Malaria entomology and vector control

GUIDE FOR **PARTICIPANTS**

World Health Organization

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Foreword

Malaria is a major global public health problem and a leading cause of morbidity and mortality in many countries. Malaria caused an estimated 219 (range 154–289) million cases and 660 000 (range 490 000–836 000) deaths in 2010. Approximately 80% of the cases and 90% of the deaths occur in Africa while the remaining cases and deaths occur mainly in the South-East Asia and Eastern Mediterranean Regions.[1]

The World Health Assembly and Roll Back Malaria (RBM) targets for malaria control and elimination aim- to achieve at least a 75% reduction in malaria incidence and deaths by 2015.

Elimination of malaria is defined as the reduction to zero of the incidence of locally acquired infection by human malaria parasites in a defined geographical area as a result of deliberate efforts. Elimination programmes require more technical malaria expertise than standard malaria control programmes, and are driven by national expertise in malaria epidemiology and entomology.

To achieve the objectives of malaria control and elimination programmes, appropriately planned and targeted delivery of essential malaria interventions is critical, including: diagnostic testing of all suspected malaria and prompt treatment of confirmed cases with effective artemisinin-based combination therapy (ACT); chemoprevention of malaria in pregnant women (Intermittent preventive treatment during pregnancy – IPTp), infants (Intermittent preventive treatment in infants – IPTi) and children (Seasonal malaria chemoprevention – SMC), where appropriate; and application of appropriate vector control interventions, particularly the use of insecticide- treated nets (ITNs/LLINs) and indoor residual spraying (IRS).

This training module on malaria entomology and vector control has been developed to support two main groups: (i) entomologists and vector control staff including technicians and (ii) programme managers/senior health officers involved in malaria control and elimination programmes.

[1.] WHO (2012). *World malaria report*. Geneva, World Health Organization. http://www.who.int/malaria/publications/world_malaria_report_2012/en/index.html

Abbreviations

ABER	Annual blood examination rate
ACT	Artemisinin-based combination therapy
API	Annual parasite rate
CSP	Circumsporozoite protein
DALY	Disability-adjusted life year
EIR	Entomological inoculation rate
ELISA	Enzyme-linked immunosorbent assay
GIS	Geographical information system
GPS	Global positioning system
GR	Geographical reconnaissance
GST	Glutathione-S-transferase
HBI	Human blood index
IEC	Information, education and communication
IRS	Indoor residual spraying
ITN	Insecticide-treated mosquito net
IVM	Integrated vector management
KAP	Knowledge, attitudes and practices
LLIN	Long-lasting insecticidal net
MCQ	Multiple choice questionnaire
PCR	Polymerase chain reaction
POPs	Persistent organic pollutants
SPR	Slide positivity rate
WHO	World Health Organization
WHOPES	WHO pesticide evaluation scheme

Acknowledgements

Development of the module

The content of the module is based on current WHO guidelines and other evidence-based technical documents.

This training module on malaria entomology and vector control builds on a previous trial version which has been updated to reflect current malarial control tools, strategies and policies. The module was revised under the guidance of technical experts representing malaria training and academic institutions, malaria researchers, country programme managers, and WHO staff from headquarters and regional offices, who guided the process of reviewing and updating the module. The process included the following steps:

► Three consultations of technical experts (7–9 April 2008, 14–16 October 2008, and 15–17 April 2009) were held to review the existing WHO training materials on malaria entomology and vector control, and to identify areas for update in view of the developments of new tools, technologies and strategies for malaria vector control.

► Technical experts were commissioned to incorporate the recommended updates in the module.

► The revised module was then reviewed for content and completeness by the technical experts, the WHO technical staff and additional external experts in entomology and vector control of malaria.

► The updated module was field-tested in several national and international courses.

► Based on feedback from field tests, and in consultation with technical experts, the text was finalized for publication.

Introduction

This module covers essential aspects of malaria entomology and vector control. It can be adapted to different specific training needs, as the depth and selection of Learning Units depend on the background of the participants and their learning objectives. It can be used to train field vector control workers, laboratory technicians, or health workers working in malaria vector control programmes at different levels. For health workers, who may not need details of the field and laboratory techniques, the training should focus on the units that deal with epidemiological application of selective vector control options and strategies. Field staff and laboratory technicians may need additional resource materials if a course is to be entirely on laboratory and field techniques.

The module can be used to train staff who have responsibility for planning, implementation, monitoring and evaluation of malaria vector control activities at national and district levels. The participants will have been asked to bring malaria data from the countries where they work for discussion during the course.

The module is in two parts, the *Guide for Participants* and the *Guide for Tutors.* The *Guide for Participants* covers basic concepts and information, and includes a series of exercises to be carried out by the participants. The *Guide for Tutors* outlines the main points to be learnt, and provides answers for the exercises which may be indicative rather than definitive; in this way it is designed to stimulate active learning.

Potential users of the training module

The module is designed for two main groups: *(i)* technical staff in entomology and vector control and *(ii)* programme managers/senior health officers. The time allocated for the first group is 120.5 h in a 7 h/training day = 17 days, and for the second group 30 h in a 7 h/training day = 4.5 days. A suggested timetable for the second group is provided in the *Guide for Tutors.*

Objectives

At the end of the training programme based on this module, participants should have acquired the skills and competence necessary to:

- understand the role of entomology in malaria control
- identify all life-cycle stages of malaria vectors
- describe sampling procedures for malaria vectors
- describe vector incrimination and malaria control
- define malaria vector control measures
- understand the basic principles in monitoring and managing insecticide resistance
- describe the epidemiological stratification of malaria and the role of vector control in different epidemiologic strata

Use of the *Guide for Participants*

This guide consists of instructional materials designed to enable the course participants to achieve the learning objectives of this module. The guide is divided into a series of Learning Units. It is necessary to acquire the skills and knowledge contained in each unit in the sequence before progressing to the next learning unit; otherwise it may prove difficult to achieve the objectives of subsequent units. Each Learning Unit includes exercises to be completed either individually or as a group, as stipulated by the tutor. The discussions during small group work and during plenary sessions with the participation of facilitators and tutors will assist this learning process.

During the course, the *Guide for Tutors* will be available only to the tutor and facilitators. Upon completion of the course/module, all participants should receive a copy of the *Guide for Tutors* so that they can use it for further training and reference.

Evaluation

Evaluation of the participant

Progress and achievement are evaluated by the tutor, the facilitators and by the participants themselves. This will be done using multiple-choice questionnaires (MCQ).

In MCQs, each question is provided with a list of possible answers from which one must be selected (i.e. considered to be correct). At the end of these sessions the tutor will analyse the results to identify topics that were not fully understood. The tutor may also explain to individual participants where mistakes were made and areas where improvement is needed.

This part of the evaluation is designed to help the participants and the tutor to assess how well the non-practical aspects of the course have been understood. Multiple-choice tests will be given at the beginning and at the end of this training.

The evaluation of the participant's progress also includes assessment of classroom, practical and field activities, degree of group participation, etc. including how the group work was presented in plenary sessions, and the degree of clarity.

The tutor is encouraged to develop a bank of questions that can be used for pre- and post-testing for subsequent training sessions. The answers are scored equally because each question is considered, in this instance, to be of equal value. The preferred answers have been provided but in some cases alternative responses are acceptable, and these have been noted.

Evaluation of the training by the participants

The entire training activity, including the organization and content of the course, the suitability of the learning methods, the quality of the teaching and training materials, and the competence of the tutors and facilitators will be assessed. Evaluation will take place at the end of the training period in order to provide as much feedback from the participants as possible. The questionnaires may be signed on a voluntary basis (as preferred by the individual participants). All participants are encouraged to make suggestions for improvement on the part of the tutor and facilitators as well as in the content of the course and the training facilities.

Feedback provided through this exercise allows the tutor to assess how well the training has been received and to make any modifications that seem necessary for improving future programmes.

Note: Participants are requested to bring malaria data (disease burden, entomological data etc) from their country/workplace, if possible covering the past 5 years. The data will be discussed during the training course.

LEARNING UNIT **1**

Introduction to malaria entomology

Learning Objectives:
by the end, participants should be able to...

- Describe how malaria is transmitted
- Describe the life-cycle of malaria parasite and mosquito
- Describe the purpose and role of entomological monitoring in malaria control

Introduction

Malaria entomology is the study of the biology and ecology of the mosquitoes that transmit malaria. The aim is to understand the relationship between the vector, its ecology and behaviour, the parasite and the host in order to develop and implement effective vector control strategies. In this unit, a brief introduction will be given about the transmission of malaria and the life-cycle of the parasite and mosquito vectors. The importance and purpose of entomological monitoring in malaria control programmes will also be discussed in detail.

1.1   Malaria transmission

Malaria is caused by the protozoan parasite *Plasmodium* which spends part of its life-cycle in humans and part in certain species of mosquitoes. Five species of *Plasmodium* cause malaria in humans: *Plasmodium falciparum, P. vivax, P. malariae, P. ovale,* and the monkey malaria parasite *P. knowlesi.* Of these, *P. falciparum* is the most important in most parts of the tropics and is responsible for most severe illnesses and deaths due to malaria.

Malaria parasites are transmitted by female mosquitoes belonging to the genus *Anopheles.* Male *Anopheles* mosquitoes only feed on plant juices and nectar and cannot transmit malaria. The life-cycle of the malaria parasite is divided into three different phases: one in the mosquito, the sporogonic cycle; and two in the human host – the erythrocytic cycle (in human red blood cells) and the exo-erythrocytic cycle (outside the red blood cells) (Fig. 1.1)

1.1.1   Life-cycle of malaria parasites

When a mosquito bites an infected human, gametocytes are ingested with the blood-meal. In the mosquito gut, the infected human blood cells burst, releasing the gametocytes, which develop further into mature sexual stages called gametes. Male and female gametes unite to form a zygote, which develops into an actively moving ookinete. The ookinete penetrates the wall of the mosquito midgut and becomes a round oocyst. Inside the oocyst, the nucleus divides repeatedly, with the formation of a large number of sporozoites and enlargement of the oocyst. When the sporozoites are fully formed, the oocyst bursts, releasing the sporozoites into the mosquito's body cavity (haemocoel). The sporozoites migrate to the salivary glands. The time necessary for the development of the sporozoites varies with temperature and to a lesser extent with the species of the malaria parasite and with humidity, but generally it is about 8–15 days.

The sporozites (the infective stage of *Plasmodium*), mixed with saliva, are injected into a human host when the mosquito feeds. The parasites enter the host's blood system and migrate to the liver where they multiply within hepatocytes. Over a period of 7–12 days, the parasite continues to multiply until the infected liver cell bursts. The parasites are then released as merozoites into the bloodstream where they invade red blood cells and multiply again. The infected red cells are destroyed, the parasites invade fresh red blood cells and the erythrocytic cycle is repeated.

A blood-meal is necessary for maturation of the mosquito's eggs. Several batches of eggs are produced during the mosquito's lifetime, providing several opportunities for mature females to transmit malaria.

Figure 1.1 Life-cycle of *Plasmodium* species in human and mosquito hosts
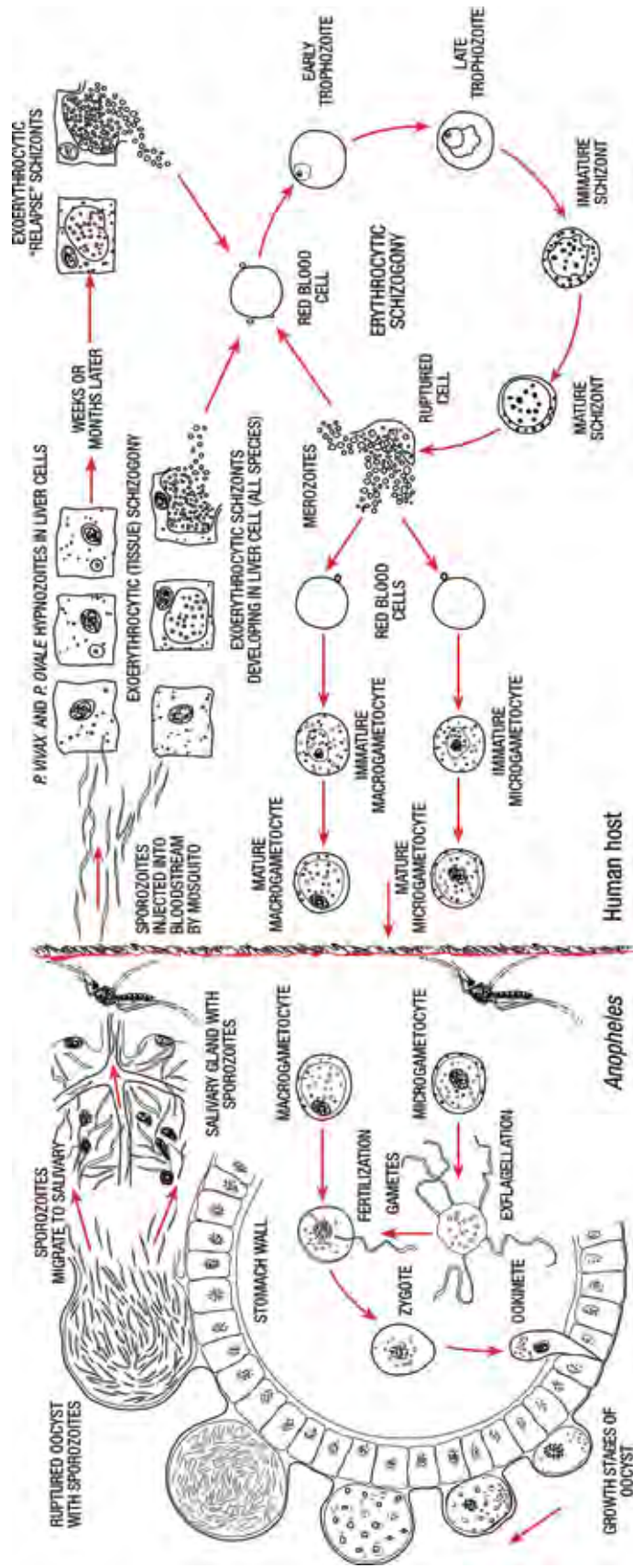
1.1.2 Life-cycle of anopheline mosquitoes

The life-cycle of mosquitoes has four distinct stages: the egg, larva, pupa and adult (Fig. 1.2). The time taken for the various stages to develop depends on temperature and nutritional factors, with development more rapid at higher temperatures.

There are about 490 species of *Anopheles* mosquitoes including sibling species. Approximately 60–70 species worldwide can transmit malaria and of these, about 30 are vectors of major importance (Fig. 1.3). Some anophelines prefer to bite animals and only rarely transmit malaria
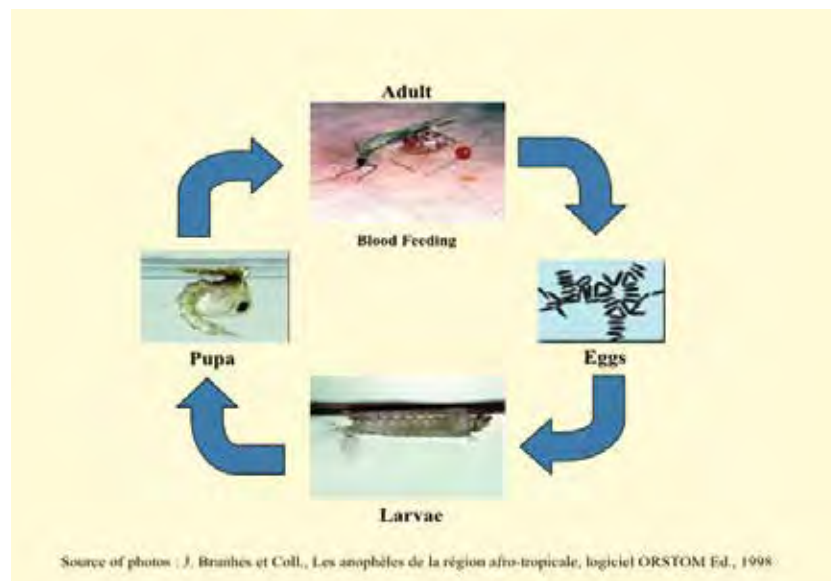
Figure 1.2 Life-cycle of an *Anopheles* mosquito
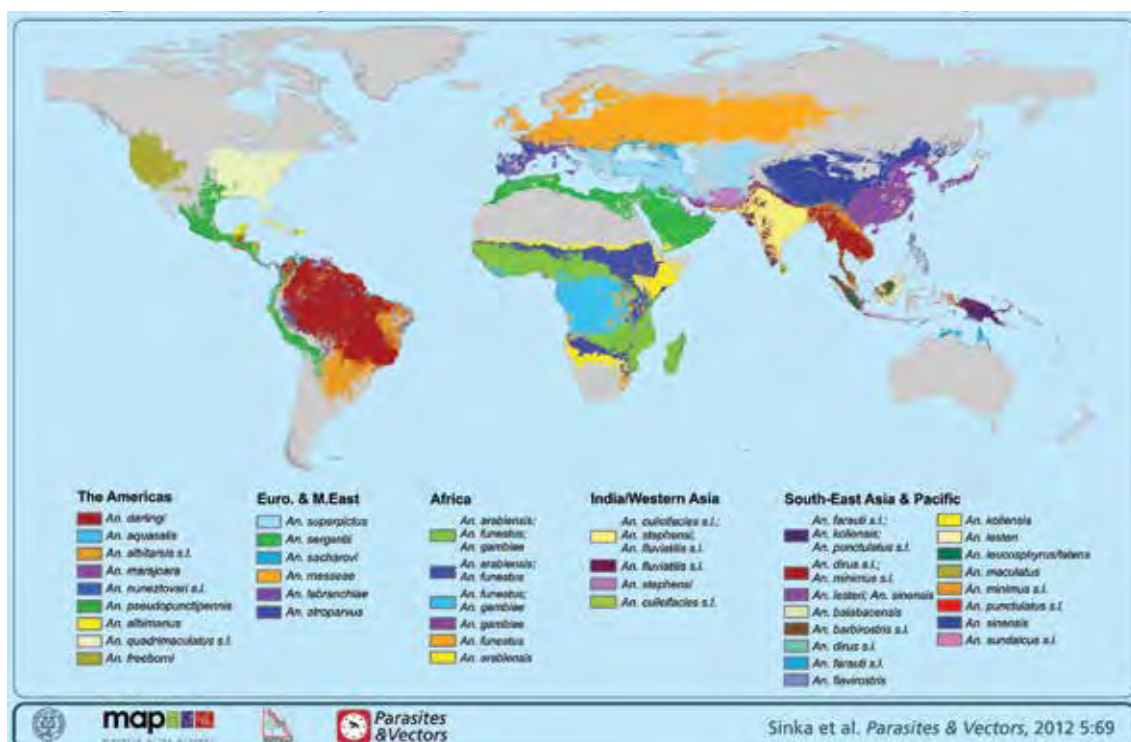
Figure 1.3 Global distribution of dominant or potentially important malaria vectors

parasites to humans. Others do not live long enough to permit development of the parasite, or the parasite does not seem to be able to develop in the mosquito.

Eggs

A female anopheline mosquito normally mates only once in the lifetime and usually requires a blood-meal after mating before the eggs can develop. Blood-meals are generally taken every 2–3 days, before the next batch of eggs is laid. About 100–150 eggs are laid on the water surface during oviposition. Oviposition sites vary from small hoof-prints and rain pools to streams, swamps, canals, rivers, ponds, lakes, rice fields, and sometimes even dirty water. Each species of mosquito prefers a particular type of habitat for oviposition.

Under the most favourable conditions in the tropics, the average lifespan of female anopheline mosquitoes is 3–4 weeks. A female mosquito continues to lay eggs throughout life and most will lay 1–3 batches of eggs, though some may lay as many as 7 batches.

Larva

A larva hatches from the egg after 1–2 days and generally floats below and parallel to the water surface, where it breathes air. It feeds by filtering food particles from the water. When disturbed, the larva quickly swims downwards but soon needs to return to the surface to breathe.

There are four larval stages or instars. The small larva emerging from the egg is called the first instar. After 1–2 days it sheds its skin and becomes the second instar, followed by the third and fourth instars at further intervals of about two days each. The larva remains in the fourth instar stage for 3–4 more days before changing to become a pupa. The total time spent in the larval stage is generally 8–10 days at normal tropical water temperatures. At lower temperatures, the aquatic stages take longer to develop. Depending on the species, larvae may be found in small pools, fresh water, rice-land, drains, ditches, running water with shade, brackish water, salt water, streams, ponds, lakes, marshes, wells, water containers, discarded tin cans, discarded tyres and hoof-prints.

Pupa

The pupa undergoes a major transformation, from living in water to becoming a flying adult mosquito. The pupa is shaped like a comma. It stays under the surface and swims down when disturbed. The pupae do not feed. The pupal stage lasts for 2–3 days after which the skin splits. The adult mosquito then emerges and rests temporarily on the water's surface until it flies.

Adult

Mating takes place soon after the adult emerges from the pupa. The female usually mates only once because sufficient sperm are received from a single mating for all subsequent egg batches. Normally the female takes the first blood-meal only after mating, but sometimes the first blood-meal is taken by young virgin females. The first batch of eggs develops after one or two blood-meals (depending on the species) while successive batches usually require only one blood-meal.

The feeding and resting habits of mosquitoes are of great importance in vector control programmes and they must be well understood. Most anopheline mosquitoes bite at night. Some bite shortly after sunset while others bite later, around midnight or the early morning.

Some mosquitoes enter houses to bite and are described as being endophagic; others bite mostly outdoors and are called exophagic.

After taking a blood-meal the mosquito usually rests for a short period. Mosquitoes that enter a house usually rest on a wall, under furniture or on clothes hanging in the house and are said to be endophilic. Mosquitoes that bite outdoors usually rest on plants, in holes, in trees or on the ground or in other cool dark places and are termed exophilic.

Host preferences are different for different species of mosquitoes. Some mosquitoes prefer to take blood from humans rather than animals and are described as being anthropophagic while others take only animal blood and are known as zoophagic. Those which prefer to take human blood are the most dangerous as they are able to transmit infection in human populations. The adults can be found on vegetation, on solid surfaces in sheltered places, in the banks of streams and in ditches, holes in rocks, culverts, cracks, caves, animal burrows, on the trunk of trees and termite mounds.

1.2 Malaria vector control

Malaria control involves the diagnosis and treatment of malaria cases, preventing mosquito bites, and killing mosquitoes. The primary methods of vector control measures in most settings are insecticide-treated mosquito nets or indoor residual spraying; larviciding, larvivorous fish, space spraying, mosquito repellents, and screening houses to prevent mosquitoes from entering are also useful in selected settings.

Eliminating breeding sites and killing larvae, pupae and adult mosquitoes will help reduce the number and, in the case of adults, the longevity of vectors. Breeding sites can be eliminated by draining or filling areas where water collects or by modifying the preferred habitats of particular vector species, for example by clearing streams so that the water flows faster. Larval breeding can be reduced or prevented by:

▶ treating the water with larvicides to kill larvae,

▶ putting fish or other predators that eat mosquito larvae in the breeding sites.

The optimal method of mosquito control in each setting depends on a detailed understanding of the behaviour and ecology of the local vectors.

