a format can be used to keep the records

- However, if there is a delay before transport a wide mouthed vacuum flask holding ice cubs should be used to keep the containers cool so that the mosquitoes remain in a suitable condition for dissection. Insulated picnic box with ice packs may be used if one is available.





## 2.3 Outdoor Collection of mosquitoes

### Objectives:

To determine

1. The species that rest outdoors
2. The relative numbers of mosquitoes resting outdoors
3. Seasonal changes in outdoor resting habits
4. Any alteration in the relative numbers of mosquitoes resting outdoors following the application of insecticides in houses and other buildings

Some mosquito species enter houses at night to bite and rest indoors. Other species do not enter buildings but bite outside and then rest in the following kinds of outdoor locations;

- On vegetation
- On solid surfaces in sheltered places, such as the banks of the streams and ditches; culverts; cracks in stone walls; caves; animal burrows;
- On the trunks or stems of larger vegetation

Outdoor collecting is performed in either the natural resting places described above or in shelters specially constructed for this purpose.

### Essential equipment:

Mouth aspirators/ mechanical aspirators

A torch (with spare bulb and batteries)

A hand net

Paper cups with net covers

Cotton wool

An insulated picnic box

A towel

Newspapers for use as packing material

A pencil

A note book

### **Choice of method for outdoor collection**

- The choice of method for outdoor collecting depend on the behavior of the vector: that is whether it prefers to rest on vegetation or on solid surfaces.
- There are three methods used to collect mosquitoes resting on vegetation:
- Mouth/mechanical aspirators and torch in natural or artificial shelters
- A hand net
- Drop net

### **Collection of mosquitoes from natural places and artificial shelters using mouth/mechanical aspirators**

- Direct collection from vegetation using sucking tube usually takes a considerable time and may enable you to find only few mosquitoes.
- Collection of both natural and artificial shelters is made using mouth/ mechanical aspirators and torch.
- After searching in vegetation/ artificial shelters, the number of collections and the total time spent should be recorded (in man hours)

### **Collection of mosquitoes from experimental outdoor shelters using mouth/mechanical aspirators**

- If the anopheline vectors prefer to rest on solid surfaces outdoors it is preferable to build a shelter to attract them. This may consist of one or more large barrels, or boxes perhaps set into river banks or they may be pits dug in the ground. Collection from outdoor shelters is carried out using mouth aspirators (sucking tube) and torches.

### **Collecting mosquitoes using hand nets**

- A hand net is used to collect mosquitoes resting on vegetation.
- The correct method of use is to move the hand net swiftly over the top of tall grasses or close to the ground around the bushes
- Make sure that you record the number of collections and the total time spent collection



Fig. 2 Hand net

### **Labeling and recording collections**

- All paper cups must be labeled in pencil with following information
- Location
- Method of collection
- Date and time of collection
  - Whether the village was treated with insecticides or with insecticides treated bed nets
  - Collectors name
  - Keep separate record in your note book or specific forms (Annexure 3)

### **Calculation of indices**

- Number of mosquitoes collected per person – hour based on use of hand/mechanical aspirators or a hand net (man-hour density= No. of mosquitoes collected/duration (E.g. 15 minutes) X 60 minutes)
- Number of mosquitoes collected per artificial shelter (No. of mosquitoes collected/No. of artificial shelters searched)



## 2.4 Cattle Baited Trap Collection

### Objective:

To determine prevalence and abundance of outdoor biting and resting Anopheline vector species.

### Essential Equipment:

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 – see picture)

Aspirators

Torches

Paper cups with net covers

Cotton wool

One calf or cow



Fig. 3 A Cattle baited trap net

### **Steps in mosquito collection using cattle baited trap**

1. A suitable place should be selected within 200 m distance from the breeding site and between the breeding place and human habitations
2. Traps should be set by 1730 hours. When setting the trap keep 6 – 8” space between the net and the ground.
3. Introduce the calf/cow by 1800 hours
4. Leave the trap overnight
5. Start mosquito collections by 0500 hours by 3 persons (lower down the net immediately after entering in to the net)
6. Collect the mosquitoes trapped in the net using aspirators and torches into paper cups
7. Identify the species using standard keys

## 2.5 Cattle Baited Hut Collection

### Objective:

To determine prevalence and abundance of indoor biting and resting Anopheline vector species.

### Essential Equipment:

Cadjan Hut

(Size of the hut: **Length x width x height (center), corners = 6' x 4' x 6.5', 5'** - see picture)

Aspirators

Torches

Paper cups with net covers

Cotton wool

Cattle



Fig. 4 A Cattle baited hut



### **Steps in mosquito collection using cattle baited hut**

1. A suitable place should be selected within 200m distance from the breeding site and the hut should be constructed between the breeding place and human habitations
2. Set the hut trap by 1800 hours by introducing the calf/cow
3. Leave the hut overnight
4. Start mosquito collections by 0500 hours by 3 persons
5. Collect all the anopheline mosquitoes trapped in the hut using aspirators and torches into paper cups
6. Identify the species using standard keys



## 2.6 Window Trap Collection (Exit Traps)

### Objectives:

1. To determine mosquito species that bite indoors but rest outdoors
2. To determine the effect of Indoor Residual Spraying and Insecticide Treated Nets on the normal movement and feeding habits of mosquitoes
3. To determine the residual effects of insecticides as indicated by the numbers of dead mosquitoes collected and by the 24-hour mortality rate of mosquitoes found alive in the traps

### Essential Equipment:

A window trap

Mouth aspirators

Paper cups with net covers

A towel

An insulated picnic box

Dark cloth or netting to block openings in rooms

### Fitting Exit Traps

- Window (exit) traps are suitable for fitting only to rooms that are well sealed and that have few exit points for mosquitoes.
- If there are any openings other than the windows to which traps are fitting they must be covered or blocked with dark cloths.
- Normally a sleeping room should be selected and the trap must be fitted to a window. Parts of the window not covered by the trap should be covered with dark cloth or hardboard.
- The trap should be fitted in a manner with the collecting sleeve is pointed to outward.
- It is also important to fix traps in position well before sun set.

### **Collecting mosquitoes from an exit trap**

- Start collection next morning just after sunrise.
- Collect all mosquitoes through the sleeve of the trap, with the use of mouth aspirator.
- Use separate containers for the mosquitoes collected from each house and keep dead and live mosquitoes separately.
- When the traps are fitted in houses that have been sprayed with insecticides or in houses using ITNs, keep the mosquitoes caught live for 24 hours.

### **Labeling paper cups**

- The cup must be clearly labeled in pencil with at least the following essential information.
  - Location
  - Date
  - Exit trap number (house number or householder's name)
  - Time of collection
  - Whether mosquitoes were found dead or live in the trap
  - Whether and when the house was last treated with insecticides
  - Availability of LLINs in the house/room
  - Name of the collector



Fig. 5 A window trap (Exit)



## 2.7 Human Landing Catches

### Objectives:

1. To determine human biting anopheline mosquito species
2. To determine the biting frequency (how often a person is bitten by a vector) of the vector
3. To determine the biting time of the day of a particular mosquito
4. To determine the peak biting period of the vector mosquitoes
5. To determine whether the vector mosquito is indoor biting or outdoor biting
6. To determine the seasonal variations of vector mosquito biting

There are two methods of human landing collections

1. Direct landing catches of mosquitoes from bait
2. Collecting mosquitoes in human baited net trap (double trap method)

Female mosquitoes are attracted to humans and/or animals to obtain blood meals. Therefore **Man Biting / Landing Frequency** (no. of mosquitoes biting humans) is a major determinant of malaria transmission.

### 2.7.1 Direct Landing Catches of Mosquitoes from Human Bait

#### Essential Equipment:

- A sucking tube (Aspirator)
- A test tube
- A torch (with spare bulb & batteries)
- Paper cups with net covers
- Cotton wool
- A plastic basin
- A towel

#### General Rules

1. Collections should not be carried out during fast winds (when wind speed is more than 10-15km/hour)
2. Collectors should not smoke cigarettes or drink alcohol while collecting

3. Team members used as human baits indoor and outdoor should be changed hourly, to minimize possible differences in their attractiveness to mosquitoes
4. Mosquito repellent substances should not be used during the work
5. Smokes should not be in the area

### **Collection of Mosquitoes on Human Baits**

- The suitable locations for the night collections should be selected. It should be closer to the vector breeding sites in the area. If there are malaria cases in the village, better to select the house with a higher number of cases.
- Direct collection of biting mosquitoes should be performed during the night as malaria vectors are active and take blood meal in the night.
- In a full night programme, hourly collections should be made during the entire period from 18.00 to 06.00 hours (dusk to dawn). As this is a very laborious activity two teams of collectors should be used each team working half of the night.
- If it is a partial night collection hourly collections should be made during the peak biting period, from 18.00 to 22.00.
- Both indoor and outdoor collections should be done to accommodate the normal resting and sleeping habits of the local people.

### **Indoor Collections**

- A house with more than one room should be preferred, allowing the inmates to stay in a one room while another is used by collectors.
- If possible the room used for collecting should be one in which the inmates usually sleep. Baits should be placed in one or two rooms.
- Collections should not be made in an open verandah, as it is considered neither inside nor outside.

## **Outdoor Collections**

- Team members selected for outdoor collection should be positioned in the outer space of the house or room selected for indoor collections.
- It is better if the space normally used by people to sit outdoors during the evening can be selected. Human baits should be positioned at least 5 meters apart from each other.