In many areas, malaria transmitted by vectors that rest indoors can be prevented or controlled by the large-scale distribution of insecticide-treated mosquito nets or spraying the insides of houses with a residual insecticide. Before, and more usually after, biting an endophilic mosquito rests on a wall, ceiling or in other dark areas inside the house. If the surfaces it rests on have been sprayed with residual insecticide, the mosquito may eventually pick up a lethal dose and be prevented from transmitting the parasite. The aim of insecticide-treated nets or residual spraying is to shorten the life of mosquitoes to less than the time it takes for the malaria sporozoites to develop, and to reduce mosquito density.

Unfortunately, mosquitoes can develop resistance to a wide range of insecticides. It is important to know when a vector species develops resistance in order to decide the most appropriate resistance management measure to adopt, such as interruption of spraying, change of insecticide to another insecticide class, or by other means.

The vectorial capacity of mosquitoes (see Unit 4) can be reduced by different methods of vector control which affect the adult density, adult survival and human biting rates. Larval control, including source reduction, use of larvivorous fish, and larviciding, mainly affect adult density. The use of housing improvements and mosquito repellents reduce human biting densities. Importantly the large-scale use of both insecticide-treated nets and indoor residual spraying is even more effective since it can reduce adult survival as well as the human biting rate.

Mathematical descriptions of vectorial capacity highlight how small reductions in mosquito lifespan can substantially reduce vectorial capacity. This is the reason that the use of LLINs and IRS are considered the key vector control strategies.

1.3   Role of entomological monitoring in malaria control

Entomological and parasitological monitoring provide information on the characteristics of malaria transmission in an area as well as the behaviour and habitats of the specific vector species. This information is an essential component of malaria control (and elimination) programmes.

Entomological monitoring have several important roles to play in malaria control, including the following:

▶ identification of the vectors responsible for transmission of the parasite;

▶ providing basic information on the behaviour and habitats of vector species for purposes of planning effective control measures;

▶ monitoring the impact of control measures (for example, by determining changes in vector population density, rates of infection, susceptibility of vectors to insecticides, and residual effects of insecticides on treated surfaces);

▶ contributing to the investigation of problem areas where control measures prove unsuccessful.

Vector control programmes should be planned on the basis of entomological monitoring. Entomological and other epidemiological studies can provide answers to the questions listed below. The skills required to address these questions will be taught in subsequent units.

▶ Is there malaria transmission in the area? If so, in which specific situation and what are the geographical limits of the disease?

▶ Are there any important mosquito-borne diseases other than malaria? If so, which ones?

▶ Which anopheline species are present in the area? Which of them are important as vectors of malaria?

▶ What proportion of the vector species feed on humans? Among the vectors that feed on humans, what proportion rest indoors?

▶ Where do most of the mosquito vectors prefer to bite humans, and where does most human-vector contact take place, indoors or outdoors? What is the peak biting time of the vector?

▶ How many infective bites are received on average per night per person?

▶ How can the duration of efficacy of an insecticide deposited on a surface (e.g. an insecticide-treated net or a sprayed wall) be determined?

▸ Which type of water bodies is preferred for breeding by a particular vector species in the area?

▸ In which epidemiological and economic situations should a vector control strategy to reduce transmission be recommended or not recommended?

▸ What proportions of the vector population are susceptible or resistant to insecticides?

▸ How do different vector control options affect malaria transmission, malaria morbidity and mortality?

▸ Which vector control options are appropriate against the specific habits and habitats of the vector species?

▸ How can the short and long-term effectiveness of a vector control strategy be evaluated?

Malaria entomology is not limited to vector control. Any malaria control strategy should be based on a thorough understanding of the transmission characteristics of the disease. Understanding the characteristics of malaria transmission will involve both theoretical studies (e.g. using mathematical models) and empirical observations. Entomological parameters form the basis of such studies. Entomological monitoring are also important in the estimation of the expected impact of the various control measures. The data allow decisions as to whether some measures are more useful than others and whether some control measures may be dangerous to implement.

Demonstration of Anopheles *life-cycle*

The tutor will demonstrate the life-cycle of *Anopheles* mosquitoes in an insectary. Participants will visit the insectary in groups of 10 and observe the live specimens of each of the stages of the *Anopheles* life-cycle. For information on the establishment of an insectary, see Annex 1.

Exercise 1.1

What is the role of entomological monitoring in malaria control?

LEARNING UNIT **2**

Identification of malaria vectors

Learning Objectives:
by the end, participants should be able to...

■ Differentiate between mosquitoes and other insects based on external morphology

■ Describe the anatomy of adults and larvae of malaria vectors

■ Describe major external morphological features of adult and larval anophelines used in species identification

■ Distinguish between male and female mosquitoes

■ Differentiate between anopheline and culicine eggs, larvae, pupae and adult

■ Use a species identification key

■ Describe the main biochemical and molecular methods used in the identification of mosquito vectors

2.1   Distinguishing mosquitoes from other insects

Mosquitoes belong to the phylum of Arthropoda and class of Insecta, order Diptera. Arthropods include (among many others) spiders, beetles, ticks, butterflies, houseflies and mosquitoes. They can be recognized by the characteristics listed below.

The body:

▸ is composed of several parts or segments, some of which may be jointed,

▸ is covered with a tough skin called an exoskeleton,

▸ normally has paired, jointed legs and antennae.

Within the Arthropoda, there are several classes, including the class Insecta – mosquitoes are members of this group. Insects have the following characteristics:

▸ the body is divided into three sections – head, thorax and abdomen,

▸ the head has one pair of antenna, and a pair of compound eyes,

▸ the thorax has three pairs of legs.

Class Insecta includes several orders; mosquitoes belong to the order Diptera. Insects in this order have the following characteristics:

▸ the thorax has one pair of visible wings,

▸ the hind wings, which are vestigial, are small movable filaments known as halters, which are mainly used for balance.

Figure 2.1 shows the main parts of the adult mosquito. The body, as in all insects, is divided into head, thorax and abdomen. Four characteristics can be used to identify adult mosquitoes: only one pair of wings; a long proboscis; maxillary palps; the body covered with scales; and wings with veins that show a defined pattern.

Figure 2.1   Main parts of the adult mosquito

Species complexes (sibling species)

Many insect vectors are members of species complexes composed of sibling species which are often morphologically identical, but differ in their behaviour and ecology. At least half of the important vectors of malaria belong to sibling (or cryptic) species complexes whose members are isomorphic or similar. The recognition that members of cryptic species complexes often differ in their capacity to transmit malaria has been, and continues to be, a driving force in the development of identification methods other than morphological criteria. An ideal method should be rapid, cost-effective, easy to implement, and applicable to both sexes and to all developmental stages.

2.2   Distinguishing anophelines from culicines

Distinguishing characteristics of anophelines and culicines are illustrated in Figures 2.2 and 2.3.

Eggs

Culicine eggs clump together in a "raft" (*Culex*) or float separately (*Aedes*); anopheline eggs float separately and each of them has "floats".

Larvae

The culicine larva has a breathing tube (siphon) which it also uses to hang down from the water surface, whereas the anopheline larva has no siphon and rests parallel to and immediately below the surface. In *Culex* larvae the siphon is longer than in *Aedes* larvae.

Pupae

Pupae of both anophelines and culicines are comma-shaped and hang just below the water surface. They swim when disturbed. The breathing trumpet of the anopheline pupa is short and has a wide opening, whereas that of the culicine pupa is long and slender with a narrow opening. However, as it is difficult to distinguish anopheline from culicine pupae in the field, it is preferable to rear them in an insectary so that the emerging adult mosquitoes can be identified.

Adults

With live mosquitoes, adult anopheline and culicine mosquitoes can be distinguished by observing their resting postures. Anophelines rest at an angle between 50º and 90º to the surface whereas culicines rest more or less parallel to the surface (Fig. 2.2).
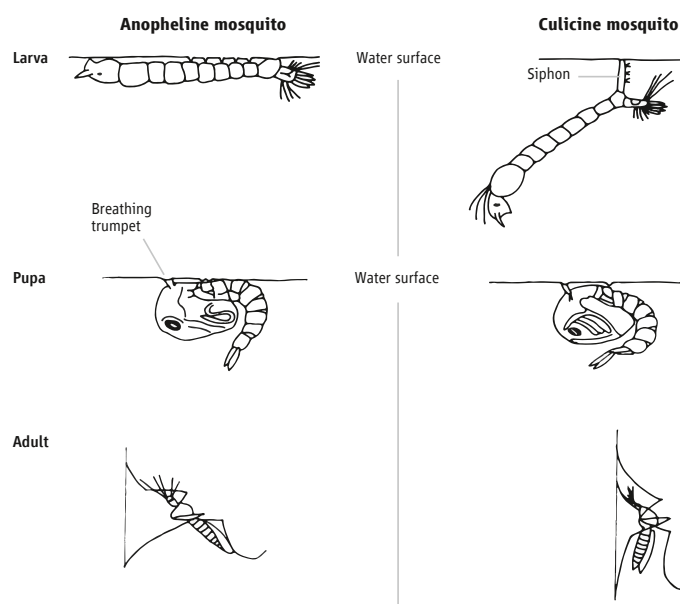
Figure 2.2   Comparison of anopheline and culicine mosquitoes
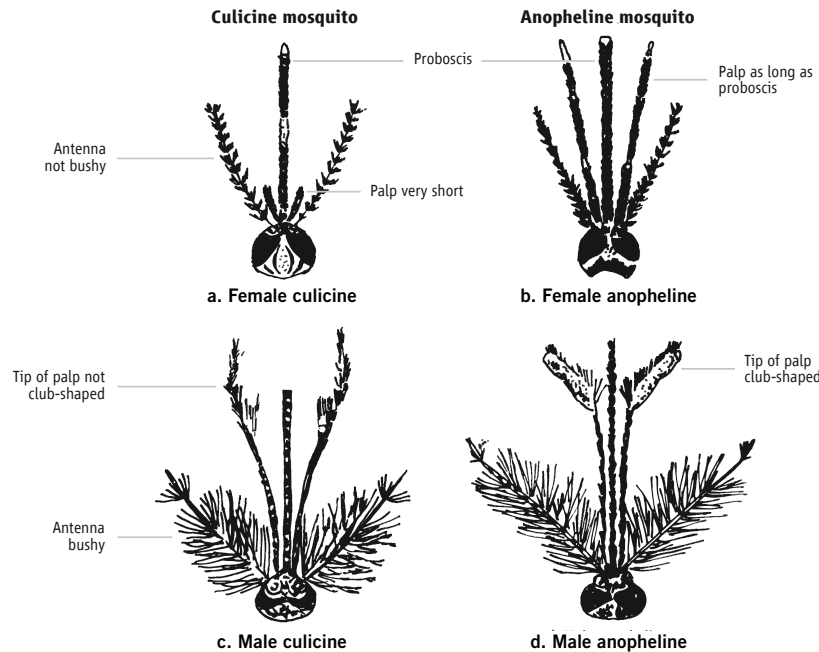
Figure 2.3   Heads of female and male culicine (a, b) and anopheline (c, d)

Anopheline mosquitoes can also be distinguished from culicines by the length and shape of the palps. The differences (Fig. 2.3) are:

▸ in female culicines, palps are very much shorter than the proboscis (Fig. 2.3a);

▸ in female anophelines, palps are as long as the proboscis (Fig. 2.3b);

▸ in male culicines, palps are longer than the proboscis, with tapered tips (Fig. 2.3c);

▸ in male anophelines, palps are as long as the proboscis and club-shaped at the tip (Fig. 2.3d).

2.3   Distinguishing between female and male Anopheles

It is important to be able to distinguish between females and males because only female *Anopheles* take blood-meals and transmit malaria.

On the antennae of the female there are relatively few hairs and these are short (Fig. 2.3b). In contrast, the male has very long hairs on the antennae, which consequently have a bushy "moustache-like" appearance (Fig.3d).

2.4   Identifying anopheline species

Participants will learn how to identify common vector species in their area using identification keys (under section 2.4.3). Information collected during mosquito surveys is only useful if the mosquitoes are accurately identified. It is therefore essential that to be able to identify the species of adults and larvae. Identification of pupae is very difficult and when pupae are obtained in the field they should be kept alive and allowed to emerge into adults because the adults can be identified more easily. Some external characteristics of adult and larval anophelines that are useful in species identification will be described.

2.4.1 External anatomy of adult *Anopheles*

Head

The head has a pair of large compound eyes. A pair of antennae is joined to the head between the eyes (Fig. 2.3). A pair of palps below the antennae is composed of five parts in anopheline mosquitoes. The palps are covered with scales which may be of different colours and used in species identification. A proboscis protrudes from the ventral part of the head and extends forward.

Thorax

The thorax has a pair of wings and a pair of halters on the upper surface and three pairs of legs on the lower or ventral surface.

The wings have several veins; each vein is given a number and/or a name (Fig. 2.4). The vein along the front edge of the wing is called the costa and the short vein behind it is called the subcosta. There are six other veins numbered 1–6 of which veins 2, 4 and 5 are forked. These veins are covered with scales. The scales are usually brown, black, white or cream in colour. The back edge of the wing has fine scales. Many anophelines have wings spotted with dark and pale areas which, together with other characteristics, are used for species identification.
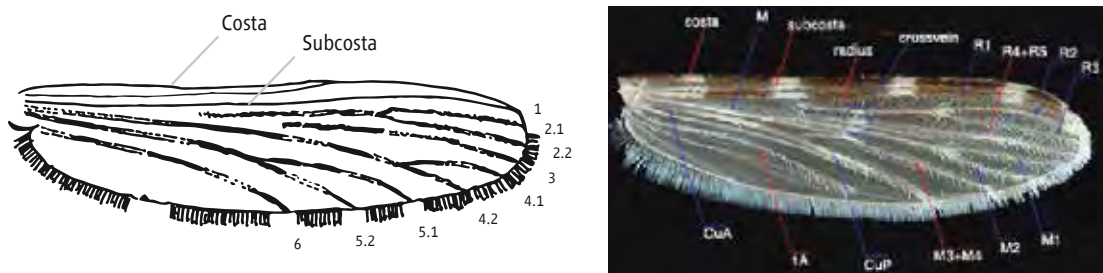
Figure 2.4 *Anopheles* wing (main veins)

The legs are long and made up of a short coxa joined to the body, followed by a short trochanter, then a long femur, a long tibia, and long tarsus which are made up of five parts (Fig. 2.5). The five parts are numbered 1–5 with segment 1 being closest to the body. At the end of the leg is a pair of claws. The legs are also covered with scales which may be of different colours and are used in species identification.
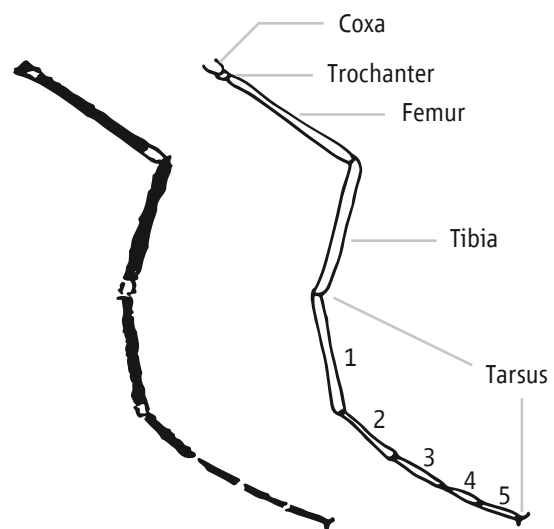
Figure 2.5 *Anopheles* leg

Abdomen

The abdomen has eight visible segments (Fig. 2.6). The upper plates are called tergites, and the lower plates are called sternites. They are joined by a membrane which allows the distension of the abdomen when the female takes the blood-meal.

2.4.2 External anatomy of *Anopheles* larva

The body of the larva is divided into three segments; the head, thorax and abdomen (Fig. 2.6a). All parts of the body have hairs attached to them.
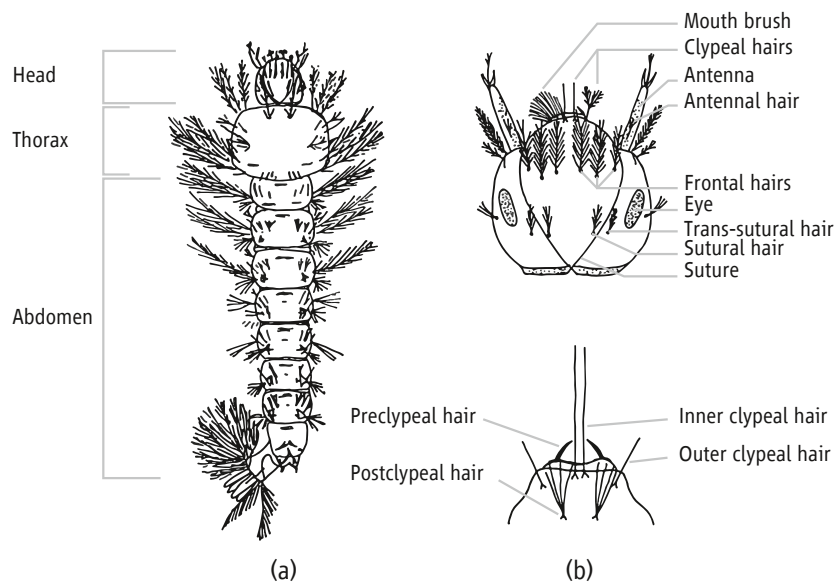
Figure 2.6 (a) body parts and (b) head of an anopheline larva

Head

The head has a pair of antennae, one on each side. The shafts of the antennae have hairs at the end and on the sides (Fig. 2.6b). A pair of mouth brushes lies at the front of the head. The upper surface of the head has several hairs; the position and shape of these hairs are important as a means of identification.

Thorax

The thorax is formed of three parts: the prothorax, the mesothorax and the metathorax (Fig. 2.7). The hairs on these parts of the thorax are called prothoracic, mesothoracic and metathoracic hairs (Fig 2.7a). Both the upper and lower surfaces have hairs. On the lower surface of the ventral part of the thorax there are several hairs including three groups on each side with four hairs in each group. These groups are the prothoracic pleural group, the mesothoracic pleural group and the metathoracic pleural group (Fig 2.7b). These hairs are also important in species identification.
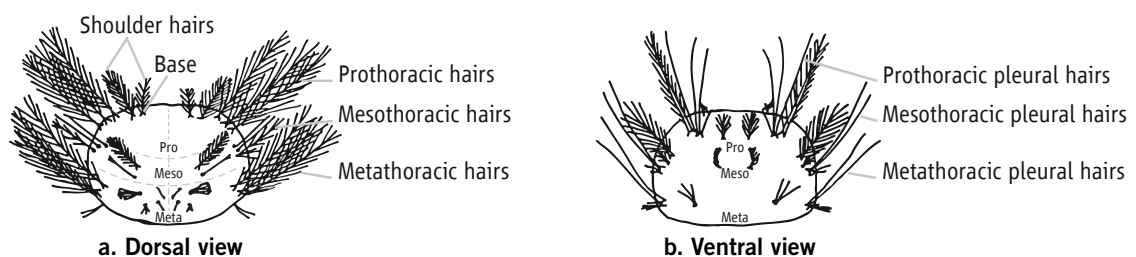
Figure 2.7 Thorax of an anopheline larva (a. dorsal and b. ventral views)

Abdomen

The abdomen has eight similar segments and two modified segments (Fig. 2.8a): the 9th segment has a pair of spiracles and the 10th is the anal part. Well-developed fan-shaped hairs, called palmate hairs, are present on segments 4–6 and sometimes also on segments 1–3. Each segment has up to four tergal plates on its dorsal side. There is usually a pair at the anterior and a second pair at the posterior of each segment, and there are also two accessory plates (Fig. 2.8b). The 9th abdominal segment is joined with the 8th segment and carries the spiracles through which the larva breathes. On each side of the 9th segment is a pectene (Fig. 2.8c), which is a triangular plate with comb-like teeth. Most of the upper surface of the anal segment is occupied by a large tergal plate called the saddle (Fig. 2.8c). Hairs may arise from the saddle or from the anal segment. On the lower surface of the anal segment is a series of hairs called the ventral brush. Four anal gills extend from the anal segment.

a. **Palmate hair (right) and abdominal plates of anopheline larva**

b. **Typical abdominal segment**

c. **Tail end of larva, side view**
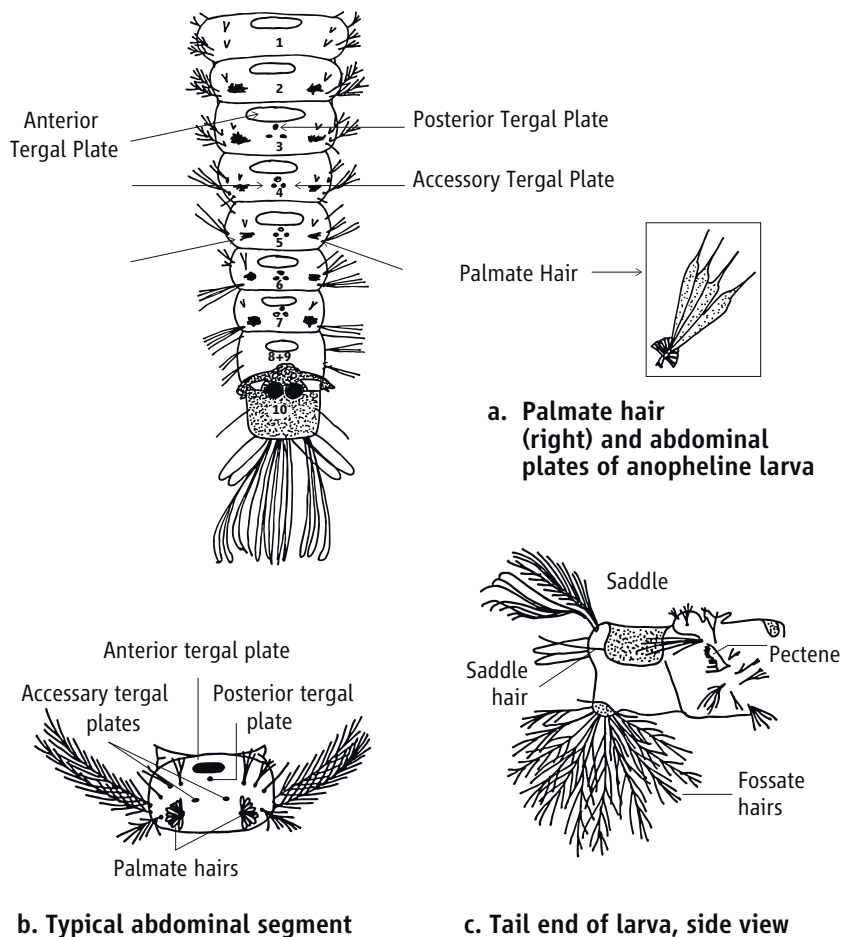
Figure 2.8   Abdomen of an anopheline larva

2.4.3   Keys for the morphological identification of *Anopheles* adults

Keys for the identification of anopheline adults and larvae have been developed for most parts of the world. Participants must first be sure to select a taxonomic key which has been developed for the geographic area concerned or as near to it as possible.