## **Collection of Landing Mosquitoes**

1. The collections of landing mosquitoes from collectors own body is the most common way of getting biting mosquitoes.
  - Collectors should be seated on chairs quietly with their legs exposed up to the knee. Lower arms should also be exposed
  - Mosquitoes attempting to feed on the collectors should be caught using the transparent glass sucking tubes and flash torches. Then they should be transferred to the relevant paper cup with a net cover. There should be separate paper cups for each test hour. The paper cups are properly labeled.
  - The information to be recorded on the cup are as follows
    - Location
    - Date
    - Whether and when the location was last sprayed
    - Site of collection (indoor or outdoor)
    - Hour of collection
    - Collectors name
2. Alternatively one person can serve as the bait while another one is collecting mosquitoes.
  - The person acting as the bait sit on a chair quietly, inside or outside the house as appropriate, adjusting clothing to expose as much as possible of the skin.
  - Using a torch and a sucking tube, the collector should check and collect landing anophelines
  - Collected mosquitoes should be transferred to the relevant paper cup



### **Transportation to the Laboratory**

Paper cups with mosquitoes should be placed in a plastic basin and covered with a wet towel. Then carefully transferred to the field laboratory where the mosquitoes are identified. Necessary dissections must be made.

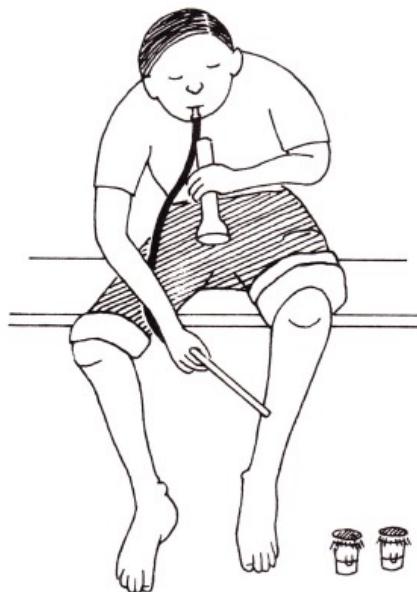


Fig. 6 Collecting mosquitoes from your own body

### **2.7.2 Landing Catches of Mosquitoes from Human Baited Trap Nets - Double Trap Method**

#### **Essential Equipment:**

- A sucking tube (Aspirator)
- A test tube
- A torch (with spare bulb & batteries)
- Paper Cups with Net Covers
- A Folding Bed
- Single Square Net
- Large Outer Net
- Five Poles / Sticks
- Cotton Wool
- A plastic basin

A towel

In the past it was considered acceptable to allow mosquitoes to bite a person engaged in a night collection. However for ethical reasons now health workers are not exposed to be bitten by mosquitoes. In fact it is not necessary to permit mosquitoes to bite the human bait. They can be collected as soon as they settle on the skin, as it can be assumed that biting would normally follow. Therefore ***Landing Rates*** are measured instead of ***Biting Rates***.

As mosquito repellants cannot be used while engage in biting collections, it is unavoidable to be bitten by mosquitoes. Therefore in epidemic situations and transmission seasons people serving as baits may easily contract malaria. To avoid this in such instances ***Human Baited Trap Net Collections*** can be conducted. Because of the protective inner net the risk to collectors can be minimized.

### **Collection Sites**

Two trap nets should be set up in positions selected, one (a sleeping room) inside the house and the other one outdoors at a site where people usually sit during the evening.

### **Collection of Mosquitoes in a Human -Baited Trap Net**

1. Folding camp beds should be set up to allow the human bait to lie on the bed.
2. A square inner net should be put up around the camp bed.
3. An outer net should be erected by tying it securely to poles or branches of trees.
4. The bottom of the outer net should be stretched and tightly tied to pegs in the ground.
5. A space of 15-25 cm height should be left between the ground and the lower edge of the outer net allowing mosquitoes to go inside to reach the bait.
6. Bait should be sent in to the inner trap and he should be lying down Mosquitoes that enter the outer net will be trapped and rest on the inner surface of the outer trap or outer surface of the inner net.
7. Alarm clock should be set to ring after one hour and hourly collections are made.
8. Hourly collected mosquitoes must be transferred in to separate cups which are labeled as above mentioned.
9. The collecting period should not exceed ten minutes.

The same procedure should be repeated throughout the night if it is a full night collection and during the peak biting period if it is a partial night collection.

### **Transportation to the Laboratory**

Paper cups with mosquitoes should be placed in a plastic basin and covered with a wet towel. Then carefully transferred to the field laboratory where the mosquitoes will be identified. Necessary dissections should be made.





## 2.8 Larval survey

### Objectives:

1. To establish the breeding habits of different species
2. To establish the geographical distribution of the vectors
3. To establish the active breeding sites
4. To evaluate the impact of anti larval measures on the larval density

### Larval collection methods

1. Dipping
2. Netting
3. Pipetting
4. Siphoning

#### 2.8.1 Dipping

The dipping method is most frequently used for the collection of anopheline mosquito larvae.

#### Essential equipment:

Ladle  
Enamel bowl  
Pipette  
Larval vials

#### Steps in dipping

1. Estimate the active breeding surface area of the breeding site.
2. Dip at the rate of 10 dips per spot of active anopheline breeding
3. During dipping, avoid shadowing (eg. dipping person, ladle etc)
4. Immerse the ladle in the breeding place (eg. River bed pools, river margins, streams, edge of swamps, rice fields, gem pits etc) at an angle of 45°.
5. Let the ladle to fill  $\frac{3}{4}$  of water (with larvae, if any) and withdraw quickly (If the dipper is immersed too slowly, the larvae get disturbed and escape).

6. Keep 2 - 3 minutes intervals between two dips to allow larvae to come to the surface again.
7. If the surface water is covered with dense floating vegetation or organic debris, the water surface should be agitated to cause the larvae to sink, clear away the vegetation and then wait for 3-5 minutes for larvae to come to the surface and dip.
8. Count the 1<sup>st</sup> and 2<sup>nd</sup> stage, 3<sup>rd</sup> and 4<sup>th</sup> stage larvae and pupae in each dip and record in the appropriate forms.
9. Collect the larvae and pupae of each habitat in separate labeled vials
10. Identify the larvae at 3<sup>rd</sup> and 4<sup>th</sup> stages using standard identification guides. Allow the 1<sup>st</sup> and 2<sup>nd</sup> stage larvae to develop to 3<sup>rd</sup> and 4<sup>th</sup> stages and then identify. In the case of pupae, allow them to emerge to adults and then identify.
11. Calculate the larval density per 100 dips for each species for each larval breeding habitat separately.

### **2.8.2 Netting**

Larvae may be collected from large stretches of water along the edge of rivers, streams, ponds, wells, and other large water bodies by netting.

#### **Essential equipments:**

Larval net

Enamel bowl

Pipette

Larval vials

#### **Steps in netting**

1. A larval net consists of a ring of iron frame (diameter 20 – 25cm) to which a nylon/ muslin cloth net (10cm long) is attached. A long wooden handle is attached to the ring.
2. For collecting larvae, the net should be held at an angle of 30° and skimmed rapidly through the surface water near emerging or floating vegetation.
3. The net should be inverted and washed out in a bowl of water
4. The larvae and pupae must be collected with a pipette
5. Larvae and pupae are identified should be recorded as in dipping
6. The larval density should be given as larvae per larval net for each species for each breeding habitat.

In the collection of larvae from wells, pond net (well net) can be used. The usual pond net is devoid of handle and provided with nylon netting attached to four points on the iron ring at equal distances.

### **Preparation and collecting larvae using a well net**

Join the four pieces of string in such a way that the ring forms an angle of  $30^{\circ}$  and attach this to a rope tied with it.

### **Steps in collection of larvae**

1. While collecting larvae from a well, put a small weight in the net to keep its bottom under the water surface.
2. Move the net around the border of the well two or three times and then withdraw
3. Invert the net in a white enamel bowl containing water.
4. Collect the larvae by a pipette.
5. Identify the larvae and make reports as previously
6. The density is given as larvae per well net for each species.

### **2.8.3 Pipetting**

#### **Essential equipments:**

Small pipettes

Larval vials

Small pipettes can be used for collection of larvae from tree holes or other small breeding sites

### **2.8.4 Siphoning**

Siphons can also be used for collection of larvae from tree holes





## 2.9 Insecticide Susceptibility test for Adult Mosquitoes

### Objectives:

The purpose of the susceptibility test is to detect the emergence of resistant individuals in a mosquito population as early as possible before it is widely established. For this purpose it is necessary;

- a) To establish the base-line susceptibility of a normal populations of mosquitoes
- b) To expose mosquito populations to discriminative dosage of insecticide at periodic intervals to detect any tolerant individuals and to monitor any changes in their susceptibility levels

#### a) Establishing the base-line susceptibility of “normal population”

“Normal population” means a population never subjected to insecticide pressure in which resistant individuals are rare. Batches of mosquitoes are exposed to different concentrations of the relevant insecticide for 60 minutes. Mortality is determined after the 24 hour holding period. The concentrations should be chosen such that at least one concentration gives 100% mortality, some give 50%-90% mortalities, and at least 2 concentrations give mortalities between 5%-50%. Base-line data is plotted on logarithmic probability paper to determine the concentration that gives 99.9% mortality. Double this concentration is taken as the discriminating or diagnostic concentration.

#### b) Monitoring of susceptibility levels by routine checks using discriminating concentration

### Insecticide impregnated papers

The insecticide papers with the discriminating concentrations of insecticides and control papers are packed in plastic boxes each contains 8 papers.

Discriminating concentrations of insecticides for malaria vectors (WHO, 1998)

Insecticide	Concentration
DDT	4%
Malathion	5%
Fenitrothion	1%
Bendiocarb	0.1%
Permethrin	0.75%
Lambda-cyhalothrin	0.05%
Deltamethrin	0.05%
Cyfluthrin	0.15%

Etofenprox	0.5%
Bifenthrin	2.0%

### Conditions of mosquitoes

Females should be used. It is recommended ideally the tests should be undertaken on non blood fed adult females of known age (24-48 hrs post emergence). Mosquitoes of known age can be obtained using larval collections or the F<sub>1</sub> progeny from wild caught females.

- Where adults obtained from larval collections are used, the type of breeding site ( *i.e.* paddy field, rain water collections, irrigation channels, river/stream bed pools etc.) should be specified.
- Where only wild caught females can be used, their physiological status ( *i.e.* unfed, blood fed, semi-gravid, gravid) should be recorded.