The type of identification key that is most commonly used comprises pairs of statements and is called a dichotomous or couplet key. In this type of key only one of each pair of statements

correctly describes the specimen. Participants must decide which statement is correct for their specimen. At the end of the statement will be either a number indicating which couplet to use next, or the correct name of the specimen. When going on to another couplet, choose the correct answer in that couplet and continue working through the key until the name of the specimen has been identified.

Example

A specimen whose wings have pale and dark scales, the legs are speckled and half of the proboscis is pale, would be identified in the following key as species E.

1. Wing scales are dark 2

   Wings with pale and dark scales 3

2. Legs with dark scales only .... Species A

   Legs with pale and dark scales .... Species B

3. Legs with dark scales only .... Species C

   Legs with pale and dark scales (speckled) 4

4. Proboscis all dark .... Species D

   Proboscis with pale scales on apical half .... Species E

2.4.4   Other techniques for species identification

Some anopheline species are similar in external morphology, but belong to different species. These are the genetically-related sibling species and are morphologically grouped under the same complex. For example, in the *Anopheles gambiae* complex (also known as *Anopheles gambiae* sensu lato or s.l.), there are seven different species: *A. gambiae* sensu stricto (s.s.), *A. arabiensis, A. quadriannulatus* species A, *A. quadriannulatus* species B, *A. bwambae, A. merus,* and *A. melas.* It is not possible to differentiate between these species by using an identification key based on external morphology. If the particular species cannot be identified by external morphology, the name of the complex, for example, *A. gambiae* s.l, should be recorded.

Note: Correct species identification is essential for epidemiological studies and control programmes. Problems in classical *Anopheles* taxonomy include not only strong morphological similarity between species, but also pronounced morphological variation within species. Accurate species identification usually requires rearing to enable correlation of adult and immature morphology.

2.4.4.1   An overview of methods and markers most widely used in *Anopheles* identification

Cytogenetics

Cytogenetics involving the karyotyping of polytene chromosomes was one of the earliest tools for the study anopheline genetics. One of the disadvantages of this technique in *Anopheles* mosquitoes identification is that the polytene chromosome preparations must be made from ovarian tissue or fourth instar larvae. This limits the samples to either adult blood-fed female mosquitoes or late instar larvae. In addition to a shortage of experienced personnel trained to read polytene chromosome preparations, markers are not abundant or particularly

informative in some species. Despite the limitations, this method remains integral for much contemporary work. Cytogenetics has proven immensely useful for differentiating sympatric taxa and chromosomal forms. In fact, cytogenetics remains the only tool to reliably differentiate between all of the *A. gambiae* s.s. chromosomal forms and until recently was the only tool to reliably differentiate between all nine members of the *Anopheles funestus* group.

Chromosomal inversions have also been used to address phylogenetic issues regarding the origin, maintenance and introgression of inversions between sympatric populations and taxa for many anopheline species groups. Such information has been used as evidence for genetic introgression of adaptive chromosomal variants between the two sibling species, *A. gambiae* s.s. and *Anopheles arabiensis.*

Isozyme analysis

Isozyme analysis is a traditional technique with proven utility, but has largely given way to PCR-based technologies. Among the drawbacks of isozyme analysis are that specimens must be fresh or kept frozen until analysis and that the procedure itself requires a relatively large amount of sample material compared to the few nanograms of DNA required for PCR. Nonetheless, isozymes have provided valuable data on which much of the contemporary work in anophelines is based.

The low polymorphism of allozymes and the apparent stability of allele frequencies among populations of *A. gambiae* s.s. led to observed discrepancies between allozyme data and karyotype designations.

Classical genetic markers

This class of markers is most often typified by targeting a defined gene or genetic fragment for analysis using a variety of techniques to evaluate genetic variability down to the resolution of Single Nucleotide Polymorphisms (SNPs). Sequencing, single-strand conformation polymorphism (SSCP) and restriction fragment length polymorphism (RFLP) are the most common techniques used for assessing polymorphism of these genetic targets.

Mitochondrial DNA

The mitochondrial genome is frequently used in phylogenetic and population genetic studies. The sequence and genome organization of the *A. gambiae* mitochondrial genome was published in 2002. This information was quickly applied in field and laboratory studies where both coding [i.e. NADH dehydrogenase subunit 5 *(ND5)* and cytochrome oxidase subunits I and II *(COI* and *COII)*] and non-coding (16S and 12S RNAs) regions are frequent targets for these analyses. Mitochondrial DNA has also been used in investigations of a wide variety of anophelines and anopheline species complexes.

Ribosomal DNA (rDNA)

The intergenic spacer (IGS) and internal transcribed spacers (ITS1 and ITS2) within the nuclear ribosomal genome have become popular targets for addressing taxonomic issues among anophelines. Researchers have discovered that the nucleotide sequence of these spacer regions are often much more polymorphic between species than within species. This region of the genome is therefore useful for delineating molecular differences between cryptic

species by length or sequence polymorphisms. As the number of recognized anopheline species complexes grows, sequence information is developed into PCR-based diagnostic tools that differentiate between cryptic taxa. Most of these molecular diagnostics fall into three categories: *(i)* tests based on sets of species-specific PCR primers that produce differential PCR products, *(ii)* rDNA diagnostics based on unique RFLP patterns resulting from enzymatic digestion of a conserved PCR product, and *(iii)* tests based on specific DNA hybridization to a species-specific sequence. Species-specific dot blots of this type have been constructed for taxa that are cryptic or difficult to differentiate from other vectors and non-vectors.

Microsatellite DNAs

Simple tandem repetitive DNA, more commonly known as microsatellite DNA, has become a popular tool for genetic studies of anophelines. Microsatellites, which have been described in both Old and New World anophelines, have proven to be especially useful for examination of population genetics and genetic mapping. The high polymorphism of these markers lends itself to study of the population structure within this complex of mosquitoes, both at the micro- and macro-geographic levels. Most of this work continues to focus on *A. gambiae* s.s. and the chromosomal forms of this species complex, but there is a growing body of work on the genetic structuring, population stability, and gene flow among populations of *A. arabiensis*.

Random Amplified Polymorphic DNA (RAPD)

Random amplified polymorphic DNA (RAPD) markers have been used to distinguish between *A. gambiae* and *A. arabiensis*. It is a potential tool to differentiate cryptic mosquito species. There are some limitations about using RAPD for species identification at the sibling level.

Demonstration

In the laboratory, live and preserved specimens of anopheline and culicine mosquitoes at the various stages of their life-cycle will be demonstrated. Participants should take time to examine the preserved or pinned specimens and observe the differences at each stage of the life-cycle.

Exercise 2.1

Describe the characteristics of anophelines that distinguish them from culicines.

You will be provided with a compound and dissecting microscope, forceps, dissecting needles and freshly pinned adult female anophelines and larval specimens on slides. Identify the specimens to the species (or species complex) level.

LEARNING UNIT **3**

Sampling malaria vectors

Learning Objectives:
by the end, participants should be able to...

■ Explain the importance of establishing entomological survey profiles

■ Identify resting places of adult mosquitoes

■ Describe the adult mosquito collection methods

■ Apply the different components of each adult mosquito collection method

■ Identify potential breeding sites of malaria vectors

■ Collect larvae and pupae using dipper and larval net

■ Handle and transport larvae and pupae collected in the field to the laboratory

■ Kill and preserve mosquitoes

3.1   Entomological surveillance

Entomological surveys are an essential component of malaria vector control programmes, operational activities and research. Four main types of survey are used in vector studies: *(i)* preliminary surveys, *(ii)* routine or trend observations, *(iii)* spot checks, and *(iv)* focal investigations.

The main aim of entomological surveillance is to gather baseline data for planning anti-vector measures, including:

- Distinguishing *Anopheles* from other insects
- Identification of malaria vector species
- Vector population density
- Rate of infection (sporozoite rate, oocyst rate)
- Longevity of vector (parous, nulliparous)
- Feeding habit (zoophilic, anthropophilic)
- Behaviour (exophilic, endophilic, exophagic, endophagic)
- Seasonal activities
- Larval habitat
- Type of water for breeding sites
- Susceptibility to insecticides
- Residual effect of insecticides

3.1.1   Preliminary surveys

Preliminary surveys are original, basic, short-term surveys used to gather baseline data for planning vector control measures. They provide information on: the identity of specific vector species; their resting and feeding habits, seasonal densities, and longevity; the types of water bodies used as breeding sites; and their sensitivity to available insecticides.

3.1.2   Regular or trend observations

These are long-term observations carried out regularly, e.g. monthly or half-yearly, for the purpose of monitoring and evaluating the impact of control measures. They provide information on changes in vector density, infection rates, behaviour, and susceptibility of vectors to insecticides.

3.1.3   Spot checks

Spot checks are carried out in localities that are chosen at random. As the fixed stations often used to monitor mosquito populations may not be representative of all areas, spot checks may be conducted randomly in selected areas to supplement routine observations or obtain a clearer indication of the effects of control measures.

3.1.4   Focal investigations

Focal investigations are undertaken in areas of new or persistent malaria transmission to determine why there is transmission or why the disease is not responding to the measures being applied, and to identify the best approaches to control.

3.2   Adult mosquito collection methods

3.2.1   Hand collection of indoor-resting mosquitoes

Many of the anopheline species which are malaria vectors rest indoors. Hand collection provides information about usual resting places, resting density, and seasonal changes in density. It also provides live specimens for susceptibility and bioassay testing and for observations on mortality among mosquitoes from houses where insecticide is present on bednets or on the walls.

Equipment

Sucking tube, flashlight, paper cups with covering net, cotton wool, rubber bands, mosquito cages, a card box container or insulated picnic box, chloroform and towels (Fig. 3.1).

How to use a sucking tube:

▶ With the mouthpiece in the mouth, hold the sucking tube with its opening 1–2 cm away from the mosquito.

▶ Move the end of the sucking tube closer to the mosquito and, at the same time, suck gently but quickly so as to draw the mosquito into the tube.

▶ Place a finger over the tube to prevent the mosquito from escaping.

▶ Place the end of the tube, with the finger still in position, near the hole in the mesh covering the paper cup. Remove the finger and quickly put the tube into the hole.

▶ Blow gently into the mouthpiece so as to transfer the mosquito to the paper cup; at the same time, tap the tube with the index finger to disturb resting mosquitoes.

Do not collect more than five mosquitoes in one sucking tube before transferring them to the paper cup.

Figure 3.1   Sucking tube (or aspirator) and paper cup for hand collection of adult mosquitoes

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Hand collecting of indoor-resting mosquitoes

Mosquitoes should normally be collected early in the morning after the house occupants are up and dressed. In any village at least 10 houses should be searched in order to provide a representative sample. The agreement of the householders should be obtained well in advance of the search, such as the night before. The occupant should not open the windows.

Mosquitoes caught alive in houses may be kept for 24 hours. This will allow a check on the 24-hour mortality rate among mosquitoes from houses with insecticide-treated nets or collected from sprayed houses.

The whole house should be examined or, if too large, spend up to 15 minutes searching room by room. Pay special attention to rooms in which people slept the previous night. With the aid of the flashlight, look for mosquitoes on walls, on the ceiling, behind and under furniture, inside large pots and jars, and under beds (Fig. 3.2). Conduct a systematic search of the house starting at the main door and searching to the left moving clockwise around the inside of the house.

Use a separate cup for each house. The cups must be clearly labelled in pencil with at least the following information: locality; date and time of collection; minutes spent on collecting; house number or householder's name; type of structure (house, animal shelter, store, etc.); whether sprayed and, if so, when; number of people and/or animals in the room during the previous night; and the collector's name. Alternatively, the label may include only the locality, date, house number and collector's name, and a collection form used (to accompany the paper cup) to fill in the complete information.

Figure 3.2   Hand collection of mosquito

Keeping mosquitoes alive in the field

If mosquitoes are to be kept for some time in the field and during transport, take the following precautions to keep them in good condition:

▶ Soak pieces of cotton wool in 5–8% sugar solution, squeeze out any excess sugar solution and place the cotton wool over the tops of the cups.

▶ Place cups holding mosquitoes upright in a cardboard box or, preferably, an insulated cold box so the mosquitoes do not become heated.

▶ Cover the cups with a damp towel and keep the towel damp until the mosquitoes reach the laboratory.

▶ Make sure that the mosquitoes are kept in places that are free from insecticide contamination and away from ants.

▶ Before transport, pack newspaper or other material between the cups to minimize movement and drive slowly and carefully.

Killing mosquitoes

Add a few drops of chloroform (or ethyl acetate) to a pad of cotton wool and place it on top of the netting of the paper cup. Cover the cup with a glass Petri dish to prevent the chloroform from evaporating. Do not use a plastic Petri dish as this will be dissolved by the chloroform. Take standard safety precautions when handling chloroform as for any other dangerous chemical.

3.2.2   Spray sheet collection of indoor–resting mosquitoes

Spray sheet collection involves using a pyrethrin space spray to knock down mosquitoes resting inside a house and collecting them on white sheets spread on the floor and other flat surfaces in the house.

It is unlikely that all the mosquitoes resting in a house would be obtained using the hand collection method. Using the spray sheet collection method, it should be possible to collect almost all the mosquitoes from a well-closed room sprayed with a fine mist of pyrethrin solution. This method of collection allows quantitative studies to be undertaken, including measurement of

- ▶ indoor resting density (the number of mosquitoes resting indoors during the day)
- ▶ human-biting density (indirectly)
- ▶ seasonal changes in indoor resting density
- ▶ number of mosquitoes remaining in a given room following a hand collection

Equipment

White cotton sheets (sizes: 2 x 1 m, 2 x 2 m and 2 x 3 m); hand sprayers; aerosol insecticides; pyrethrin solution; kerosene; small Petri dishes; paper cups; hand lens; forceps; a container (or preferably a cold box) for transporting mosquitoes; cotton wool; filter paper and a torch.

The hand sprayers should be of the double-action type with an air valve (Fig. 3.3). The pyrethrin solution should be prepared at a concentration of 0.2%–0.3% in kerosene. Take the necessary safety precautions when handling pyrethrin, and always keep it out of the reach of children.

Figure 3.3   Hand sprayer

Preparation of rooms for spray sheet collection

It is usual for the work to be carried out by a team of three or four people so that collections can be made in 8–10 rooms in each locality.

Ensuring that any resting mosquitoes are disturbed as little as possible, prepare a room for spraying as follows:

► Remove all people and animals.

► Remove or cover all food, water and equipments.

► Remove all small items of furniture.

► Cover all openings and eaves with cloth or mosquito netting.

► Spread the white sheets so that they completely cover the floor and all flat surfaces of the remaining furniture; sheets should also be spread under tables, beds and other places where mosquitoes may hide.

► Close all windows and doors.

Carrying out space spraying and collection of mosquitoes

One of the team members should walk round the outside of the room and spray in open spaces or holes in the walls and eaves. The same person or another member of the team should then enter the room, close the door and, moving 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.

Starting from the doorway, pick up the sheets one at a time by their corners. Carry the sheets outside. Collect the knocked down mosquitoes outside in daylight using forceps. Place collected mosquitoes in a labelled Petri dish with a layer of damp cotton wool and filter paper on top of the cotton wool. Use separate Petri dishes for each house, and label the dishes with all the essential information (Fig. 3.4).

Figure 3.4   Spray sheet collection used for sampling indoor mosquitoes

3.2.3   Window trap collection (exit traps)

Some mosquito species bite indoors, but leave the house soon after biting (endophagic and exophilic). Exit or window traps are commonly used to capture mosquitoes leaving houses. These traps are used to determine:

► the mosquito species that bite indoors but rest outdoors

► the effect of indoor residual spraying and insecticide treated nets on the normal movement and feeding habits of mosquitoes

► 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

Equipment

Window trap, mouth aspirators, paper cups with net covers, towel, insulated picnic box and dark cloth or netting to block openings in rooms.

Fitting window 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.

Figure 3.5   Window trap (Exit)

3.2.4   Outdoor collection of adult mosquitoes

Some mosquito species do not enter houses but bite outside and then rest on vegetation or on solid surfaces in sheltered places such as the banks of streams and ditches, holes in rocks, culverts, cracks in stone walls, caves, animal burrows, on the trunks or stems of larger trees, and in old termite mounds.

Data from outdoor collections is important in evaluating the impact of vector control measures. It provides information on:

► the species that rest outdoors

► the relative numbers of mosquitoes resting outdoors

► seasonal changes in outdoor resting habits

► any alteration in the relative numbers of mosquitoes resting outdoors following the application of insecticides in houses and other buildings

Outdoor collection is carried out in either the natural resting places described above or in shelters specially constructed for that purpose. Artificial shelters have the advantage of providing

concentrated sites for collections and more representative samples that can be used for quantitative work.

Equipment

The equipment required for outdoor collection is the same as that listed under hand collection of indoor-resting mosquitoes. In addition, a hand net and a drop net may be used. Since the preparation or construction of artificial shelters will be undertaken during field practice, also required are: a barrel, two spades, a pickaxe, and an axe.

Outdoor collection methods

The common methods used to collect mosquitoes resting on vegetation involves the use of a sucking tube, a hand net, or a drop net. Anopheline species that normally rest on solid surfaces are collected with the aid of a sucking tube from natural or artificial shelters. Artificial shelters may consist of large barrels or boxes, perhaps set into riverbanks, or they may be pits dug in the ground (Fig. 3.6, a and b). Well-placed shelters normally yield more mosquitoes than natural environments.

(a) (b)
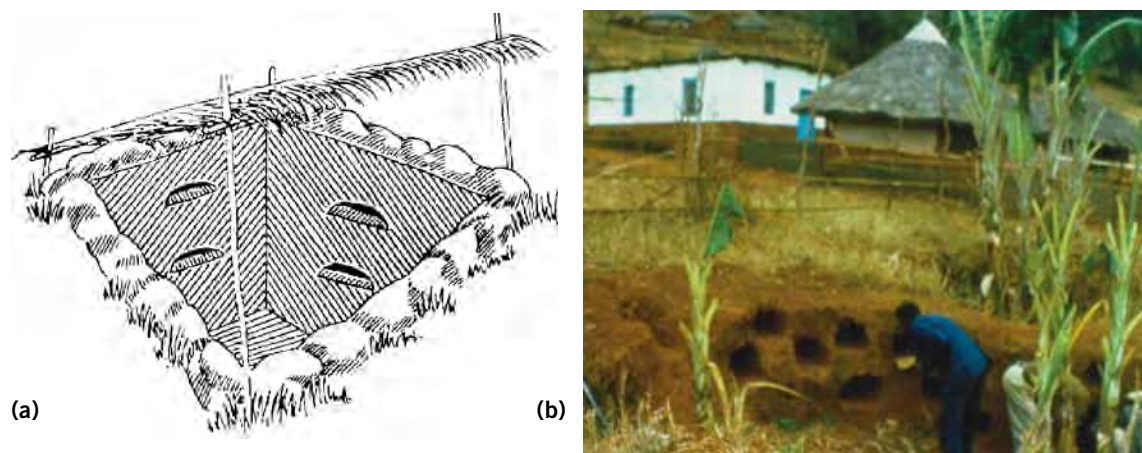
Figure 3.6   (a) Pit shelter with roof and (b) Natural pit shelter

A hand net or sweep net is used to collect mosquitoes resting on vegetation (Fig. 3.7). The correct method of use is to move the hand net swiftly over the tops of tall grasses or close to the ground around bushes. Make sure that the type of shelter, the number of collections, and the total time spent collecting are recorded.

Figure 3.7   A hand net

3.2.5   Direct catches of mosquitoes from baits

Female mosquitoes are attracted to humans and/or animals to obtain blood-meals. The number of vectors biting humans is therefore a major determinant of malaria transmission, and it is important to know:

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> ▸ which anopheline species bite humans and which prefer to bite animals,

> ▸ which of those that bite humans are vectors of malaria,

> ▸ how often a person is bitten by a vector,

> ▸ whether the vectors bite indoors or outdoors,

> ▸ their peak biting time,

> ▸ the seasonal variations in the numbers of mosquitoes biting humans.

Equipment

Sucking tube, flashlight, paper cups with net covers, alarm clock, wooden pegs and a rope (to tether the animal being used as bait), wooden pegs (for the tether), hammer, cotton wool, towels, card box container or an insulated picnic box.

Human baits

Although collection of mosquitoes of human baits is useful as a direct measurement of human biting rates, there are ethical concerns because the individuals involved are at risk of infection. Due consideration must be given to this concern and ethical clearance obtained before using the technique. It is recommended to avoid using this technique unless it is absolutely essential, especially if other safer techniques are available to provide proxy estimates of human biting rates. These alternative techniques will be described in subsequent sections. All the collectors should be trained for appropriate communication and ways of mosquito collection.

Persons serving as human baits should take appropriate antimalarial prophylaxis to avoid contracting malaria during collection of biting mosquitoes. It is not necessary to allow mosquitoes to feed; they should be collected as soon as they settle on the skin, since it can be assumed that biting would normally follow. Landing rates should therefore be measured instead of biting rates.

If possible, a house should be selected in the area of the village with the greatest number of cases of malaria. A human collector is seated indoors and another is seated outdoors. In some situations it may be better to have two collectors indoors and two outdoors in order to reduce the possibility of collectors falling asleep during the night. Collectors should switch sites every hour. The collections are often made during the entire night (if necessary) or during part of the night. Collectors may also work in shifts during the night.

The person serving as human bait should adjust his/her clothing so that the legs are exposed as far as the knees and sit quietly. When a mosquito is felt, quickly turn on the torch, collect the mosquito[1] with the sucking tube and transfer it to a paper cup. Use one cup for each hour of collecting. Do not smoke while collecting.

Alternatively, one person can serve as bait and another as collector. The person acting as bait sits or lies in a quiet place, inside or outside the house as appropriate, with the clothing adjusted to expose as much skin as is acceptable. The collector checks for and collects biting anophelines every two or three minutes. Note the habitual sleeping time of the local people;

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[1]. Unless the collector is an extremely experienced entomologist, collect all mosquitoes and sort out anophelines afterwards rather than trying to select anophelines during bait-catching.

this information will be used to study whether most human-vector contact occurs indoors or outdoors, and the average number of bites that villagers receive at each site during the night (Fig. 3.8).

Animal baits

Collecting from animal bait is normally carried out in the same location and at the same time as collecting from human bait. Before sunset, select a tame animal from the village, usually a cow. The collecting site should be near the place where the animal usually passes the night. Tie the animal securely. Examine the animal every 2–3 minutes and collect all the mosquitoes that are found. Keep each hour's collection in a separate paper cup (Fig. 3.9).

Figure 3.8 Collection of mosquito from human bait

3.2.6 Collecting mosquitoes in baited trap nets

In this section, participants will learn how to use trap nets; the purposes of collection by this method are the same as those for direct collecting.

Figure 3.9 Collection of mosquito from animal bait

Animal-baited trap nets generally produce more mosquitoes than can be collected by direct capture from animals; the opposite, however, is true for human-baited trap nets. For this reason, standard night collecting from bait usually involves direct collection from humans indoors and outdoors, and collection from animal-baited trap nets outdoors.

Equipment

Sucking tube, torch, paper cups with net covers, cotton wool, towels, an insulated picnic box, an alarm clock, two camp beds, two small mosquito nets with frames to fit the camp beds, two trap nets for human bait, one trap net for animal bait, wooden pegs and a rope (for tethering the animal), hammer, pegs and string (for securing trap nets), and a needle and thread (for repairing trap nets).