### Conditions of test

- The experiments should be done indoors, if possible the buildings free from insecticidal contamination and extremes of temperature, humidity, illumination and wind.
- Temperature and relative humidity should be recorded during the test (both the exposure and the holding periods). Ideal temperature for testing is 25±2°C. ; Never at temperature higher than 30°C. Relative humidity should be 70-80%

### Test Kit

- WHO standard susceptibility test kit for adult mosquitoes is used.

The components of a test kit as follows:

a) 12 plastic tubes (125mm length and 44mm in diameter), with each tube fitted at one end with 16-mesh screen. The 12 tubes include:

- Five (5) marked with a red dot use as exposure tubes
- Two (2) marked with a green dot for use as control tubes
- Five (5) with a green dot for use as holding tubes, for pre-test sorting and post-exposure observation

- b) Seven (7) slide-units, each with a screw-cap on either side, and provided with a 20mm filling hole
- c) 40 sheets of clean paper (12x15cm) for lining the holding tubes
- d) 14 spring wire clips to hold the papers in position against the walls of the tubes. Of these, the 7 steel clips are for use only for the holding and the control exposure tubes, and the 7 copper clips are for use in the insecticide exposure tubes.
- e) Two (2) glass (or plastic) aspirator tubes of 12mm internal diameter, together with 60cm of tubing and mouth-piece.
- f) One roll of self-adhesive plastic tape
- g) Instruction sheets.

### **Test procedure**

- It is recommended a minimum number of 100 mosquitoes should be tested for any insecticide concentration, with 4-5 replicates of 20-25 mosquitoes per test. Where possible collect this number from the same locality and monitored over time to examine the trends.
  - Test chambers should be kept vertically.
  - When testing pyrethroids, timed observations of the rate of knock down (kd) should be made routinely after 10, 15, 20, 30, 40, 50 and 60 minutes of exposure.
- 
- a) Into each of the holding tubes, insert a piece of clean white paper rolled into a cylinder to line the wall and fasten it in position with a steel spring-wire. Attach the slides to the tube.
  - b) Mosquitoes should be collected in lots, with each individual lot consisting of more than ten mosquitoes who are gently transferred to the holding tubes through the filling-hole in each side.
  - c) A pre-test holding period may be necessary to guard against including damaged specimens in the test. For this purpose, the holding tubes are set upright, screen end up, for 1 hr. At the end of this time the damaged mosquitoes are removed.

- d) Introduce into each exposure tube a sheet of insecticide impregnated paper, rolled into a cylinder to line the wall and fastened into position with a copper clip.
- e) Similarly introduce a control paper impregnated with oil alone into control tubes and fasten it in position with a steel clip.
- f) Introduce the mosquitoes into exposure tubes by attaching it to the vacant screw-top in the slide. The slide should be pulled out to appoint beyond the filling-hole so that no part of it occludes the tube openings. The mosquitoes are then blown gently down into the exposure tube. Close the slide. Detach the holding tube and set it aside.
- g) Leave the exposure tubes standing upright with screen end up for the required exposure period under conditions of moderate, diffuse illumination and adequate humidity.
- h) At the end of the exposure period, transfer the mosquitoes to the holding tubes by reversing the procedure. Attach the holding tube, 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 moist cotton-wool on the screen.
- i) Keep the holding tubes for 24 hrs in a secluded, shaded place. If necessary, the tubes should be protected from ants by placing them on a platform standing in a pan of water. If conditions are very hot and dry a moist chamber may be prepared by suspending damp toweling in a container.
- j) Mortality counts are made after 24 hours adults should be considered as live if they are able to fly, regardless of the number of legs remaining.
- k) The results should be recorded on the forms provided.

### **General remarks**

- It is recommended that impregnated papers especially pyrethroid papers should not be used more than 5 times. After the impregnated paper has been removed the package should be

resealed carefully with the plastic tape provided. The paper should be left in the tube, with the open end well wrapped and placed in the kit box and should be kept in a cool place.

### **Interpretation of susceptibility test results**

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

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

- 98-100% mortality indicates susceptibility
- 80-97% mortality suggests possibility of resistance that needs to be confirmed
- <80% mortality suggests resistance



## 2.10 Insecticide Susceptibility test for Mosquito Larvae

- a) In order to detect the emergence of an insecticide resistance strain of a mosquito, it is necessary to establish a base-line for the species to a given insecticide with samples from an untreated area. Where regular larviciding operations are used, the normal susceptibility levels of the larvae should be determined as early as possible. For this several tests should be carried out in different localities and seasons. Tests should be done at regular intervals to determine the reduction in susceptibility levels.
  
- b) Previous use of insecticide in the area both for public health and agriculture should be noted.

WHO standard larval susceptibility test kits could be used

### Materials required for testing

- One (1) pipette delivering 100-1000 $\mu$ l
- Disposable tubes (100 $\mu$ l, 500  $\mu$ l) for measuring aliquots of dilute solutions
- Five (5) 1ml pipettes for insecticides and one for the ethanol
- Three droppers with rubber suction bulbs
- Strainer
- Disposable cups or, glass bowls/beakers capacity of 100ml and 250ml
- Measuring cylinder
- Log-probit papers
- Data record forms

### Test Procedure



a) For a complete test with one insecticide, sufficient larvae should be collected from the field. 300 individuals from the same species should be selected; they should be in their 3<sup>rd</sup> or early 4<sup>th</sup> instar and should be retained in the water in which they were collected until selection for testing.

b) Batches of 20-25 larvae should be transferred by means of a strainer to beakers each containing 200ml of water. The average temperature of the water should be 25°C.; it must not be below 20°C or above 30°C

c) Prepare the test concentration by pipetting 1ml of the standard insecticide solution under the surface of the water in each of the beakers and stirring vigorously for 30 sec. with the pipette. In preparing a series of concentrations, the most dilute should be prepared first. Four or more replicates are set up for each concentration and equal numbers of controls are set up simultaneously with water in which 1ml ethanol is added.

d) Within 15-30 minutes of the preparation of the test concentration larvae should be added.

e) After 24 hr exposure, larval mortality is recorded. For slow acting insecticides, 48hr reading may be required. Moribund larvae are counted and added to dead larvae for calculating percentage mortality. Dead larvae are those that 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 or not showing the characteristic of diving reaction when the water is disturbed.

f) Discard the larvae that have pupated during the test. If more than 10% of the control larvae pupate in the course of the experiment, the test should be discarded and repeated.

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

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

g) It is important to obtain three mortality counts between 10% -90%. It is not possible to do this with standard concentrations in the test kit. Therefore, it is necessary to prepare additional concentrations by diluting a portion of a standard solution with pure ethanol.

### **General remarks**

- a) The accuracy of the concentrations provided will be affected if the alcohol is allowed to evaporate from the standard solutions. Therefore, the bottles should be tightly capped after use.
- b) Test beakers should be carefully cleaned after use to remove traces of insecticides. They should be thoroughly rinsed, scrubbed with detergent and water. Pipettes should be thoroughly cleaned with acetone or alcohol.

## **2.11 Bio Assay Test for Insecticide Deposits on Wall Surfaces**

### **Objectives:**

- (1) To assess the potency of an insecticide deposits to adult mosquitoes with proven susceptibility at various time intervals after application on different surfaces
- (2) To detect the decline of toxic effect of the deposit.

### **Composition of the test kit**

- Twenty four (24) conical chambers of transparent plastic, 8.5cm in diameter at the base and 5.5cm high. Of these 20 are marked with red dot for carrying out the test and 4 with a green dot for use in the controls.
- Four (4) glass aspirator tubes with ends bent to facilitate reaching all parts of the exposure chamber, together with 60cm of flexible rubber or plastic tubing. Two tubes are marked with a green dot for use with the controls only.
- Four rolls of plastic sponge tape-two thick and two thin
- One box of pins

### **Test Procedure**

- a) The exposure chamber is fastened to the selected surface with an appropriate device that will hold the chamber tight against the surface.

- b) Ten (10) female mosquitoes are collected with the aspirator tube and introduced into the chamber by blowing gently. Care should be taken that the end of the tube does not contact the test surface.
- c) The chamber is left undisturbed for a period of 30 minutes.
- d) At the end of exposure period the mosquitoes are collected carefully by means of a bent transfer tube introduced through the aperture of the chamber without contacting the test surface and are transferred immediately to the holding container. Paper cups or suitable containers can be used.
- e) At the time of performing each test room temperature and relative humidity are recorded.
- f) The holding containers are kept for 24 hrs in a secluded, shaded place, where the temperature does not exceed 30°C if feasible. 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.
- g) The exposure chambers and transfer tubes are carefully washed in detergent after each use, rinsed and allowed to drain dry.

## **Results**

- a) After 24 hrs, counts of dead and live mosquitoes are recorded. It is essential that the percentage of observed mortalities be recorded for each individual test. If it is found that the control mortality is above 10% it is recommended to increase the number of controls in subsequent tests. When control mortalities exceed 20% the series of tests should be considered unsatisfactory and repeated.
- b) Mortalities on one type of surface are averaged. Where the control mortality is between 5-20% the average observed mortality is corrected by Abbott's formula:

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

### **General remarks**

It is of great importance to carry out the test on an adequate scale at regular intervals. It is necessary to test and evaluate separately the potency of the insecticide deposit on each type of mosquito resting surface requiring investigation. On a given type of surface not less than 10 points should be chosen for the bioassay tests on a given day. They should be distributed in several houses, not more than three points being in any one house. Controls must be run at a rate of at least 2 controls for every 10 bioassay tests. The control runs are carried out in a suitable situation away from the sprayed premises and as unsprayed surface.