Collecting by means of human-baited trap nets

As previously noted, the direct catch of mosquitoes using human baits is not generally recommended because of ethical concerns over the exposure of collectors to malaria infection. In this case an alternative method of collection must be used which gives a representative sample of the vector population that would bite humans.

One technique uses two trap nets set up so that one covers a sleeping area and the other forms the outer trap net. The inner net is put up around a camp bed to protect the person acting as

Figure 3.10 Trap net with human as bait

bait (Fig. 3.10). The bottom of the outer net is stretched tightly and tied to pegs in the ground, leaving 15–20 cm between the ground and the lower edge of the net. At sunset, the collector enters the trap and lies on the bed and sets the alarm clock to ring after one hour. When the alarm rings, all the anophelines in the trap net are collected. The collecting period should not exceed 10 minutes. The collector then returns to the bed and sets the alarm as before. The procedure is repeated throughout the night.

Collecting by means of human-baited CDC light traps

A CDC[1] light trap is installed in the bedroom beside a bed with a mosquito net. The human bait sleeps under the net, while the light trap attracts female anophelines that have entered the room to bite the person under the net. The trapped mosquitoes can be used as a proxy to estimate the biting rate. In the house, one volunteer acting as human bait sleeps alone during the night. One CDC light trap is positioned indoors, fitted with incandescent bulbs, and placed close to the human volunteer sleeping under an untreated bednet in his/her usual sleeping place. The light trap is installed at about 1.5 m above the floor next to the foot of the bed. Trapped mosquitoes are removed the next morning (Fig. 3.11).
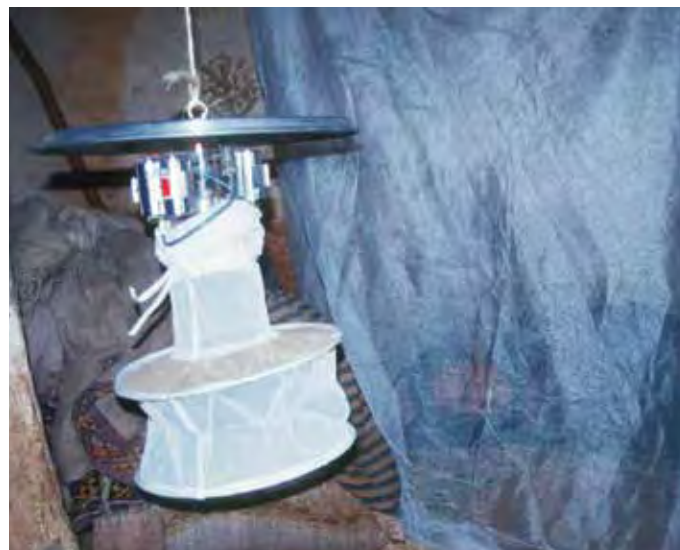
Figure 3.11 Light trap positioned next to a human-bait under an untreated bednet

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[1.] US Centers for Disease Control and Prevention.
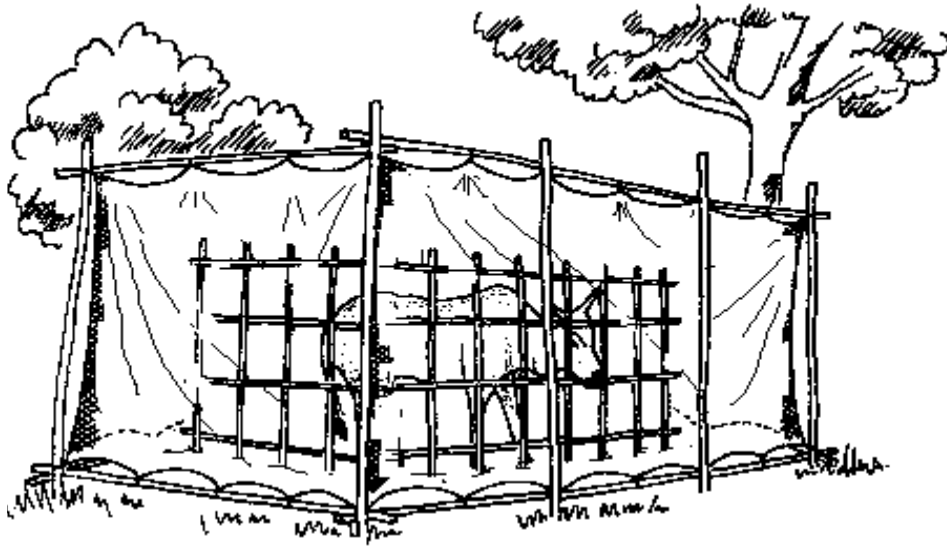
Figure 3.12 Animal-baited trap net

Collecting by means of animal-baited trap nets

An animal-baited trap net is situated close to where the animal is customarily kept overnight. Animal-baited traps are normally used outdoors. The trap net (Fig. 3.12) is similar to that used for collecting mosquitoes attracted to human bait. The animal must be securely tethered so that it cannot break free and damage the trap or harm itself.

If there is to be repeated collection at the site, a small enclosure may be built to confine the animal. Place the animal in the trap at sunset and collect mosquitoes every three hours.

3.3 Methods for collecting larvae and pupae

3.3.1 Where to look for anopheline larvae and pupae

Each type of mosquito prefers to lay its eggs in a particular kind of water. Some will lay only in fresh, clear, water with some shade, others only in brackish water; some may lay eggs in very small quantities, such as water in a hoof-print.

It is most important to know the preferred breeding sites of the anopheline mosquitoes that transmit malaria in the area, and the densities of larvae and pupae at these sites. Collecting from different types of breeding site in an area will allow to:

► determine the species present,

► ascertain the preferred breeding sites of each vector species,

► make an assessment of the effectiveness of a vector control programme.

To identify the preferred breeding sites, it is essential to be systematic and check all possible breeding places, even those that are difficult to reach. This will indicate the type of site most likely to harbour the larvae of anopheline mosquitoes.

3.3.2.1 Potential breeding sites

They include:

▶ Small rain pools, hoof-prints, drains and ditches, where the entire surface of the water should be examined.

▶ Brackish water (where fresh water and salt water mix).

▶ Streams, which should be searched at the edges where there is vegetation and the water moves slowly.

▶ Ponds, lakes and marshes where larvae usually occur in vegetation around the edges, but can sometimes be found far from the shore among floating vegetation.

▶ Special sites, such as wells and water containers made of cement, where the entire surface of the water should be examined.

Whatever the collecting method used, the breeding place must always be approached cautiously, facing the sun: if the larvae are disturbed by shadows and movement many of them will swim downwards and disappear from view. It will then be necessary to wait quietly for several minutes until they return to the surface of the water. Particular care should be taken to search where small amounts of debris accumulate on the water surface or where vegetation rises from the water. Both clean and polluted water should be searched, since anophelines may be present in polluted water.

Equipment

Dipper, larval net, large tray, pipette, specimen tubes (vials), 70% alcohol solution, cotton wool, a pencil, and safety match or lighter. If live specimens are required for insecticide testing, larger bottles or a wide-mouthed vacuum flask will also be needed.

Use of the dipper

A white enamelled or plastic dipper is preferable, because this allows the larvae to be seen most easily (Fig. 3.13).

▶ Lower the dipper gently into the water at an angle of about 45º, until one side is just below the surface.

Figure 3.13 Dipper

▶ While dipping, care should be taken not to disturb the larvae and thus cause them to swim downwards – if they are disturbed, wait for a minute or two until they come up to the surface again, and then continue dipping.

▶ Move along the breeding site, skimming the surface of the water with the dipper.

▶ Lift the dipper out of the water, making sure that the water containing the larvae and pupae is not spilled.

▶ Hold the dipper steady until larvae and pupae rise to the surface of the water.

▶ Collect the larvae and pupae by means of a pipette and transfer them to a bottle or vial.

▶ Do not throw the residual water back into the breeding place as this may further disturb the larvae and pupae.

▶ Make sure to note whether the larvae are immature (1st and 2nd instars) or mature (3rd and 4th instars), since mature larvae are of more concern in larviciding programmes.

Estimation of larval density

Count the number of dips in each type of breeding place – this helps in calculating larval density in each type of water body. The larval density in each breeding site can be calculated by instar or as the number of 3rd and 4th instar larvae of each species collected per dip or 10 dips (or per 100 dips if the density is too low). Also note the time spent in minutes while collecting in each type of breeding place (see Fig. 3.14).

Figure 3.14 Larva collection in breeding sites

Use of the larval net

A larval net for collecting larvae and pupae in ponds and lakes consists of a fine mesh net mounted on a wooden handle and a plastic bottle or tube tied to one end (Fig. 3.15). To collect larvae and pupae, sweep the water surface by holding the net at an angle and moving it through the water. Larvae and pupae on the water surface will be swept into the net and will be collected in the plastic bottle or tube.
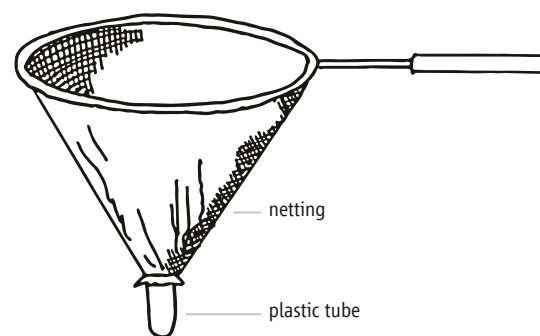
Figure 3.15 Larval net

A simple net with no attached bottle or tube can be used. After sweeping, the net should be inverted into a bowl of water and its contents dislodged. The water in the bowl is then searched for larvae and pupae, which are picked up and transferred to a bottle or vial by means of a pipette.

The net used for sampling from wells is similar to the larval net but does not have a wooden handle; instead, it is held at an angle by four strings and controlled by a long string or rope.

Larval collection by pipette

In some circumstance where the breeding places are limited and shallow, a pipette can be used for larval collection.

3.4   Transporting live larvae and pupae

Place all the specimens from a particular breeding place in one bottle or vial and label it. The label must be written in pencil on a piece of paper and dropped into the specimen bottle. Do not use a ballpoint pen as the ink dissolves in water.

The larvae and pupae collected must arrive alive and undamaged at the laboratory. Cap each bottle or vial tightly so that water cannot spill. Make sure that there is air in the top 1–2 cm so that the larvae and pupae can breathe for a few hours. If a larger air space is left, the water will become agitated during transportation and the specimens will suffer damage, particularly loss of hairs.

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. Pack bottles and vials carefully so that they are not jolted during transport. 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. For colonization of larvae see Annex 1.

3.5   Killing and preserving larvae and pupae

Hold the vial containing the larvae over a flame of a burning cotton wool soaked with alcohol (placed on a rock) for about 30–60 seconds. Alternatively, larvae may be transferred to hot water (50 °C–70 °C) using a pipette.

- ▶ Carefully pour the water until as much water as possible has been removed from the vial while keeping the killed larvae in the vial.

- ▶ Add 70% alcohol (ethanol) to the vial.

- ▶ Add a plug of loose cotton wool to the vial.

- ▶ Prepare a label with all of the following information written in pencil (do not use a pen): locality, type of breeding site, number of dips taken, time spent in minutes, date of collection, and name of collector.

- ▶ Put the label inside the vial above the cotton wool.

- ▶ Close the vial tightly (Fig. 3.16).
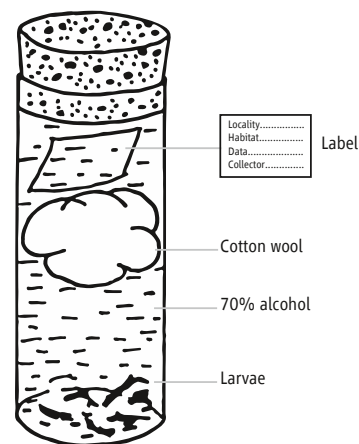
Figure 3.16 Preserving larvae in vials

Exercise 3.1

Working in small groups, participants will discuss the different methods of collecting mosquitoes. Using the schematic representation of the malaria vector life-cycle below (Fig. 3.17), indicate the points for the different methods of mosquito collection.
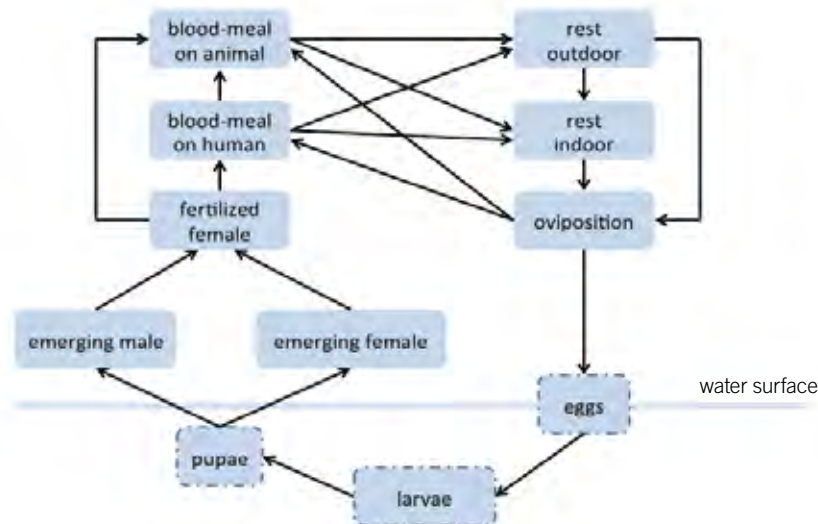
Figure 3.17 Schematic representation of the malaria vector's life-cycle

Exercise 3.2

In the laboratory, participants should practise the following:

► Picking adult mosquitoes from a cage and put them in paper cups.

► Picking live larvae and pupae using droppers and put them in vials.

► Killing and preserving adults, larvae, and pupae.

The applications of different methods for entomological surveys are presented in Table 3.1.

Exercise 3.3

In the field, participants will work individually and in groups to carry out the following activities:

► Each participant will search for indoor-resting mosquitoes in three houses.

► Each participant will spend at least 20 minutes searching for outdoor-resting mosquitoes.

► In groups of four, participants will carry out spray-sheet collections in one house per group.

► Before sunset a light trap will be set next to a person sleeping under an untreated bednet, 1–2 m above the floor, near the foot end of the bed.

► Each participant will collect larvae and pupae from natural breeding sites for at least 30 minutes.

► Participants will practice the correct ways of sitting with bare legs indoors and outdoors during night-landing collections (due to shortage of time, this will be done during the daytime for the sake of practice and demonstration).

Table 3.1   Applications of different methods for entomological surveys

Method of collection	Mosquito density	Species identification	Biting time	Mosquito behaviour (exophilic, endophilic, exophagic, endophagic)	Parity rate	Human blood index	Sporozoite rate	Insecticide susceptibility test	Irritancy of insecticides
Hand collection of indoor-resting mosquitoes	+	+	−	+	+	+	+	+	−
Spray sheet collection of indoor-resting mosquitoes	+	+	−	+	+	+	+	−	−
Outdoor collection of adult mosquitoes (shelter pits)	+	+	−	+	+	+/−	+	+	+
Human bait collection	+/−	+	+	+/−	+	−	+	+	−
Animal bait collection	+/−	+	+	+	+	−	+	+	−
Human-baited trap net	+	+	+	+	+	+	+	+	−
Animal-baited trap net	+	+	+	+	+	−	+	+	−
Light trap	+	+	−	−	+	+	+	+	−
Larval collection	+	+	−	−	−	−	−	+	−

+   appropriate
−   not appropriate
+/−   in some circumstances

▸ Participants will transport live specimens to the laboratory.

▸ Setting window trap and collecting mosquito.

Exercise 3.4

Working in pairs, participants will *(i)* learn to kill anophelines and *(ii)* determine their abdominal conditions.

LEARNING UNIT **4**

Vector incrimination and malaria control

Learning Objectives:
by the end, participants should be able to...

■ Describe the methods used to incriminate malaria vectors

■ Describe the methods and applications of mosquito age grading and salivary gland dissection

■ Identify the entomological indicators of malaria transmission

■ Calculate the entomological indicators associated with resting and feeding habits, human-vector contact, and entomological inoculation rates for malaria

■ Measure the components of the vectorial capacity model and understand its value for malaria transmission and control

■ Interpret the entomological measurements and their implications for malaria vector control

Introduction

Malaria entomology is the study of the biology and ecology of the mosquitoes that transmit malaria. The aim is to understand the relationships among the vector, its ecology and behaviour, the parasite and the host in order to develop and implement effective vector control strategies. In this unit, a brief introduction will be given about the transmission of malaria and the life-cycle of the mosquito vectors. The importance and purpose of entomological monitoring in malaria control programmes will also be discussed in detail.

4.1   Vector incrimination

The entomological data used to incriminate a vector include:

▸ Presence, abundance and proportion of mosquitoes of a given species infected with sporozoites.

▸ Age or parity of the vector.

▸ Feeding behaviour of the vector:

1. where a mosquito bites
2. when a mosquito bites
3. what host is preferred

From these data it is possible to calculate and compare several entomological indicators concerning the vector:

▸ the human-biting rate of the vector,

▸ its resting habits,

▸ the longevity of the population of vectors,

▸ their infectivity,

▸ the proportion of blood-meals taken on people (human blood index),

▸ the entomological inoculation rate,

▸ the vectorial capacity.

4.2   Vector incrimination techniques

In order to calculate a vector's capacity to transmit malaria it is important to measure a number of key parameters which require close examination of the vectors. These include determining the abdominal condition or blood digestion stages of vectors, which can be used to determine how frequently mosquitoes feed. Dissection and examination of ovaries is required in order to determine longevity and the age of a vector population. Both these parameters are important in calculating vectorial capacity (see below).

Another important parameter that can be measured in the field is the proportion of infective vectors (infection rate in vectors). To do this it is necessary to dissect the salivary glands and examine them for the presence of sporozoites, or to use an immunological method (e.g. ELISA) or a DNA-based method for sporozoite detection.

In this learning unit, participants will learn to carry out these techniques.

Important structures within a female mosquito

Before dissecting an adult mosquito, it is essential to know the position of the different organs within its body. Figure 4.1 shows the structures inside a female mosquito as if the mosquito were cut in half vertically along the middle of the body. The positions of the various structures are as follows:

▶ The salivary glands lie inside the thorax, but are joined to the head by salivary ducts.

▶ The stomach or midgut lies in the abdomen, and the Malpighian tubules are at the bottom end of the midgut.

▶ The ovaries lie on either side of the gut in the posterior part of the abdomen and join at the ampulla to form a common oviduct.

▶ A single spermatheca where the male sperm is stored is attached to the common oviduct.
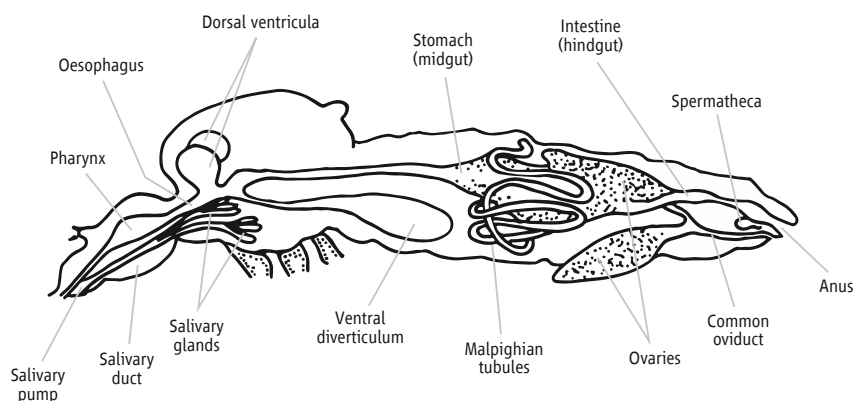
Figure 4.1   Internal anatomy of a female mosquito

Recognizing blood digestion stages

The blood digestion stage refers to the appearance of the abdomen of the female *Anopheles* as the result of blood digestion and ovarian development. In anophelines, ovary maturation (egg development) occurs at the same time as blood digestion. Based on their blood digestion stage or abdominal condition, anophelines can be grouped as unfed, freshly fed, half-gravid, and gravid (Fig. 4.2).

1. *Unfed* – The abdomen is flattened.

2. *Freshly fed* – The abdomen appears bright or dark red from the blood in the midgut. The ovaries occupy only a small area at the tip of the abdomen and this part is not red; it includes only two segments on the ventral surface and at most five segments on the dorsal surface.

3. *Half-gravid* – The blood is dark in colour, almost black, and occupies three to four segments on the ventral surface and six to seven on the dorsal surface of the abdomen. Ovaries occupy most of the abdomen.

4. *Gravid* – The blood is reduced to a small black patch on the ventral surface or may be completely digested. The ovaries occupy all the rest of the abdomen.
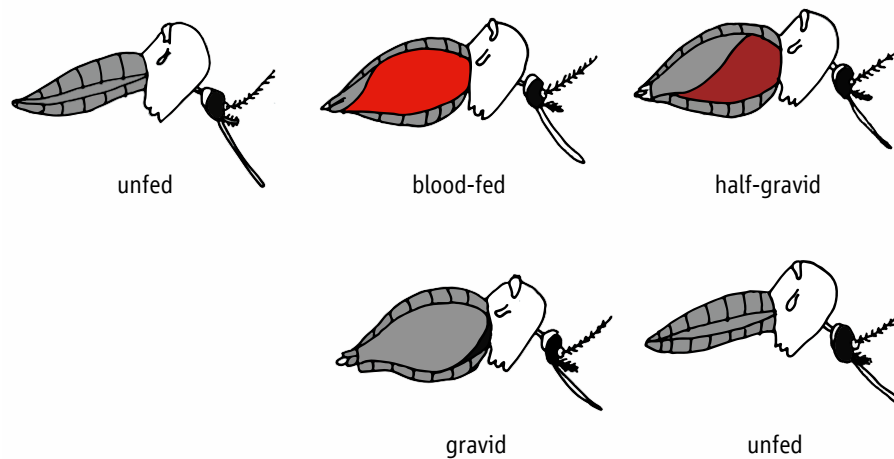
Figure 4.2   Abdominal conditions of a female

Dissecting ovaries and determining parity

Equipment needed to dissect ovaries: dissecting (or stereoscopic) microscope, compound microscope, dissecting needles, fine forceps, slides, dropper and distilled water.

Dissection of the female mosquito to obtain ovaries for parity determination

Parity determination is done by dissecting out the ovaries and examining them to see whether 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 and have not laid eggs).

Only females which are unfed or freshly fed are suitable for this method of parity determination. To dissect out ovaries, proceed as follows:

▸ Kill the female and remove legs and wings.

▸ Place the mosquito on a slide and add a drop of distilled water (Fig. 4.3).

▸ 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.
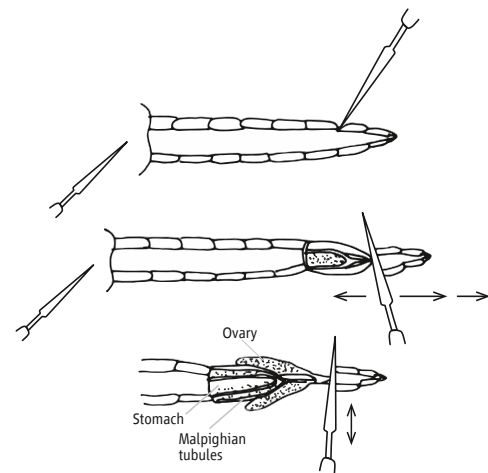
Figure 4.3   Dissecting ovaries

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. 4.4a).
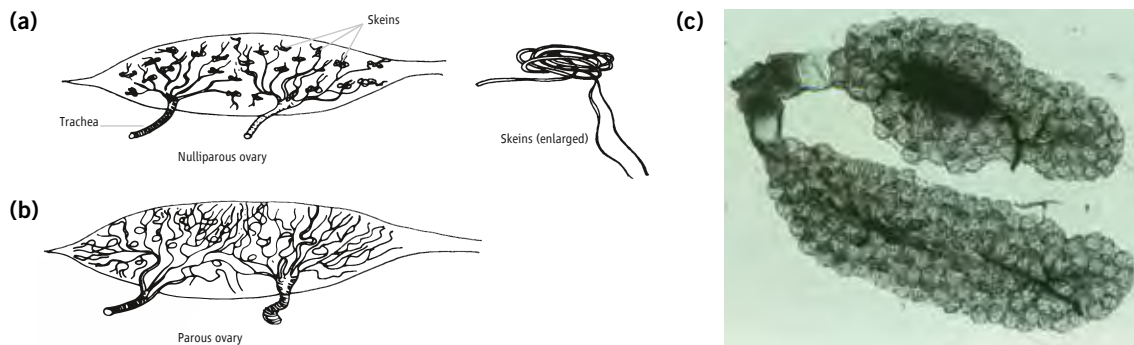
Figure 4.4 Appearance of nulliparous (a), parous (b) and freshly dissected ovaries (c)

▶ Ovaries in which the tracheoles have become stretched out are parous (Fig. 4.4b). Fig. 4.4c shows freshly dissected ovaries.

▶ 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.

Parity as an indicator for longevity

By measuring the proportion of parous mosquitoes in a vector population, changes in vector populations can be monitored and the impact of an intervention evaluated. For example, if a population is increasing, this is usually because more nulliparous adults are emerging, therefore the parous rate decreases. Conversely, as a population gets older, with fewer mosquitoes emerging, the parous rate increases.

The aim of residual insecticide spraying is to reduce malaria transmission by killing mosquitoes that enter dwellings to rest before or after feeding and hence reduce their longevity and their ability to transmit malaria. If residual spraying is effective, there will be fewer parous mosquitoes compared to nulliparous mosquitoes after spraying than before spraying, or if compared to areas that were not sprayed. Parity is an entomological indicator used to determine whether malaria transmission has been reduced.

A nulliparous mosquito cannot transmit malaria because it has not yet acquired the *Plasmodium* parasite. Even a female that has laid eggs once (or twice) may not be old enough to transmit malaria parasites because the gonotrophic cycle – the time from the first blood-seeking to the second blood-seeking – averages only three days whereas sporozoite development takes 10–12 days. A mosquito may therefore need three gonotrophic cycles before it is able to transmit malaria.

The dissection of ovaries and their examination are essential tools in entomological analysis and assessment of the impact of vector control interventions.

Note: In some anopheline species it is possible to observe the scars that form on the common oviduct after each oviposition. Therefore, the age of the mosquito can be estimated by counting the number of scars and multiplying this number by the gonotrophic cycle. This method is difficult and is usually only done in special research projects.

$$\text{Parity rate} = \frac{\text{Number of parous females}}{\text{Number of females examined}} \times 100$$

Dissecting and examining salivary glands for sporozoites

The salivary glands are examined for sporozoites in order to determine which mosquito species carry malaria parasites and the proportion of each species that is infected. 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 to assess the impact of malaria control interventions. The dissection technique indicates whether or not the mosquito is infected with *Plasmodium*, but does not distinguish the species of parasite.

Equipment needed to dissect the salivary glands: dissecting microscope, compound microscope, dissecting needles, fine forceps, slides, dropper, 0.65% saline solution.

Procedure for dissecting salivary glands:

▸ Kill the mosquito, identify the species and remove legs and wings. The salivary glands of nulliparous females do not need to be dissected because they are not infected.

▸ Place the mosquito on a slide, lying on its side with the head pointing to the right (Fig. 4.5) for right-handed participants, or left, for left-handed participants.

▸ Place a small drop of saline solution close to the front of the thorax.

▸ Hold the thorax firmly with a blunt dissecting needle in the left hand for right handed participants or right for left handed individuals.

▸ Place the needle held in the right (or left for left-handed participant) hand on the neck of the mosquito without cutting the neck.

▸ Gently pull the head away from the thorax – the glands will come out of the thorax, attached to the head.

Figure 4.5   Dissection of female mosquito for sporozoites

▸ 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.

Examining freshly dissected salivary glands for sporozoites

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. The glands should be examined under a high-power 40x 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.

Staining sporozoites:

▶ Place a drop of adhesive on the top side of the cover slip and take it off carefully, by using the tip of a dissecting needle, and turn it over with the wet side up; fix it temporarily to one end of the slide. In this way the sporozoites that stick to the cover slip can be saved and stained.

▶ Draw a circle around the salivary glands and sporozoites with a grease pencil on the reverse side of the slide (this makes it easy to locate the specimen later).

▶ Allow the preparation to dry and protect it from ants and flies.

▶ Fix it by immersing the whole slide for a few seconds in methanol.

▶ Stain for 30 minutes with 5% Giemsa stain in buffer solution. The slide may be left face up and the stain applied with a dropper to flood the specimen and cover slip.

▶ Wash well with buffer solution and examine under the high power of a compound microscope.

Enzyme-linked immunosorbent assay (ELISA) for sporozoites

It is now common to use an ELISA method rather than dissection to detect sporozoites in mosquitoes. The tests are based on monoclonal antibodies to circumsporozoite proteins (CSP) and a colour-forming enzyme reaction; the use of species-specific antibody enables the species of *Plasmodium* to be identified. When a large number of specimens are to be tested, this is the preferred method.

$$\text{Sporozoite rate } = \frac{\text{Number of mosquitoes with sporozoites}}{\text{Number of females examined}} \times 100$$

Preparation of blood-meals for identification (Anthropophilic Index)

For a mosquito to be able to transmit malaria, it must bite humans at least twice during its lifetime: once to pick up gametocytes and a second time to inject the sporozoites. Some mosquitoes (both groups and individuals) prefer to feed on humans, some on animals and some to varying degrees on both. Thus, all mosquitoes in a group may not feed on people and not be potential transmitters of malaria. In malaria surveys it is therefore often necessary to know the sources of the mosquitoes' blood-meals. The most anthropophagic mosquitoes are the most efficient vectors.

Equipment

A killing chamber, filter paper (10–15 cm in diameter), plain papers, glass rods or slides or needles, and recording form.

Preparing filter papers for blood-meal squashes (see Fig. 4.6)

▶ Fold the filter paper in half, then fold again and fold twice more.

▶ Unfold the paper, which will now be marked by folds into 16 parts.

▶ Draw pencil lines along the folds from the edge of the filter paper towards the centre, but leave the central 5 cm blank.
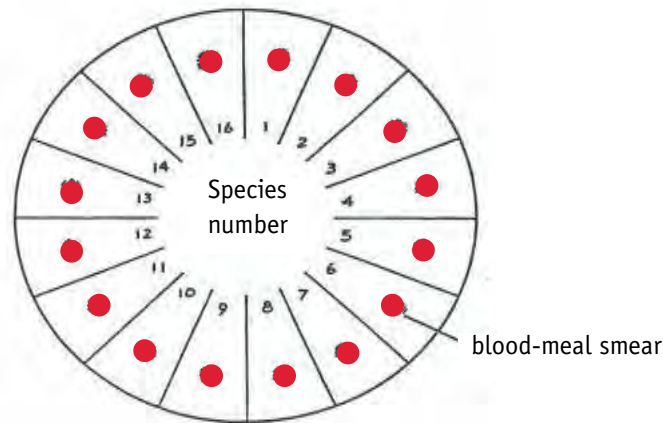
Figure 4.6   Filter paper containing blood meals for identification

▶ On the inner edge of the filter paper, number the divisions from 1 to16.

▶ When the filter paper is used, write a number in the centre of each paper and record the species of mosquito from which the blood was taken, where and when.

Making the blood-meal squashes

Blood is in a suitable condition for identification when the mosquito is freshly fed or, for most anopheline species in the tropics, within 24 hours after a blood-meal has been taken. The abdominal condition of the mosquito is half-gravid by this time. Older blood-meals are not suitable.

▶ Each filter paper must be used for only one species of mosquito that has been obtained from the same type of resting site, for instance inside houses, inside animal sheds and preferably outdoor natural resting sites. Use one filter paper for each specimen from a single collection, because this reduces the possibility of error in carrying out the test.

▶ Label the filter paper with a number in the centre and enter the name of the mosquito species, the place of collection, the date and time.

▶ Kill or anaesthetize the freshly-fed mosquitoes.

▶ Place a female mosquito on the filter paper about 1 cm from the edge and inside the area labelled with the number 1.

▶ Squash the abdomen using a blunt needle or the corner of a slide or glass rod.

▶ Make sure that the squashed abdomen remains inside the area labelled 1.

▶ Place a second female mosquito in the area labelled with the number 2 and squash it.

▶ Each female must be squashed with a clean needle, the corner of a slide or a glass rod. If using a slide, use each corner in turn and after four squashes, discard the slide. Ensure that blood from one specimen is not transferred to another (i.e. contamination) from the rod or slide. Alternatively squash the blood-meals using the round end of a pair of forceps, covered with clear adhesive tape. To avoid contamination the tape can be changed for each squash preparation.

▶ Continue in this way until all 16 areas of the filter paper have been used.

▶ Write the details that are asked for on the recording form and make two copies of it.

▶ Allow blood-meal squashes to dry, making sure that the papers are protected from ants and humidity.

▶ Store filter papers by placing them on top of each other with a piece of plain paper between each set of papers on which there are squashed mosquitoes.

▶ Place filter papers in a dessicator or refrigerator.

▶ When all the required squashes have been made, the filter papers should be packed into a self-sealing plastic envelop. If self-sealing envelops are not available, the papers can be placed in a plastic bag and the end sealed with a hot iron.

▶ Send one copy of the record form to the laboratory.

Calculation of the human blood index (HBI)

$$\text{Human blood index} = \frac{\text{Number of mosquitoes which have fed on humans}}{\text{Total number of mosquitoes whose blood-meals have been identified}}$$

ELISA technique for detecting source of blood meal

ELISA technique for sporozoite detection is the most widely used method for determining infection rates in mosquitoes. In this technique, the thorax and head parts of dried mosquitoes of known species are ground in a specific solution. Samples are then placed in the wells of microtitre plates coated with *Plasmodium* species-specific antibodies. If the corresponding antigen from the same species is present in the sample, it will bind to the wells. Enzymes and substrates which form colour reactions are then used to recognize positive wells. This technique saves time, particularly for large numbers of samples, and permits the identification of the particular *Plasmodium* species that caused the infection in the mosquito.

A similar ELISA technique is used to identify the source of blood-meals of mosquitoes. In this case, blood samples collected on filter papers by squashing freshly fed mosquitoes are tested using antibodies prepared from several known animal hosts. Figure 4.7 shows the different steps for the ELISA test.

PCR-based technique for blood-meal identification

Recent improvements in DNA-based methods for mosquito species identification have also been usefully applied to improving the efficiency and reliability of blood-meal identification in arthropods. One of the appropriate methods is the Restriction Fragment Length Polymorphism (RFLP-PCR) assay.

Exercise 4.1

Working in pairs, participants will: *(i)* learn to kill anophelines and determine their abdominal conditions and *(ii)* practice in dissecting ovaries and salivary glands of mosquitoes.

Indirect ELISA

Numbers correspond to steps in text

Marker protein
Primary antibody
Secondary antibody

Add sample in buffer (2)

Marker becomes attached to well surface (2)

Block unoccupied sites on well surface with neutral protein (4)

Incubate (2)

Wash to remove unattached marker (3)

Add 1° antibody & incubate –1° attaches to sample (5)

Wash to remove unattached antibodies (6)

Add 2° antibody & incubate –2° attaches to 1° (7)

Wash to remove unattached 2° antibody (8)

Add substrate & incubate – peroxidase enzyme breaks down substrate and turns blue (9)

Figure 4.7   Different steps for ELISA test

Exercise 4.2

Participants (working in pairs) will dissect ovaries of unfed and freshly fed females. This should be repeated until parous and nulliparous mosquitoes can be distinguished with confidence.

Exercise 4.3

After tutor's demonstration on how to dissect out the salivary gland, participants should practise the technique and examine the glands under the compound microscope.

Exercise 4.4

There will be a field trip to allow participants to practise the various mosquito collection techniques taught in Learning Unit 3 and the dissection techniques demonstrated in this unit. In the field, participants will work individually and in groups to carry out the following activities:

▸ Search for indoor-resting mosquitoes in three houses.

▸ Spend at least 20 minutes searching for outdoor-resting mosquitoes.

▸ In groups of four, carry out spray-sheet collections in one house per group.

▸ Collect larvae and pupae from natural breeding sites for at least 30 minutes.

▸ Practise sitting with bare legs indoors and outdoors during night landing collections (because time is short, this will be done during the day to allow practice and demonstration). If carried out at night, ensure that participants are taking antimalarials.

▸ Transport live specimens to the laboratory.

Exercise 4.5

Participants (working in pairs) will kill the mosquitoes that have been collected during the field trip and identify the abdominal conditions and species, then practise dissecting ovaries and salivary glands of the mosquitoes.

Exercise 4.6

Participants (working in pairs) will prepare the filter paper for ELISA test.

4.3   Entomological indicators of transmission

This section addresses the use of the techniques discussed above to obtain information of importance for malaria control. Participants will learn skills required to correctly interpret entomological information.

To illustrate most of the important concepts, an example is taken from an entomological study carried out to gather base-line data on local anophelines in an upland valley in Ethiopia in 1964–1965.[1] The study was designed to investigate the characteristics of malaria transmission and the habits and habitats of the local vector species in order to plan an effective control programme.

Some of the results of the study have been re-analysed in the light of current knowledge and new control tools. The objective is to illustrate how entomological information is used for vector control.

4.3.1   Study design and sampling techniques

Selection of study villages and description of the area

The area lies in central Ethiopia within the Great Rift Valley. The terrain is relatively flat and the altitude is 1600–1800 metres. The population (about 420 000), was largely rural. The people engage in agriculture and stock herding, living in scattered clusters of "tukuls", which are the common type of rural housing. Cattle are herded in open enclosures close to human habitations or herded for the night into a section partitioned off from the rest of the house by a loose framework of posts and twigs.

The main rainy season usually extends from June until the end of October and a short rainy season occurs in March and April. The hottest months are March, April and May. The coldest months are November and December.

Six villages were selected as observation posts, three in Awasa sector and three in Adamitulu sector (now Zway sector). A sector is an area delineated for the purpose of malaria control. The sectors were chosen primarily on entomological grounds, but the malaria endemicity and accessibility throughout the year were also considered. The area had never been sprayed with insecticides when the study was conducted. Table 4.1 and Figure 4.8 show parasite and spleen rates in the selected villages. Of the infections recorded, 6.6% were mixed *P. vivax* and *P. falciparum* infections, 61.8% *P. falciparum,* 25.0% *P. vivax,* and 6.6% *P. malariae.*

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[1]   Rishikesh N (1966). *Observations on anopheline vectors of malaria in an upland valley in Ethiopia*. Unpublished document of the World Health Organization, WHO/Mal/66.554.

U4

Table 4.1   Parasite and spleen rates in selected villages

Village	Month and year	Blood films examined	Parasite rate (%)	Spleen examinations	Spleen rate (%)
Abella Wondo	Jun. 1964	59	0	55	13.0
Galle	May 1964	49	4.1	37	35.1
	Oct. 1964	194	13.4	–	–
	May 1964	92	5.4	–	–
Awasa Tabor	May 1964	52	13.5	45	26.7
	Nov. 1964	37	8.1	–	–
Bulbula	May 1964	40	15.0	30	50.0
Woldia	Nov. 1964	181	2.6	–	–
	Dec. 1964	206	2.1	–	–
Ajiti Washgula	Nov. 1964	47	31.9	–	–
	May 1965	75	4.0	–	–
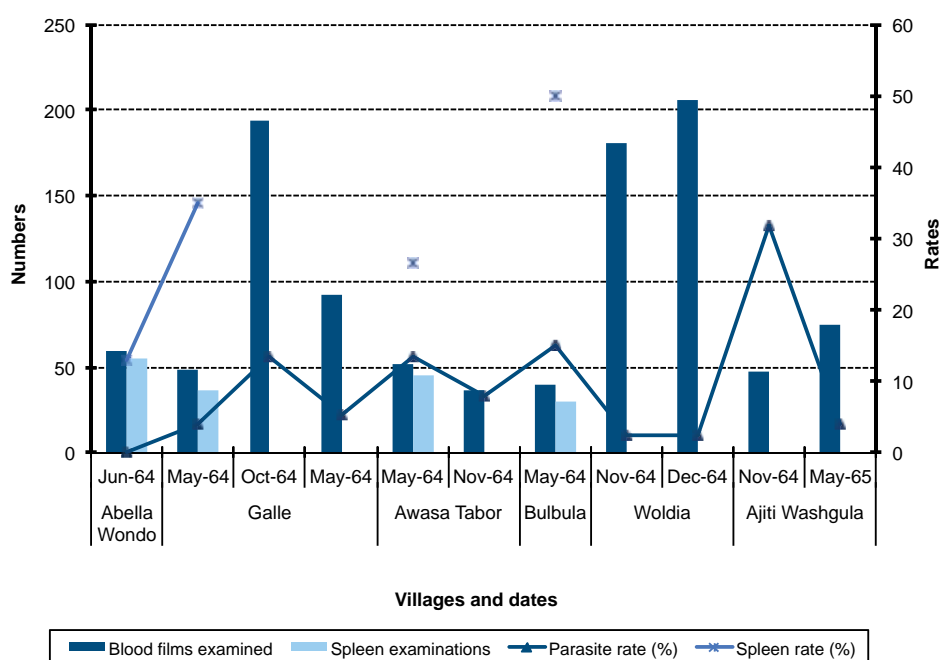
Figure 4.8   Parasite and spleen rates in selected villages

4.3.2   **Entomological sampling techniques**

Indoor resting collections

Indoor resting mosquitoes were sampled in the six villages once a month using the spray sheet collection method. The collections were analysed according to species and abdominal condition (see Table 4.2 and Figure 4.9 a,b,c,d). The salivary glands were dissected to establish infection rates.

Table 4.2   Results of indoor resting collections in Awasa Sector (1964–1965)

Month and year	No. of houses	No. of occupants	A. arabiensis[a]				A. pharoensis				A. funestus			
			Unfed	Fed	Half-gravid	Gravid	Unfed	Fed	Half-gravid	Gravid	Unfed	Fed	Half-gravid	Gravid
Jun 64	8	35	11	135	59	96	0	36	21	21	1	0	0	0
Jul 64	17	75	91	904	378	141	4	102	46	43	7	18	12	11
Aug 64	15	66	458	1041	459	678	11	60	19	14	2	26	7	14
Sept 64	18	79	149	586	270	236	8	101	45	45	1	25	8	5
Oct 64	18	79	185	802	438	340	14	46	16	15	3	80	9	9
Nov 64	23	101	8	65	51	38	0	10	9	7	3	47	13	34
Dec 64	24	106	2	25	13	9	2	13	4	4	3	43	2	4
Jan 65	24	106	1	9	6	4	1	3	3	4	0	4	1	2
Feb 65	23	101	0	0	0	0	0	3	2	1	0	0	0	2
Mar 65	23	101	0	1	0	0	0	5	6	3	1	0	0	0
Apr 65	23	101	1	5	3	6	5	28	8	12	0	17	2	2
May 65	23	101	2	34	19	22	2	29	13	15	1	12	1	0

[a]   From later studies it has established that the particular species referred to here as *A. gambiae* s.l. is *A. arabiensis.*
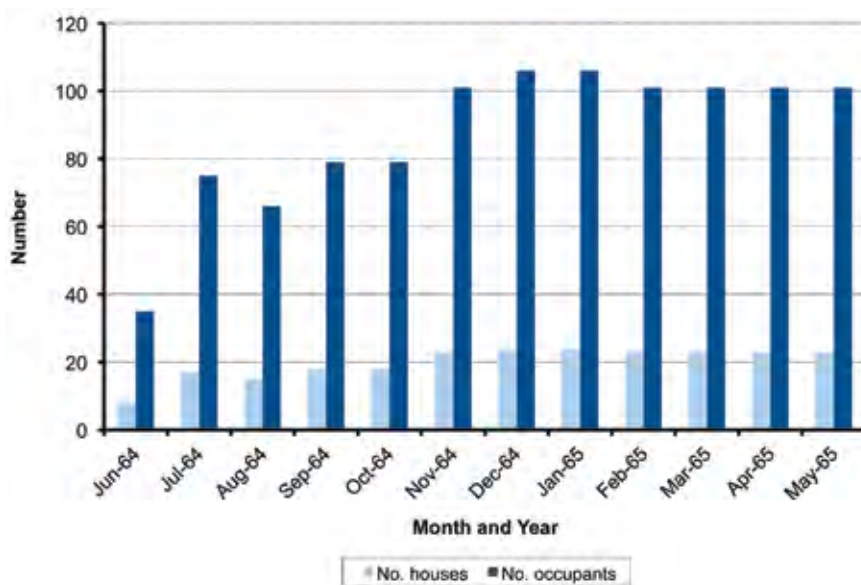
Figure 4.9a Results of houses and occupants in Awasa Sector (1964–1965)
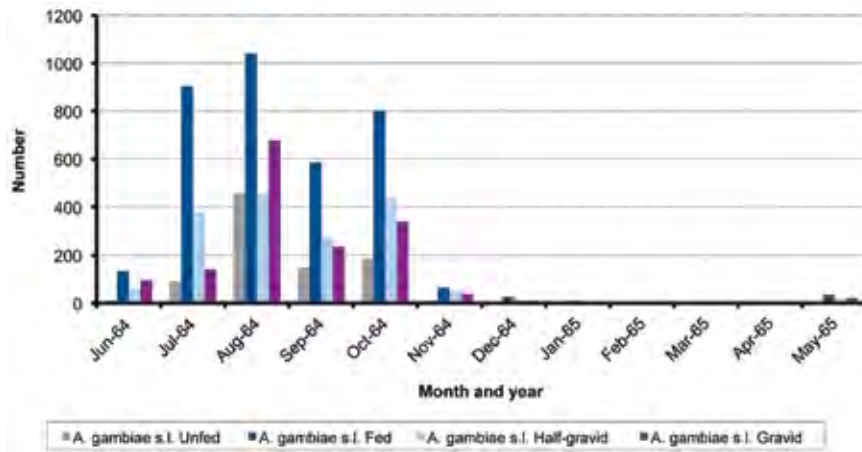
Figure 4.9b Abdominal stages of *A. gambiae* in the study area
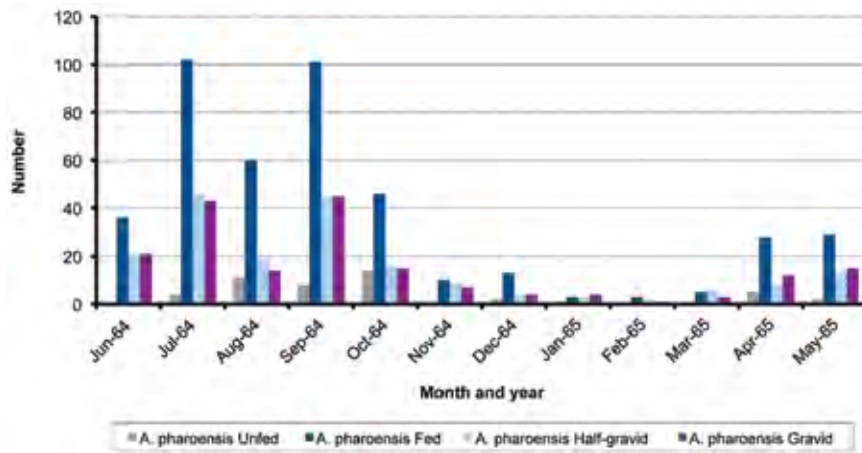
Figure 4.9c Abdominal stages of *A. pharoensis* in the study area
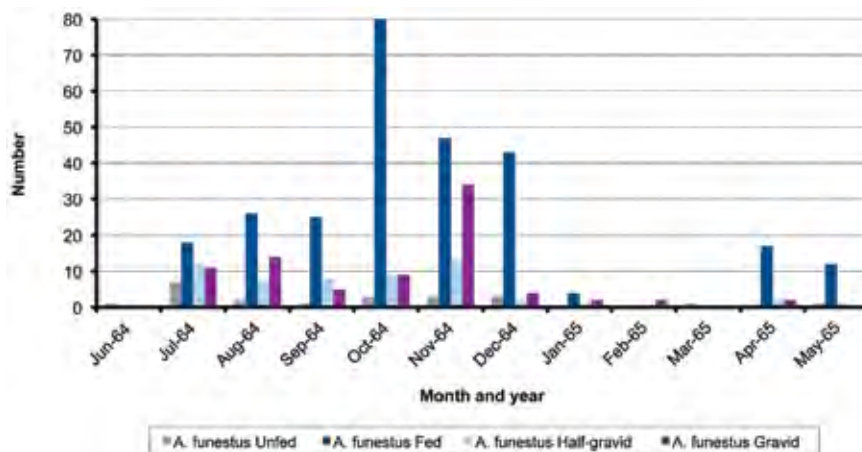
Figure 4.9d Abdominal stages of *A. funestus* in the study area

Night landing collections

Night landing catches were usually made twice a month (at Abella Wondo). Human baits were employed to catch the anophelines landing on their bare legs. Indoor catches were carried out throughout the night from 18h00 to 06h00, while outdoor catches were limited to the period 18h00–22h00, as none of the inhabitants were normally found outdoors after 22h00, except for one set of concurrent indoor and outdoor catches that were carried out in order to understand the feeding habits of the vectors if given equal opportunity throughout the night at both sites (Table 4.3 and Figure 4.10). Two collectors each were stationed indoors and outdoors and worked in 4-hour shifts. The indoor capture stations also contained their normal occupants at the relevant times. The collected samples were identified in the morning and dissected to examine the salivary glands for sporozoites. The ovaries were also dissected to determine the parity rates.