## 2.12 Bioassay Test for Insecticide Treated Mosquito Net Surfaces

### **Objectives:**

To assess the potency of an insecticide deposit on treated mosquito nets to adult mosquitoes with proven susceptibility after number of washes (LLINs) / various time intervals after impregnation

### **Test procedure**

Susceptible wild caught female mosquitoes (preferably non blood fed and aged 2-5 days if available) are introduced in to WHO plastic cones for a period of 3 minutes. To minimize the chances of mosquitoes disturbing each other during the short exposure period on netting, batches of only 5 females are introduced in to three cones that are applied to the same net. Cones can be fitted at upper middle and lower part of the net. A total of 10 replicates of 5 mosquitoes is used for each sample tested, giving a total of 50 mosquitoes per sample. Results are pooled for analysis.

After exposure, females are placed in 150ml plastic cups (10 individuals per cup) with sucrose solution provided, and maintained in a suitable climatic condition for 24hrs. Ideal temperature for testing is 27±2°C. Relative humidity should be 70-80%.

Percentage knock-down after 60 minutes and percentage mortality after 24 hrs are recorded.

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

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















### **3.0 Entomological Laboratory techniques**

1. Salivary gland dissection for sporozoites
2. Ovary dissection for parity determination

### 3.1 Dissection of salivary glands for sporozoites

#### Objectives:

The salivary glands are examined for sporozoites in order to determine which mosquito species carry malaria parasites. Determination of sporozoite rates is necessary to confirm the role of a particular mosquito species as a vector to determine intensity of malaria transmission (inoculation rate) and assess impact of malaria control methods. The dissection technique indicates whether or not the mosquito is infected with *Plasmodium*, but does not distinguish the species of parasite.

#### Essential equipments:

Dissecting microscope

Compound microscope

Dissecting needles

Fine forceps

Glass slides

Droppers

0.65% saline solution.

#### Procedure for dissecting salivary glands:

- Kill the mosquito, identify the species and remove legs and wings.
- Do not dissect the salivary glands of nulliparous females because they are not infected.
- Place the mosquito on a slide, lying on its side with the head pointing to the right (Fig.7).
- Place a small drop of saline solution close to the front of the thorax.
- Hold the thorax firmly with a blunt dissecting needle in your left hand.
- Place the needle held in your right hand on the neck of the mosquito without cutting the neck.
- Gently pull the head away from the thorax and the glands will come out of the thorax, attached to the head.

- If the glands do not come out with the head, they may be obtained by gently squeezing the thorax.
- Separate the glands with the other needle, and place them in a drop of saline solution.
- Cover the salivary gland with a standard 18 x 18 mm cover slip and observe under a microscope.

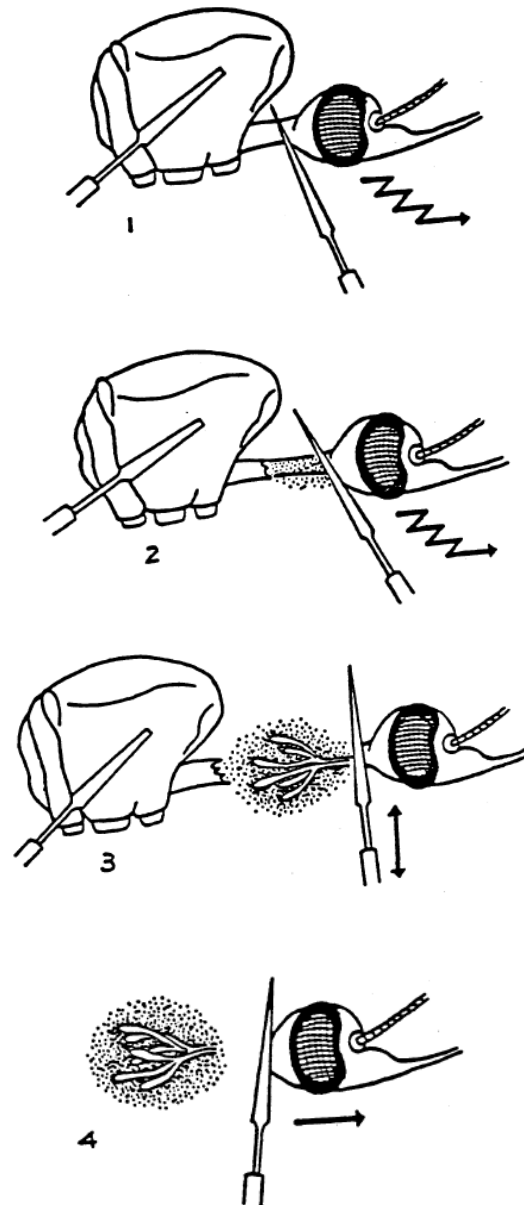


Fig.7 Salivary gland dissection

### 3.2 Ovary dissection for parity determination

**Objectives:**

To see if they are **parous** (those that have taken a blood meal at least once and laid eggs at least once) or **nulliparous** (mosquitoes that have not taken a blood meal yet and have not laid eggs).

Only females which are unfed or freshly fed are suitable for this method of parity determination.