Artificial outdoor shelters for outdoor resting mosquitoes were installed and inspected monthly.

Results

Table 4.2 shows the indoor resting collections for each anopheline species per house per day.

Exercise 4.7a

Using Table 4.2, participants should calculate the indoor resting density per house per day for *A. gambiae* s.l. and *A. pharoensis* for the month of October 1964.

4.3.3 Feeding habits

Feeding habit refers to whether the vectors prefer to feed indoors (endophagy) or outdoors (exophagy) and the times of feeding during the night (nocturnal biting cycle).

$$IRD = \frac{\text{No. of females of a particular species}}{\text{No. of houses inspected}}$$

IRD: indoor resting density

Degree of endophagy/exophagy and nocturnal biting cycle were estimated by concurrent whole night indoor and outdoor landing collections (Table 4.3 and Figure 4.10).
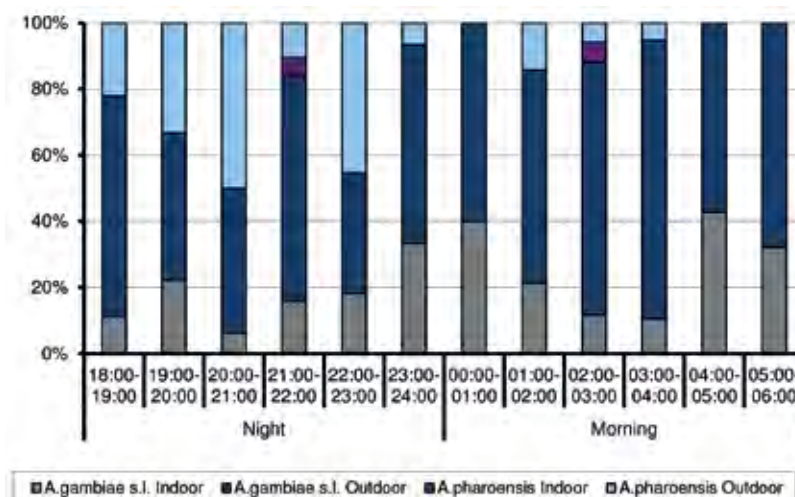
Figure 4.10 Concurrent indoor and outdoor night landing collections

Table 4.3   Concurrent indoor and outdoor night landing collections

Time		A.gambiae s.l.		A.pharoensis	
		Indoors	Outdoors	Indoors	Outdoors
Night	18:00–19:00	1	6	0	2
	19:00–20:00	2	4	0	3
	20:00–21:00	1	7	0	8
	21:00–22:00	3	13	1	2
	22:00–23:00	4	8	0	10
	23:00–24:00	5	9	0	1
Morning	00:00–01:00	6	9	0	0
	01:00–02:00	3	9	0	2
	02:00–03:00	2	13	1	1
	03:00–04:00	2	16	0	1
	04:00–05:00	3	4	0	0
	05:00–06:00	18	38	0	0
Total		50	136	2	30

Exercise 4.7b

Participants should calculate the ratio of indoor versus outdoor biting for each species using Table 4.3. Which species is exophilic? Which is endophilic?

4.3.4   Human-biting rates

The human-biting rate refers to the average number of bites per person per night by a vector species, and depends on both the feeding habits of the vector and the night-time habits of the local people. The human-biting rate (per night) is obtained by dividing the total number of fed mosquitoes by the total number of human occupants who spent the night in the houses used for collection:

$$M = \frac{F}{w}$$

> *where* F = total number of freshly fed mosquitoes of the particular species
> w = total number of human occupants in houses used for collection

Direct calculation of the human-biting rate

To calculate the human-biting rate, the night-time habits of the local people need to be taken into consideration. In the present survey, on average about one hour was spent outdoors between 18h00 and 22h00 and the remaining hours of the night indoors. Almost all the villagers were indoors by 22h00. Night landing catches were undertaken from 18h00 to 06h00 indoors whereas outdoor catches were restricted to the period 18h00–22h00 (Table 4.4).

Table 4.4 also shows some of the calculated values of the human-biting rates. The indoor and outdoor components of the human-biting rates are calculated separately and the total human-biting rate is obtained by adding the two rates together.

Table 4.4   Results of the night landing collections in Awasa Sector (Abella Wondo station), 1964–1965

(a) *A. gambiae* s.l.

Month & Year	No. of nights of catch	No. of baits		Total catches indoors		Total catch outdoors	Human-biting rate		
		Indoors	Outdoors	18.00–22.00	22.00–06.00	18.00–22.00	Indoors (3+8 h)	Outdoors (1 h)	Total (12 h)
Jul 64	2	2	2	12	84	16	23.3	1.0	24.3
Aug 64	2	2	2	81	340	76			
Sep 64	1	2	2	5	7	12			
Oct 64	2	2	2	4	21	34			
Nov 64	2	2	2	2	1	9	0.6	0.6	1.2
Dec 64	2	2	2	0	0	4	0.0	0.3	0.3
Jan 65	2	2	2	0	0	2	0.0	0.1	0.1
Feb 65	2	2	2	0	0	0	0.0	0.0	0.0
Mar 65	2	2	2	0	0	0	0.0	0.0	0.0
Apr 65	1	2	2	0	0	0	0.0	0.0	0.0
May 65	2	2	2	0	0	0	0.0	0.0	0.0

(b) *A. pharoensis*

Month & Year	No. of nights of catch	No. of baits		Total catches indoors		Total catch outdoors	Human-biting rate		
		Indoors	Outdoors	18.00–22.00	22.00–06.00	18.00–22.00	Indoors (3+8 h)	Outdoors (1 h)	Total (12 h)
Jul 64	2	2	2	19	17	92	7.8	5.8	13.6
Aug 64	2	2	2	37	83	74	27.7	4.6	32.3
Sep 64	1	2	2	23	31	143	24.1	17.9	42.0
Oct 64	2	2	2	12	12	105	5.3	6.6	11.9
Nov 64	2	2	2	2	1	33	0.6	2.1	2.7
Dec 64	2	2	2	0	1	42	0.3	2.6	2.9
Jan 65	2	2	2	3	7	44	2.3	2.8	5.1
Feb 65	2	2	2	7	0	9	0.3	0.6	1.9
Mar 65	2	2	2	0	1	8	0.0	0.5	0.8
Apr 65	1	2	2	0	0	11	2.6	1.4	1.4
May 65	2	2	2	11	2	19	2.6	1.2	3.8

U4
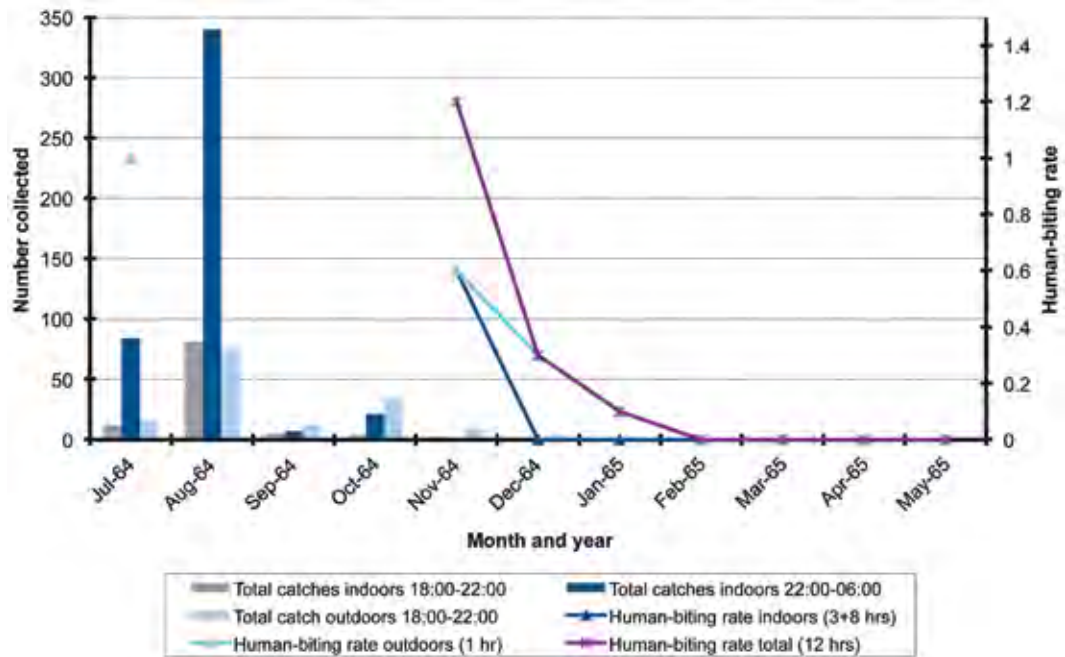
Figure 4.11a Results of the night-landing collections of *A. gambiae* s.l. in Awasa Sector (Abella Wondo station), 1964–1965
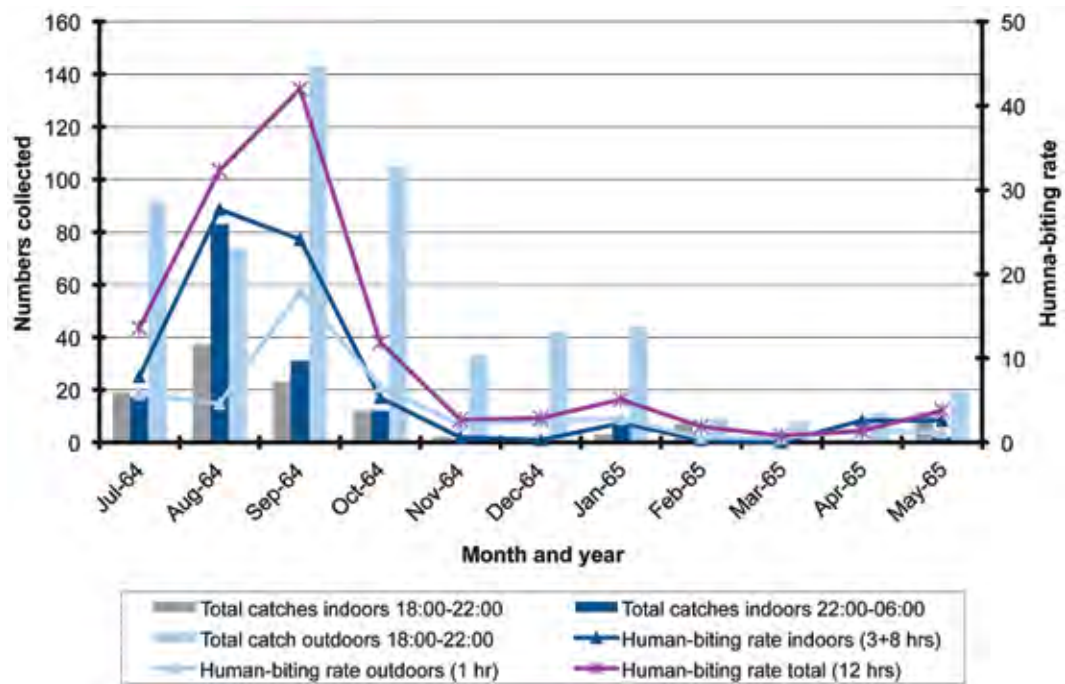
Figure 4.11b Results of the night-landing collections of *A. pharoensis* in Awasa Sector (Abella Wondo station), 1964–1965

The outdoor component M_y is the average number of bites per bait per hour during the period 18h00–22h00.

$$M_y = \frac{ty}{uTc_y}$$

where $T =$ number of hours between 18h00 and the latest time after which all villagers stay indoors (here $T = 4$)

$t =$ average number of hours spent by each villager outdoors after 18h00 (here $t = 1$)

$y =$ number collected outdoors during period T

$c_y =$ number of collectors outdoors

$u =$ number of nights of collection

For July 1964, for instance, the denominator of the outdoor human-biting rate M_y is 16 (two nights for two collectors $\times T$); the numerator is $(t)(y)$, with $t = 1$ and y number of outdoor captures $= 16$; M_y is therefore $16 / 16 = 1$

The indoor component (M_x) is the average number of bites per indoor bait in 4 hours between 18h00 and 22h00, plus the average number of bites per indoor bait in 8 hours between 22h00 and 06h00.

$$M_x = \frac{(1 - \frac{t}{T})x_1 + x_2}{uc_x}$$

where $T =$ number of hours between 18h00 and the latest time after which all villagers stay indoors (here $T = 4$)

$t =$ average number of hours spent by each villager outdoors after 18h00 (here $t = 1$)

$x_1 =$ number collected indoors during period T (18h00–22h00)

$x_2 =$ number collected indoors after period T (22h00–06h00)

$c_x =$ number of collectors indoors

$u =$ number of nights of collection

For July 1964, the denominator of the indoor human-biting rate (M_x) is 4 (2 nights for two collectors); the numerator $= [1 - (t / T)]x_1 + x_2$ with $t / T = 0.25$, x_1 the number of indoor captures during the period 18h00 to 22h00 (12) and x_2 the number of indoor captures during the period 22h00 to 06h00 (84), thus the numerator of $(M_x) = [(1 - 0.25)\,12] + 84 = 93$ and $(M_x) = 23.3$.

The total human-biting rate (M) is given as:

$$M = M_x + M_y = 23.3 + 1 = 24.3$$

The results for July 1964 indicate that an average villager would be bitten by 24.3 *A. gambiae* s.l. per night during that month. Of these bites, 23.3 were received indoors while only 1 was received outdoors even though the vector has an exophagic habit. The results demonstrate that the sleeping habits of the people influence where human-vector contact occurs, i.e. indoors.

Exercise 4.7c

Using Table 4.4, participants should calculate the human-biting rates for *A. gambiae* s.l. and *A. pharoensis* for the months of August and September 1964. From the results of this calculation, where does most human-vector contact take place, indoors or outdoors? Which species is endophilic? Do these results differ from the ones calculated in Exercise 4.7a?

Indirect calculation of the human-biting rate from spray sheet collections

This method uses spray sheet collections to estimate the human-biting rate. The human-biting rate (per night) is obtained by dividing the total number of fed mosquitoes by the total number of human occupants who spent the night in the houses used for collection:

$$M = \frac{F}{w}$$

> *where* F = total number of freshly fed mosquitoes of the particular species
> w = total number of human occupants in houses used for collection

The above estimation of the human-biting rate makes two implicit assumptions:

1. All fed mosquitoes found in the houses used for collection took their blood-meals from the occupants of the same houses; and

2. No fed mosquitoes left the houses after taking their blood-meals until the time of collection.

If these assumptions are more or less correct, this method is more efficient for endophilic species and less labour intensive for estimation of the human-biting rate.

Nevertheless, in some vector species such as *A. arabienisis,* a significant proportion (up to 30%) can feed on animals and may be found resting in human dwellings. The results may need to be adjusted accordingly by multiplying "*M*" by the proportion of females found that had fed on human blood.

4.3.5   Host preference

Host preference is usually determined by analysing the sources of the mosquito blood-meals. The proportion of mosquitoes with human blood, the human blood index (HBI), in a vector species can then be used as an indication of the degree of anthropophily of that particular species.

In the example study, the HBI was not determined so an estimate of 0.6 will be used for both *A. gambiae* s.l. and *A. pharoensis* for this exercise.

4.3.6   Resting habit

An important index is the proportion of blood-meals taken on humans followed by resting indoors. One element of the success of indoor residual spraying (IRS) in interrupting transmission is the proportion of the vectors that rest on the sprayed surface before and after feeding on humans. The aim of residual spraying is to reduce the probability of infected vectors reaching an infective age.

The proportion of blood-meals taken on humans and followed by indoors resting is calculated as:

$$f = \frac{kHD}{NPM}$$

where
$k =$ a correction value of 1.16

$H =$ human blood index – not calculated during the Ethiopian survey, but for which the arbitrary value 0.6 is used

$D =$ indoor resting density (total number of females collected divided by number of houses used for the spray-sheet collection)

$M =$ human-biting rate for October (see exercise 5.6)

$P =$ duration of resting indoors after feeding, in days; $P = 1 + G / F$, where G is the total number of half-gravid and gravid females (spray-sheet collections) and F is the number of freshly fed females (spray-sheet collections)

$N =$ average number of persons per house (household size)

For October 1964 the values that are the same for both *A. pharoensis* and *A. gambiae* s.l. are:

$k =$ 1.16
inhabitants = 79
houses = 18

$$N = 79 / 18 = 4.4$$

and separately,

	A. gambiae s.l.	*A. pharoensis*
H (human blood index)	0.6	0.6
Total number of females	1765	91
D (indoor-resting density)	1765 / 18 = 98.06	91 / 18 = 5.06
fed females	802	46
half-gravid females	438	15
gravid females	340	16
P (indoor resting post feeding)	1 + [(340 + 438) / 802] = 1.97	1 + [(16 + 15)] / 46 = 1.67
M (human-biting rate)	8.1[a]	11.9[a]

[a] see the results: Unit 5, Exercise 5.6 and Table 5.4

$$f = 1.16 \ (D)H \ / \ (N)(M)(P)$$

thus,

for *A. gambiae* s.l. $f = (1.16) \ (98.06)(0.6) / (4.4) \ (8.1) \ (1.97) = 0.972$

for *A. pharoensis* $f = (1.16) \ (5.06) \ (0.6) / (4.4) \ (11.9) \ (1.67) = 0.040$

Exercise 4.8

Participants will write a brief description of the results shown above. Compare your results with those of the facilitator.

4.3.7 Longevity and infectivity

Two other factors affect the likelihood of being bitten by an infective mosquito:

1. **The survival of a female mosquito after a blood-meal (probability of surviving one day after blood-meal, denoted as p and expectation of life for n days (n being the number of days for a sporogonic cycle to be completed).**

The result of ovarian dissections between July and December 1964 in Awasa Sector was as follows:

$$
\begin{array}{ll}
\textit{A. gambiae}\ \text{s.l.} & 72\ /\ 108 = 0.667 \\
\textit{A. pharoensis} & 107\ /\ 276 = 0.388
\end{array}
$$

Given an interval of two days between blood-meals, the probability of surviving one day (denoted as p) can be estimated as:

$$p = \sqrt{\text{Proportion parous}}$$

Thus,

for *A. gambiae* s.l. $p = \sqrt{0.667} = 0.817$ and

for *A. pharoensis* $p = \sqrt{0.388} = 0.623$

If a 3-day interval is assumed, we have

for *A. gambiae* s.l. $p = \sqrt[3]{0.667} = 0.874$ and

for *A. pharoensis* $p = \sqrt[3]{0.388} = 0.729$

The above formula for p assumes that the mosquito population has a stable size and age structure, and the death rate is independent of age. For this reason, the proportion of parous mosquitoes is usually averaged over the whole population cycle, to eliminate the effect of seasonal fluctuation in population size and age structure.

It is also possible to calculate the probability of surviving through n days. As p is the probability of surviving one day, p^n is the probability of surviving n days. For example, at an average daily temperature of 27 °C, it would take about 10 days for *P. falciparum* to complete the sporogonic cycle in the vector.[1] The probability that this parasite can be transmitted by *A. gambiae* s.l. and *A. pharoensis* is 0.874^{10} (= 0.26) and 0.729^{10} (= 0.042), respectively.

The life expectancy for each species can also be calculated as:

$$1\ /\ -\ln p \qquad [2]$$

Using that formula and the data of the previous paragraphs, expectation for *A. gambiae* s.l. is 7.4 days and 3.2 days for *A. pharoensis*.

[1.] The duration of sporogony as a function of temperature can be calculated by the formula $n = T\ /\ (t - t_{min})$, where n = duration of sporogony; T = 111, 105 and 144 for *P. falciparum*, *P. vivax* and *P. malariae*, respectively; t = actual average temperature in degrees centigrade and t_{min} = 16 for *P. falciparum* and *P. malariae* and 14.5 for *P. vivax*.