**Essential equipments:**

Dissecting microscope

Compound microscope

Dissecting needles

Fine forceps

Glass slides

Droppers

Distilled water

**Procedure for dissecting ovaries:**

- Kill the female and remove legs and wings.
- Place the mosquito on a slide and add a drop of distilled water (Fig.2).
- While holding 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. The ovaries will come out of the abdomen.
- Cut through the common oviduct and separate the ovaries from the rest of the specimen.
- Transfer the ovaries to a drop of distilled water on another slide and allow them to dry.



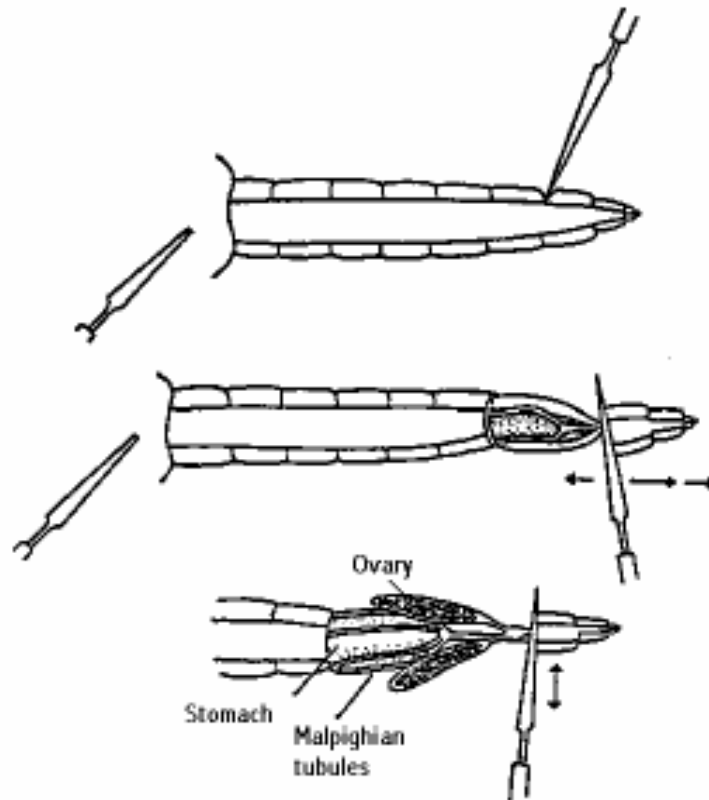


Fig. 8 Ovary dissection

### Differentiating between nulliparous and parous ovaries

- Examine the dried ovaries under a compound microscope using the 10x objective, and if necessary, confirm using the 40x objective.
- Females in which the ovaries have coiled tracheolar skeins are nulliparous (Fig.3).
- Ovaries in which the tracheoles have become stretched out are parous.
- In some females not all developed eggs are laid; if some eggs (usually less than five) are retained in the ovaries, the female is parous.

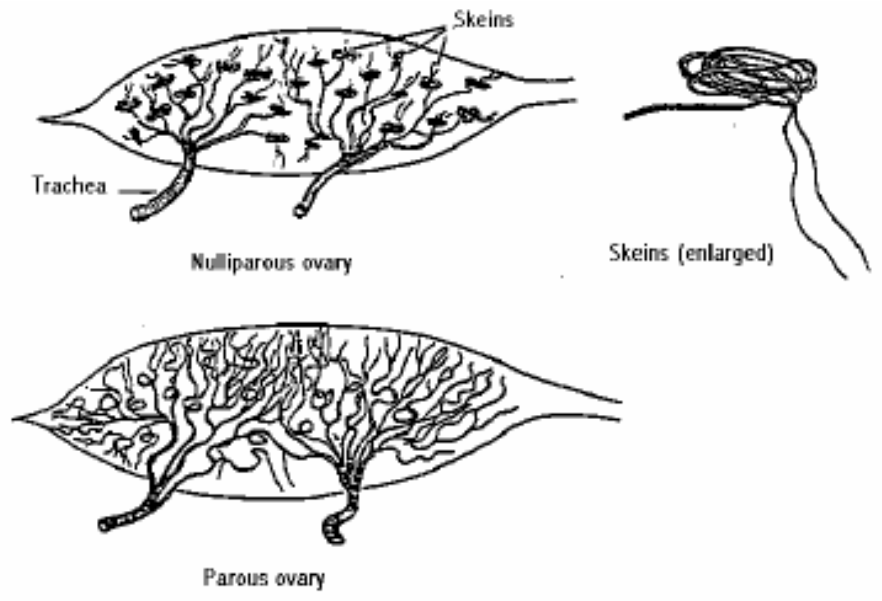


Fig. 9 Appearance of parous and nulliparous ovaries

#### 4.0 References

1. Entomological field techniques for malaria control. Part I, Learners guide. Geneva, World Health Organization, 1992.
2. Entomological laboratory techniques for malaria control. Part I, Learners guide. Trial edition. Geneva, World Health Organization, May 1994.
3. Malaria entomology and vector control. Part I, Learners guide. Trial edition. HIV/AIDS, Tuberculosis, Malaria. Roll back Malaria. Geneva, World Health Organization, July 2003 (WHO/CDS/CPE/SMT.18 Rev.1).