[2.] *ln* means "natural logarithm" and *p* is the daily survival rate of a mosquito (which must be defined in the text). The formula is the inverse of minus the natural log of *p*, that is, 1/-ln (*p*).

At Abella Wondo, the average daily temperature during the months of July–December is usually 20 °C. At this temperature, it takes approximately 28 days to complete the sporogonic cycle for *P. falciparum*. The probability of transmission of *P. falciparum* infection by *A. gambiae* s.l. is therefore 0.874^{28} (= 0.023 or 2.3%). During the same period, out of 2434 females of this species, 3 were found infected, i.e. 0.1%. The low sporozoite rate (or the low probability of transmission) in the study area could be the result of both the survival probability of the vectors and the ambient temperature.

2. **The sporozoite rate of female mosquitoes and the number of infective bites per night.**

The sporozoite rates for the Awasa Sector are shown in Table 4.5 and Figure 4.12.

Table 4.5　Salivary gland dissection, *A. gambiae* s.l., Awasa Sector (1964–1965)

Month & Year	No. dissected	No. sporozoite positive	Sporozoite rate (%)
Jun 1964	128	0	0.00
Jul 1964	212	0	0.00
Aug 1964	580	0	0.00
Sep 1964	630	0	0.00
Oct 1964	803	2	0.25
Nov 1964	162	1	0.62
Dec 1964	47	0	0.00
Jan 1965	20	0	0.00
Feb 1965	0	–	–
Mar 1965	0	–	–
Apr 1965	0	–	–
May 1965	38	0	0.00
Total	2620	30	0.11
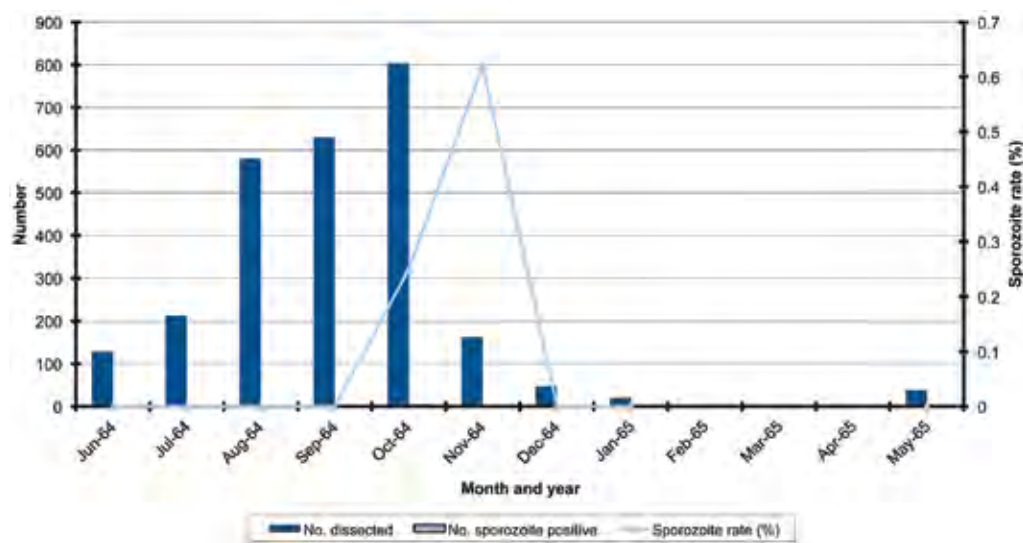
Figure 4.12 Salivary gland dissected, *A gambiae* s.l., Awasa Sector in 1964–1965

In Awasa Sector, 3/2620 (0.11%) *A. gambiae* s.l. were found positive and during the same period, 6/1918 *A. gambiae* s.l. (0.31%) were sporozoite positive in Adamitulu Sector. In both sectors, of a total of 2577 *A. pharoensis* collected none were found positive.

Example

For an inhabitant of Abella Wondo (in Awasa Sector) in 1964, how many infective bites of *A. gambiae* s.l. would be expected during the month of October 1964 if no protection against mosquito bites had been used?

This question can be re-phrased as: among all *A. gambiae* s.l. females that could have taken their blood-meals on the person during October 1964, how many would have been infective? To answer this question, two measurements are needed:

- ▶ the sporozoite rate, and
- ▶ the human-biting rate

The sporozoite rate was 0.25% (Table 4.5). On average, the person would be bitten by 8.1 *A. gambiae* s.l. per night. The number of infective bites per person per night, known as the Entomological Inoculation Rate (EIR) and is calculated as:

$$EIR = \frac{\text{human-biting rate x sporozoite rate (\%)}}{100}$$

The EIR is therefore 8.1 x 0.0025 = 0.0203 per person per night. Assuming that the same number of females would bite every night in October 1964, a total of 0.0203 x 31 days = 0.63 infective bites would be expected during the month.

Alternatively, the same solution can be derived as follows. If 8.1 *A. gambiae* s.l. bite a person every night, 8.1 x 31 = 251.1 could have bitten him/her during the whole month. From the sporozoite rate, 0.25% of these mosquitoes are expected to be infective; thus the expected infective bites would be 0.0025 x 251.1 = 0.63 infective bites per person per month (i.e. less than one infective bite). When expressing the EIR, the time dimension (whether it is per night, per month or per year) must always be noted. Incidentally, the number of infective bites is low in this particular area. In hyperendemic areas of Africa, a person could receive one infective bite every night.

A similar calculation for *A. gambiae* in November 1964 (Tables 4.4 and 4.5) shows a human-biting rate of 1.2 per person per night (lower than in October) and a sporozoite rate of 0.62% or 0.0062 (higher than in October); the EIR is 1.2 x 0.0062 = 0.00744 infective bites per person per night or 0.00744 x 30 = 0.22 infective bites per person per month. It is likely that in November the remaining vectors were older mosquitoes (likely to be infected) but the lower biting rate reduced the EIR.

Exercise 4.9

Working in groups, participants will answer the following questions:

a) *From the results of earlier observations and of calculations on life expectancy and on human-vector contact, which of the two anopheline species is the most important vector of malaria in the area? Why? (Give three reasons).*

b) *If a decision is taken to use residual spraying to control A. gambiae s.l., what would be the timing for the application of an insecticide with six months residual efficacy? Refer to indoor resting densities and human-biting rates.*

The results will be presented in plenary.

4.3.8   Vectorial capacity

Vectorial capacity is an index (or model) that is defined as the capacity of a vector population to transmit malaria in terms of the potential number of secondary inoculations originating per day from an infective person. The formula of the vectorial capacity (C) is given as:

$$C = m\,a^2\,p^n\,/ - \ln p$$ [1]

where m = density of vectors in relation to humans
a = number of blood-meals taken on humans per vector per day
(= human blood index multiplied by 0.5, if a gonotrophic cycle of two days is assumed)
p = daily survival probability (or proportion of vectors surviving per day)
n = incubation period in the vector (days)

The formula can be derived as follows: a person is bitten by ma vectors in one day; a fraction p^n of these vectors survive the incubation period; they survive $[1 / (- \ln p)]$ days, during which time they feed on a persons per day; multiplying ma by a, and then by p^n and $[1 / (- \ln p)]$ yields the above formula. It is difficult to measure all these parameters correctly and several assumptions have to be made. Nevertheless, the vectorial capacity is one of the most important concepts in the theoretical studies of the epidemiology and control of malaria. For example, using this concept, it can be shown that halving the survival p (by using residual spraying) produces a much greater reduction in vectorial capacity than halving a, which is itself more effective than halving the density m.

Closing discussion

The participants will review the key concepts of vector biology and how they relate to malaria transmission and vector incrimination. The tutor will raise the questions listed below. Participants will develop a list of the components of a vector's biology that increase the risk of malaria. The class results can be placed on a flip chart.

1. What characteristics of the vector aquatic habitats contribute to the risk of malaria?

2. What life history characteristics of the adult vector increase the likelihood that it will transmit malaria?

[1.] *ln* means "natural logarithm" and *p* is the daily survival rate of a mosquito (which must be defined in the text). The formula is the inverse of minus the natural log of *p*, that is, 1/-ln (*p*).

3. What biting activity behaviours increase the potential for malaria transmission?

4. What human activities and behaviours put them at risk of malaria?

5. Is it necessary to measure all the components of the vectorial capacity model in order to monitor the entomological component of malaria transmission? Explain.

6. The EIR has become an important entomological indicator of malaria transmission for comparing regional differences. Why has this happened?

LEARNING UNIT **5**

Malaria vector control

Learning Objectives:
by the end, participants should be able to...

■ Discuss the role and objectives of vector control in malaria prevention and control

■ Describe vector control options, their advantages and limitations

■ Describe the formulations of different insecticides (organochlorines, organophosphates, carbamates and pyrethroids)

■ Demonstrate competence in applying insecticides using indoor residual spraying (IRS), insecticide-treated mosquito nets (ITNs/LLINs), larviciding and space spraying

■ Demonstrate competence in operation, storage and maintenance of vector control equipment (the Hudson pump sprayer, fogging machines, ultra-low volume)

■ Describe different methods used for biological control of malaria vectors

■ Describe geographical reconnaissance and its place in vector operations

■ Explain the options for Integrated Vector Management (IVM)

Introduction

Vector control is a cornerstone of malaria control and it remains the most generally effective measure to prevent malaria transmission and therefore is one of the strategic approaches to malaria control.

The objectives of malaria vector control are two-fold:

▶ to protect individual people against infective malaria mosquito bites, and

▶ to reduce the intensity of local malaria transmission at community level by reducing the longevity, density and human-vector contact of the local vector mosquito population.

Vector control methods vary considerably in their applicability, cost and sustainability of their results. They target against the adult mosquito and/or its larvae.

5.1   Vector control methods

Interventions using vector control methods are related to the following three major control measures:

1. Reducing human-vector contact

▶ Insecticide-treated mosquito nets
▶ Improved housing
▶ Repellents and mosquito coils

2. Adult mosquito control

▶ Insecticide-treated mosquito nets
▶ Indoor residual spraying
▶ Space spraying

3. Larval control

▶ Larviciding
▶ Source reduction
▶ Larvivorous fish

Not all of these methods are applicable to all of the diverse malaria epidemiological and operational situations that can occur. The two most powerful and most broadly applied vector control interventions are insecticide-treated mosquito nets and indoor residual spraying. These interventions work by reducing human-vector contact and by reducing the lifespan of female mosquitoes (so that they do not survive long enough to transmit the parasite).

Other interventions, such as larviciding or environmental management, can be useful in a specific set of conditions, depending on the target vector and the local situation. Due to logistical and operational limitations, these methods cannot be efficiently implemented in all areas but can in specific settings play a complementary role to insecticide-treated mosquito nets and indoor residual spraying. Larviciding is useful only where breeding sites are few, fixed and findable.

Exercise 5.1

Working in small groups, participants will write on the diagram below (Fig. 5.1) the potential vector intervention methods for each life-cycle stage of the vector. Classify them as *(i)* reducing human-vector contact, *(ii)* adult mosquito control, *(iii)* larval control. Refer to the text about vector control methods at the end of this learning unit when developing this exercise. Each group will present the information in plenary session and discuss their conclusions.
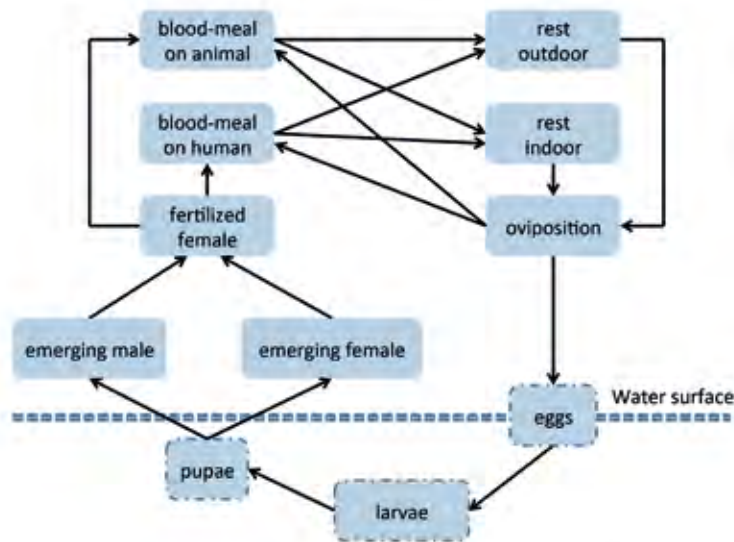
Figure 5.1   Schematic presentation of the life-cycle of mosquito

Exercise 5.2

Working in small groups, participants will complete Table 5.1 on the expected effects of the various vector control methods on different aspects of the vector population. The following signs may be used in the blank cells of the table: (+) reduction expected; (–) no effect; and (+/–) effect doubtful or conditional on other factors. Present the group's results in plenary. Refer to the text on vector control methods at the end of this learning unit when developing this exercise.

Table 5.1   Aspects of the vector (and components of vectorial capacity) that are likely to be affected by various vector control methods

Method	Larval density (m)	Adult density (m)	Adult survival (p)	Human biting habit (a)
Reducing human-vector contact				
Insecticide-treated nets, other materials				
Improved housing				
Repellents and mosquito coils				
Adult mosquito control				
Insecticide-treated nets, other materials				
Indoor residual spraying				
Space spraying				
Larval control				
Source reduction				
Larvivorous fish				
Larviciding				

+ reduction expected; – no effect ; +/– effect doubtful or conditional on other factors

Demonstration

The commonly used vector control methods, equipment and chemicals will be demonstrated. Participants will practise the methods and discuss their use, maintenance, operations and safety.

5.1.1 Selection of vector control methods

Vector control methods vary in efficacy, resource requirements, potential delivery systems, and personnel required to implement the method. Some methods are highly specific and others broad ranging. Figure 5.2 demonstrates the complexity of the vector control selection process. The central point revolves around a particular epidemiological situation. This unit focuses on the impact of different vector control methods on the vector population.
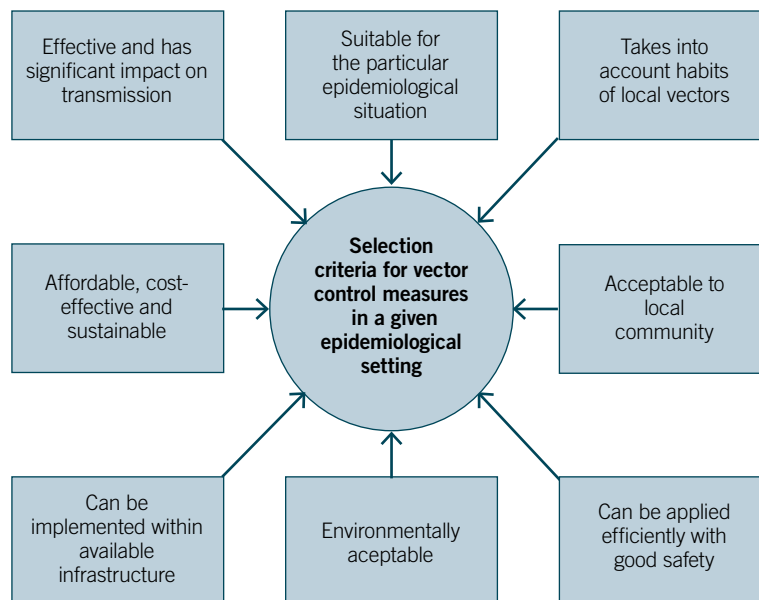
Figure 5.2   Criteria for selection of appropriate vector control option(s) for a given epidemiological situation

Exercise 5.3

An important component of the selection process for vector control interventions is knowledge of the advantages and limitations of each method. Working in groups, participants will develop a list of advantages and limitations for the methods listed in Table 5.1. Reference can be made to the information provided at the end of this learning unit as the list is prepared.

Exercise 5.4

The tutor will assign each group one of two interventions to be used in the case study for Ethiopia. In this exercise it is assumed that the government has been given conflicting opinions on whether to use ITN or IRS. The group will justify the selection of the appropriate method, develop the intervention plan, and promote it in plenary. The reading at the end of this module can be used as a guide in the development of the plan.

5.2   Reducing human–vector contact

5.2.1   Insecticide-treated mosquito nets

An insecticide-treated net is a mosquito net which is impregnated with an insecticide. It repels, disables and/or kills mosquitoes coming into contact with insecticide on the netting material. There are two categories of insecticide treated nets as described below:

▸ A conventional insecticide treated net (ITN) is a mosquito net that has been treated by dipping in a WHO-recommended insecticide. To ensure its continued insecticidal effect, the net should be re-treated after three washes, or at least once a year.

▸ A long-lasting insecticidal net (LLIN) is a factory-treated mosquito net made with netting material that has insecticide incorporated within or bound around the fibres. The net must retain its effective biological activity against vector mosquitoes for at least three years in the field under recommended conditions of use, avoiding the need for regular insecticide treatment. For this reason, WHO stresses the use of the long-lasting insecticidal nets instead of the conventional ITNs.

Insecticide treated nets work both by (i) protecting the person sleeping under the net (individual level – personal protection) and by (ii) extending its effect to an entire area (community level – mass effect). Personal protection operates by preventing contact between the mosquito and the person under the net. The 'mass effect' occurs when the insecticide in the net actually kills the mosquito that touches it, therefore affecting the vector population and lowering the overall intensity of transmission in the targeted area. However, when comparing people sleeping outside the net within the same household to those sleeping under the net, the protective effect of the insecticide treated nets is lower. Therefore, WHO recommendation is to achieve 'universal coverage', rather than reaching a pre-determined number of insecticide treated nets per household.

5.2.1.1   Netting material and mosquito net models

The following terms are used to characterize netting materials:

Material

Nets are made of polyester, polyethylene or plypropylene (see Fig. 5. 3).

Polythylene   polyester   polypropylene

Figure 5.3   Different materials of bednets

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Mesh

The number of holes per square inch, e.g. a mesh count of 156 has 12 x 13 holes per square inch. The 156 mesh count is considered a standard for untreated or field-treated netting on condition that the netting has holes of reasonably homogenous size. Because of their retention of insecticidal activity against mosquitoes, LLINs may have a different minimum mesh count, which may be product-specific.

Bursting strength

Defined as the maximum pressure that can be applied to a given surface area of netting before it bursts under the strain. It is measured on areas of netting material measuring 7.3 cm². The minimum bursting strength for acceptable netting materials is 250 kPa.

Denier

An indication of the weight (and therefore the strength) of the thread. It is defined as the weight in grams of 9000 m of a single thread. A denier of 100 and more is strong and often recommended. Nets of 75 denier are also used, but are fragile.

Colour

Blue, green or pink are commonly used because they show less dirt and avoid cultural issues associated with white. In some areas however white nets are preferred.

Shape and size

Nets are made usually in two shapes: rectangular and conical (or circular). Large-scale programmes often use rectangular nets. Table 5.2 summarizes the most commonly used mosquito nets. The shape and size of nets used depend on local preference.

Table 5.2   The most commonly used mosquito nets

Size	Width (cm)	Length (cm)	Height (cm)
Single	70	180	150
Double	100	180	150
Family	130	180	150
Large family	190	180	150

The conical nets in use are approximately 8.76 m² for the single nets, 10.20 m² for double nets, 11.64 m² for the family size, and 14.52 m² for large-family size.

5.2.1.2   Treatment procedures for ITNs

From a programmatic perspective, treatment and retreatment of nets will be needed until coverage with LLINs is more widespread and replace all the conventional ITNs already delivered and distributed. Furthermore, certain communities in malaria endemic settings might continue to use nets that need treatment and re-treatment. Treatment procedures are described in Annex 2.

5.2.1.3   Source of nets

Potential sources of nets are:

▸ Commercial manufacturers of ready-made nets and netting outside the country
▸ Local commercial manufacturer of ready-made nets
▸ Small-scale production of nets, e.g. produced by local tailors
▸ Home-made nets produced by the household owner

5.2.1.4 Long-lasting insecticidal mosquito nets

Long-lasting insecticidal mosquito nets (LLINs) are ready-to-use pre-treated mosquito nets, which do not require any re-treatment during their expected lifespan (generally 2–3 years). Data on durability (physical integrity) from the field suggest that they last not more than 24 months. This has huge implications on cost for replacement. Current work is ongoing to develop longer lasting nets that may allow for longer intervals between replacement cycles. In addition, if there is evidence of premature net failure then replacement should be undertaken sooner than 3 years.

LLINs have several important advantages over conventional mosquito nets. These include eliminating the need to re-treat the nets (one of the main obstacles to the use of ITNs in many countries), avoiding problems associated with the storage and handling of insecticides by non-professionals and in the community, reducing insecticide use, and minimizing the environmental hazards caused by the release of insecticide into natural water bodies.

The WHO Pesticide Evaluation Scheme (WHOPES) provides recommendations regarding LLINs specifications. Purchasing authorities should only procure LLINs that comply with the WHOPES specifications. All batches of LLINs should be quality assured. There are 13 LLIN products currently recommended by WHO as indicated in Table 5.5.

Table 5.5   WHO recommended long-lasting insecticidal mosquito nets

Product name	Product type	Status of WHO recommendation
DawaPlus® 2.0	Deltamethrin coated on polyester	Interim
Duranet®	Alpha-cypermethrin incorporated into polyethylene	Interim
Interceptor®	Alpha-cypermethrin coated on polyester	Interim
LifeNet®	Deltamethrin incorporated into polypropylene	Interim
MAGNet™	Alpha-cypermethrin incorporated into polyethylene	Interim
Netprotect®	Deltamethrin incorporated into polyethylene	Interim
Olyset®	Permethrin incorporated into polyethylene	Interim
PermaNet® 2.0	Deltamethrin coated on polyester	Full
PermaNet® 2.5	Deltamethrin coated on polyester with strengthened border	Interim
PermaNet® 3.0	Combination of deltamethrin coated on polyester with strengthened border (side panels) and deltamethrin and PBO incorporated into polyethylene (roof)	Interim
Royal Sentry®	Alpha-cypermethrin incorporated into polyethylene	Interim
Yorkool® LN	Deltamethrin coated on polyester	Full

1. Reports of the WHOPES Working Group Meetings should be consulted for detailed guidance on use and recommendations. These reports are available at http://www.who.int/whopes/recommendations/wgm/en/); and

2. WHO recommendations on the use of pesticides in public health are valid ONLY if linked to WHO specifications for their quality control. WHO specifications for public health pesticides are available at http://www.who.int/whopes/quality/newspecif/en/.

5.2.1.5 Advantages and limitations of ITNs/LLINs

The advantages and limitations of ITNs/LLINs are presented in Table 5.6.

Table 5.6 The advantages and limitations of ITNs and LLINs compared to IRS

Characteristics	ITNs or LLINs
Advantages of ITNs/LLINs	
• No need for special equipment and training	ITNs and LLNs
• Few organization and logistical problems	ITNs and LLNs
• Low amount of insecticide needed	ITNs and LLNs
• Protection against dust, rats and snakes	ITNs and LLNs
• Community-based	ITNs and LLNs
• No re-treatment (2–3 years)	LLINs
Limitations of ITNs/LLINs	
• Exophilic and exophagic mosquitoes decrease the effectiveness	ITNs and LLNs
• Lack of participation of groups at risk may limit the effectiveness	ITNs and LLNs

5.2.1.6 Essential components of ITN/LLIN programme implementation and evaluation

There are technical, socio-cultural, economic and operational issues which are likely to influence the effective implementation of the ITN/LLIN strategy. Some of the questions that need to be considered when planning the implementation of an ITN/LLIN programme are:

▶ What are the behavioural patterns of the vectors? Are they mostly exophagic or endophagic and what are the peak biting periods, especially in relation to peoples' sleeping patterns? Are people outdoors (outside ITNs/LLINs) at times when mosquitoes bite most?

▶ What are the night-time movements and habits of people likely to affect exposure to vectors, including the time they go to bed? (This will vary with age, sex, and occupation).

▶ What are the attitudes of the people towards the use of nets?

▶ Is there any preference for size, shape and colour of the nets?

▶ Who uses nets already? From where do they get the nets and at what cost?

▶ Are there seasonal variations in net use patterns?

▶ What is the economic status of most people? (This will affect net ownership, the ability to pay for insecticides and net (re)treatments.)

ITNs/LLINs are indicated as a long-term intervention in most situations, especially in the following:

Epidemiological situations

▶ In a wide range of transmission conditions where long-term protection is needed;

▶ In areas with a relatively long season of malaria transmission, or perennial transmission, such that more than one IRS cycle would be required;

▶ In areas where IRS cannot be used and only personal protection can be achieved (e.g. forest malaria or among nomadic populations).

Socioeconomic situations

▸ In places where IRS may face problems of acceptability for one reason or another.

Access and programmatic situations

▸ In areas where routine ITN/LLIN distribution can easily be integrated into existing health systems such as routine EPI or ANC;

▸ In areas where the specialized skills and programme infrastructure needed for IRS have not (yet) been developed, an ITN/LLIN distribution campaign can rapidly achieve high levels of coverage;

▸ To protect hard-to-reach populations, where repeated IRS spray-cycles are not feasible (a one-time distribution of ITNs/LLINs can provide relatively long-term protection, compared to the shorter-duration of protection given by one IRS spray cycle);

In every country, there is a different range of local situations and eco-epidemiological settings. Therefore, there it will often be justifiable to use IRS in some settings and ITNs/LLINs in others or both in other situations.

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Target population for ITN/LLIN programme

Population at risk – universal coverage

ITNs/LLINs should be provided in sufficient numbers to cover everyone exposed to transmission in target communities. When supplies are constrained, however, ITNs/LLINs should be used to provide personal protection to the most vulnerable groups including:

Children under five years old

Young children have not developed a protective level of immunity against malaria because they have had limited exposure to infection. In areas of high transmission, this group is vulnerable to severe malaria. This age group is usually one of the main target groups for malaria control interventions.

Pregnant women

Pregnancy increases the vulnerability of women to malaria. In nonimmune women in areas where malaria transmission is unstable, cerebral and other forms of severe and complicated falciparum malaria are more common in pregnancy, particularly in primiparae. Even in some hyperendemic areas, clinical symptoms and parasitaemia are worse in primiparous than in multiparous women and other patients. In addition, malaria during pregnancy is a risk factor for low birth weight and other adverse preganancy outcomes.

Refugees and internally displaced people

This group often moves from areas of no malaria transmission or low transmission to highly endemic areas. Because they lack protective immunity, the people are a high risk group with all ages at risk of malaria.

Choosing between conventional ITNs and LLINs

WHO encourages national malaria control programmes and their partners involved in insecticide treatment mosquito nets interventions to procure only long-lasting insecticidal

nets. Furthermore LLINs are more cost-effective (as it can be used for 2–3 years) than distribution of conventional bed nets and treating them with insecticide once or twice a year.

However, in areas where ITNs have been distributed in the past or acquired by the population through other delivery channels and where significant proportion (at least 50%) of the community is using bed nets, re-impregnation of bed nets may still be cost-effective. It is expected eventually that LLINs will become the main malaria vector control intervention in most areas, and the need for ITNs and re-treatment of nets should be limited.

Only LLINs recommended by the WHO Pesticide Evaluation Scheme (WHOPES) should be procured by national malaria control programmes and partners for malaria control. Since quality of LLIN products could be variable, it is highly recommended that pre- and post-shipment quality testing should be requested and potential suppliers should cover the cost for testing.[1]

Delivery of ITNs/LLINs

High coverage rates are needed to realize the full potential of ITNs/LLINs. Therefore, WHO recommends full coverage of all people at risk in areas targeted for malaria prevention through ITNs/LLINs.

In general, rapid scale-up in the coverage of target populations can be achieved most efficiently through the distribution of free or highly subsidized ITNs/LLINs. Cost should not be a barrier to making ITNs/LLINs available to all people at risk, especially young children and pregnant women.

Universal access to ITNs/LLINs is best achieved and sustained by a combination of delivery systems such as catch-up and keep-up delivery mechanisms. Catch-up means mass distribution campaigns, which can rapidly achieve universal coverage of ITNs/LLINs. However it is essential that such campaigns are complemented by continuous 'keep-up' delivery systems, particularly that routine delivery to pregnant women through antenatal services and to infants at immunization clinics.

Delivery of ITNs/LLINs to pregnant women through antenatal care is practiced in many countries and can be done in two ways:

▶ giving a free or subsidized ITNs/LLIN (i.e. direct product);

▶ giving a voucher or coupon that can be exchanged for an ITNs/LLIN at a distribution point such as a commercial outlet.

Delivery of ITNs/LLINs to children may be done through:

▶ routine immunization;

▶ child health days/weeks, which target children under 5 years of age with a package of interventions including ITNs/LLINs, vitamin A supplementation and deworming;

▶ health facilities or by mobile teams as part of monthly outreach services.

In emergency situations in areas with unstable malaria, campaign-like delivery as part of relief efforts can help to achieve rapid coverage of the entire population.

[1.] WHO (2012). *Guidelines for procuring public health pesticides.* WHO/HTM/NTD/WHOPES/2012.4.

The social marketing techniques employed in some countries use a range of distribution approaches, including public health facilities and a combination of community-based and private-sector distribution, the latter mainly in urban centres. Commercial markets are valuable sources of nets. Where strong commercial markets exist or are developing, they should be encouraged.

Estimation of the number of nets needed in a particular area

It is currently proposed that one ITN/LLIN should be distributed for every two persons. At the household level, the distribution of one ITN/LLIN for every two members of the household will entail rounding up in households with an odd number of members (e.g. 3 ITNs/LLINs for a household with 5 members, etc). Because of this rounding up, the achievement of "one ITN/LLIN for every two people" at household level requires an overall ratio, for procurement purposes, of 1 ITN/LLIN for every 1.8 people in the target population.

▶ In settings where ITNs/LLINs had not been distributed yet, estimation of nets required should be based on 1 LLIN to 1.8 persons calculation (same as 550 nets/1000 population), reflecting the need to assure universal coverage for all households including those with odd numbers of occupants.

▶ In settings where ITN/LLIN household ownership levels is low (< 30%), or where ITN/LLIN household ownership cannot be estimated through an existing net tracking system but is assumed to be low, it is recommended that the existing ITNs/LLINs not to be taken into account. Instead, estimation of nets needed should be based on 1 LLIN to 1.8 persons calculation as above.

▶ In settings where ITN/LLIN household ownership levels are estimated to be above 30%, a more detailed quantification for net procurement could increase efficient use of resources. A strong quantification methodology would consider the following factors when determining ITNs/LLIN procurement estimates:

  ▶ Pre-existing undistributed stocks of ITNs/LLINs available for use;

  ▶ Estimated numbers of previously distributed ITNs/LLINs nets over the past 3 years, by year, preferably by district;

  ▶ Efficacy and likely condition of ITNs/LLINs accounting for loss at the rates described in the table below based on the age of the net (number of years since distribution).

Loss rates of nets

Several studies on the durability and effective life of different nets in different settings are currently underway. Countries are encouraged to prospectively monitor the durability of LLINs using methodology outlined in the WHO publication entitled *Guidelines for monitoring the durability of long-lasting insecticidal mosquito nets under operational conditions.*[1] Countries that have specific data on the viability or durability of nets in their country can use this to estimate number of viable nets and remaining life. Rates of loss can also be calculated at 8% for year 1 (0–12 months) since distribution, 20% for year 2 (13–24 months) since distribution, and 50% for year 3 (25–36 months) since distribution. These rates of loss are based on data available to

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[1.] WHO 2011. *Guidelines for monitoring the durability of long-lasting insecticidal mosquito nets under operation conditions.* http://www.who.int/whopes/guidelines/en/

date, and may change over time as more data become available. The examples below show how these numbers would be used to calculate LLIN losses since the time of distribution.

Example: calculation of LLINs for 2013

Year nets distributed	2011	2012	2013
Quantity distributed	50 000	100 000	10 000
Quantity lost	50 000*0.5 = 25,000	100 000*0.2 = 20 000	10 000*0.08 = 800
Proportion available	1–0.5 = 0.5	1–0.2 = 0.8	1–0.08 = 0.92
Nets existing from each year	25 000	80 000	9 200
Total existing nets 2013	114 200		

Distribution of ITNs/LLINs to households

The allocation of ITNs/LLINs at household level will depend on the existing functional nets in the community as follows:

▶ In communities with household ownership of ITNs/LLINs below 30% or unknown or where nets had not been distributed yet, allocation of nets through mass distributions is recommended targeting all households regardless of existing ITNs/LLINs.

▶ In communities with household ownership above 30%, household allocation to achieve universal coverage taking in to account the existing nets is recommended, assuming that there are sufficient numbers of nets in good condition.

▶ In communities where data suggest that most households (65%–85%) own multiple ITNs/LLINs and near universal coverage levels have been reached, national ITN/LLIN distribution campaigns may not be necessary. However, mini campaigns may be used to scale up household ownership in areas with low household ownership within regions / districts. In such situations emphasis can be put on establishing and strengthening distribution via routine systems to ensure sufficient ITNs/LLINs are reaching households to maintain universal coverage in areas where it has been achieved, and to take the final steps to achieving universal coverage where it has not.

Existing coverage is irregular, with some households having more than one net and many others with none, in which case it may be necessary to perform a household and net census as part of the household registration process in order to avoid local surpluses and stock-outs during the distribution. One method for household level allocation is to use a household registration or ITN/LLIN census process to identify needs in each house based upon current household ownership of functional nets and number of household members or sleeping spaces. In some countries coupons or bracelets for the number of nets needed for each household are given at this point to a representative of the household to bring to the distribution point on the day of the campaign. Distributions will thus target households where nets are needed.

Household registration and net censuses are an important step in the campaign process, and should be done following procurement of ITNs/LLINs, but before actual distribution. Planning for logistics (transportation, storage and prepositioning of ITNs/LLINs) is often based on the household registration and net census, so it is likely to benefit countries to leave enough time to feed household registration data into logistics planning and allow time for moving the ITNs/LLINs down the supply chain to the community distribution points.

Ensuring proper use of ITNs/LLINs

In order to be protected, households must not only own ITNs/LLINs but also use them. Behaviour change interventions including information, education, and communication (IEC) campaigns are strongly recommended. Distribution of ITNs/LLINs should be systematically accompanied by provision of information on how to hang, use and maintain them properly.

Basic information needed for implementation of LLINs as a vector control intervention

Insecticide

► Vector susceptibility to insecticides

► Efficacy of insecticides

Demographic data

► Population estimates

► Age, sex, income, number of people using nets

► Target groups: children, pregnant women (only when resources for universal coverage are not available)

Socio-behavioural data

► Sleeping pattern (outside, inside)

► Current use of nets

► Cultural attitudes

► Acceptable colours

► Preferred sizes given housing and sleeping conditionss

Vector bionomics

► Main vector species

► Biting habits (Exophilic vs endophilic, zoophilic vs anthropophilic, biting times)

► Vector density

► Seasonality of transmission

Climatic data

► Temperature

► Rainfall

► Humidity

Geographical data

► Area, rivers, roads, houses, type of houses

Burden of malaria

► Annual confirmed malaria cases per 1000 population

► Annual inpatient malaria deaths per 1000 population

▶ Annual parasite incidence (API)

▶ Slide positivity rate (SPR)

Health facilities

▶ Access to health facilities

5.2.1.7 Monitoring and evaluation of ITN/LLIN

The goal of most ITN/LLIN programmes is to reduce malaria mortality and morbidity by a specific amount during the planned years. Table 5.7 lists the main indicators (input, process, output, outcome and impact) used in evaluating ITNs/LLINs intervention.

Table 5.7 Process (operational), output, outcome (coverage) and impact (entomological) indicators for evaluation of insecticide treated nets (ITNs) or long-lasting insecticidal nets (LLINS)

Indicators	Control Method: LLINs
Process indicators	Number of LLINs procured for distribution (R)
Output indicators	Number of LLINs distributed to the target population (R)
Outcome indicators	Proportion of population with access to an ITN/LLIN within their household (R))
	Proportion of population at risk potentially covered by ITNs/LLINs distributed (R)
	Proportion of population sleeping under ITN/LLIN the previous night (R)
Impact (entomological) indicators	Human blood index (T)
	Insecticide susceptibility status (R)
	Human biting rate (T)
	Sporozoite rate (S)

R: indicators may be monitored regularly; S: selectively for specific purpose; T: for detecting trends

Following are descriptions of the indicators:

a. *Input indicators:* measure the level of resources available for use by the programme/ intervention, e.g. funding obtained to purchase ITNs/LLINs.

b. *Process indicators:* help to check that the ITN/LLIN intervention is implemented as planned, e.g. verifies that ITN/LLIN have been purchased and is ready for distribution and resources were available to deliver the nets.

c. *Output indicators:* measure bench marks of programme-level performance. The most important outputs to monitor include delivery of ITNs/LLINs by the programme and by the commercial market, e.g. the number of ITNs/LLINs distributed to the target population.

d. *Outcome (coverage) indicators:* measures population-level coverage of the ITN/LLIN intervention. The indicators used for ITN/LLIN coverage are the proportion of households with at least one ITN/LLIN for every two people, proportion of the population with access to an ITN/LLIN in their household, proportion of individuals sleeping under an ITN/ LLIN the previous night. The same definition of coverage should be used in baseline surveys

and post-programme evaluation. As well as coverage, re-treatment and appropriate use, all programmes should monitor and evaluate access and affordability in order to assess equity.

e. ***Impact indicators.*** The goal of most ITN/LLIN programmes is to reduce mortality and morbidity due to malaria through the use of ITNs/LLINs. Impact indicators therefore include reduction in mortality and morbidity. However, it is difficult to assess the specific contribution of ITN/LLIN programmes to any reduction in malaria mortality and morbidity as other antimalarial interventions such as diagnostic testing and treatment are used concomitantly. Impact of a vector control intervention can be measured in entomologic terms as well.

Other indicators for monitoring and evaluating ITN/LLIN programmes are described below:

LLINs durability indicators. The durability of LLINs distributed to the targeted populations should be monitored as part of the programme activities to assist in decisions on procurement, replacement, disposal or recycling, in product improvement and to further guide programme planning and practices. The elements to be considered in assessing the durability of LLINs are *(i)* net survivorship/attrition; *(ii)* fabric integrity and *(iii)* insecticidal activity (bioefficacy).[1] These components of durability are determined partly by factors intrinsic to the manufacture of the net (e.g. material composition, knitting or weaving pattern, quality of finishing, insecticide type and content, additives, LLIN technology) and partly by extrinsic factors that cause wear and tear. The following definitions, descriptions and indicators are proposed for the elements of durability:

Survivorship is the proportion of distributed nets still available for use as intended in the households to which they were given after a defined period, e.g. 1, 2, 3 or more years.

Attrition (opposite of survivorship) is the proportion of nets no longer in use as intended after a defined period following their distribution to the households. Attrition can be categorized as decay (e.g. destroyed, so torn and worn out that it is considered useless for protection against mosquitoes), absence (e.g. stolen, given away, moved) or used for other purposes.

Fabric integrity reflects the number, location and size of holes in each net. When possible, the assessment can also be categorized by type of hole (burn, tear, seam failure, nibbled or chewed by animals).

Insecticidal activity (bioefficacy) is the degree of knock-down, mortality or inhibition of blood-feeding induced in susceptible mosquitoes, as determined by standard WHO test procedures and criteria. Insecticidal activity is associated with the type and content or availability of insecticide. The insecticide content is expressed as g/kg or mg/m^2 of the LLIN and is determined by the method outlined in WHO specifications for LLINs (http://www.who.int/whopes/recommendations/en/).

5.2.2   Other insecticide-treated materials

Curtains and hammocks can also be treated with pyrethroid insecticides and used to reduce human-vector contact. Pyrethroid-impregnated blankets, canvas tents, chador, and plastic tents are all potentially protective against malaria; although generalizable data are limited they

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[1.]   WHO (2011). *Guidelines for monitoring the durability of long-lasting insecticidal mosquito nets under operational conditions.* Geneva, World Health Organization. http://whqlibdoc.who.int/publications/2011/9789241501705_eng.pdf

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can be used in some special circumstances, such as refugee camps. Curtains on doors and windows could also be useful supplementary interventions to ITNs in areas with a significant vector biting rate in the early evening, before people sleep, provided people are indoors at this time.

5.2.3 Other approaches for reduction of human-vector contact

5.2.3.1 Improved housing and location of settlements in relation to breeding sites

Household and community actions to improve the quality of housing (design, construction, alteration including screening/mosquito proofing) and to deter mosquito entry and indoor resting can have more permanent effects than insecticide-related control methods. Improved housing also improves the living condition and general health of the population. These are also relevant considerations in planned settlements including in development projects.

Poor housing is linked to higher risk of human-vector contact. For example, incomplete houses with open walls, wide or unscreened eaves, and houses with open windows and doors or without ceilings, favour mosquito entry. Houses with damp walls and floors favour indoor resting. House protection with screening of windows, eaves and doors is an effective method of reducing human-vector contact if properly implemented and maintained. New settlements should be carefully planned, selecting the correct design, structure, construction material, and location in relation to breeding sites, to prevent malaria.

5.2.3.2 Repellents, mosquito coils and protective clothing

The use of repellents and protective clothing are useful for people who are outdoors during peak vector biting periods. Most repellents have a very short duration of effect (8 hours, see Fig. 5.6).

Repellents. Repellents are available as creams, lotions and aerosol soaps. These may be applied either directly on the skin or on clothes. The use of repellents is a measure for individual protection. They complement ITN/LLIN and house protection and can be used after dark before retiring under the mosquito net or by people who stay outdoors during part of the night. In epidemics, repellents have sometimes been distributed for malaria control, although their cost-effectiveness is doubtful.

Figure 5.6   Mosquito coil and electric insecticide vaporizers

Mosquito coils. Some insecticides kill or repel mosquitoes at a distance when vapourized with a heating device. Mosquito coils are among the most popular and widely used insecticide vaporizers. Once lit, the coils smolder, releasing insecticide into the air at a steady rate for 6–8 hours.

Protective clothing. Clothes that cover most of the body, i.e. long-sleeved jackets and shirts, trousers and socks can provide a certain level of personal protection from mosquito biting.

5.3   Adult mosquito control

5.3.1   Indoor residual spraying (IRS)

IRS consists of the application of residual insecticides to the inner surfaces of dwellings, where many vector species of anopheline mosquito tend to rest after taking a blood meal. The main effect of IRS is killing the mosquitoes entering houses and resting on sprayed surfaces. Therefore, IRS is not useful for the control of vectors which tend to rest outdoors, although it may be effective against outdoor biting mosquitoes which enter houses for resting after feeding. IRS, when implemented properly, is a highly effective intervention providing protection to communities through a rapid mass effect on vector populations, reducing densities and longevity of vectors and their "vectorial capacity" to transmit malaria parasites.

5.3.1.1   Essential components of IRS planning, training, implementation and evaluation

Conditions for use and effectiveness of IRS

IRS is recommended only where:

▶ majority of the vector population is endophilic,

▶ the vector population is susceptible to the chosen insecticides,

▶ a high percentage of the houses or structures in the operational area have adequate sprayable surfaces,

▶ spraying is done correctly.

Mosquitoes rest in various locations during the gonotrophic cycle. Resting takes place indoors in human habitations, in animal shelters and outdoors on vegetation. The preferred vector resting sites in houses are walls, eaves, under furniture, and cool, dark, humid places. Vectors resting on sprayed surfaces are more likely to encounter a lethal dose of the insecticide and die than those that do not rest on sprayed surfaces.

Criteria for selective IRS

The considerable resource requirements, import needs, environmental concerns in their use, and the potential for development of vector resistance, compel highly selective targeting of IRS. As with any control intervention, the selection of IRS requires the definition of the population to be protected and the areas where the measure should be applied. The epidemiological situation determines which areas receive total coverage for a relatively long period of time, and which areas are covered only after the detection of certain risk factors.

In the areas to be sprayed, IRS requires, in principle, the coverage of all potential places where the vector might rest, at least for the first few hours after feeding and while searching for a host within an epidemiological unit. An epidemiological unit is the area where the vector circulates freely between breeding places and blood sources. It may be as small as an isolated group of houses together with several breeding places. The extent and intensity of the malaria problem and the mobility of the population affected will determine the size of the unit of intervention. The unit can be as large as an entire valley or even vary between certain altitudes.

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IRS is best indicated as a means of rapidly reducing malaria transmission in the following conditions:

▶ the control of epidemics detected in the early stages of development, where spraying can be done early enough to cut off peak transmission (i.e. areas where the seasonal peaks can be covered with one spray cycle per years);

▶ the control of seasonal transmission in areas with high malaria mortality, morbidity, and disease severity in order to reduce peaks of incidence;

▶ the prevention of epidemics following significant alarm signals of emerging risks in specific epidemic-prone areas (e.g. abnormally heavy rains or drought leading to an increase in vector breeding sites, high humidity or high temperatures, and the migration of large numbers of non-immune people into endemic areas);

▶ special risk situations, (e.g. non-immune population groups temporarily exposed to transmission risks) such as refugee camps, settlers in development project areas, labour camps, and army and police posts ;

▶ the reduction of transmission and the curtailment of the spread of drug-resistant parasites in areas with major drug resistance problems ;

▶ to respond to areas of identified insecticide resistance especially in areas where LLINs are the existing vector control intervention.

Planning for IRS

Planning for IRS involves stratification and delineation of areas to be covered, with more precise definition of the operational boundaries and the frequencies and times of applications (i.e. macro-, micro-analysis of information to select targets). The community should understand the importance of IRS including its safety.

Issues to be considered in planning IRS:

▶ transmission and burden of malaria are often focal and may vary with malaria endemicity and vector density even within a small area;

▶ aggregate indicators such as annual parasite incidence rates should not be the only criteria for undertaking IRS. Micro-stratification of malaria epidemiology, at the smallest unit used in planning control activities, is necessary for IRS targeting;

▶ the size of operational areas is influenced by vector distribution, distance from important breeding sites, vectors' flight range, demographic features, and distribution of malaria;

▶ IRS may be limited to specific geographical areas, villages, and times of the year.

The first decision to be made is whether IRS is a suitable intervention for the malaria problem in a particular area. The choice should be based on an evaluation of the results of previous vector control activities. To improve the interpretation of existing records it is necessary to collect information on local vector bionomics and behaviour.

For IRS to be effective, apart from the identification of an effective insecticide, a number of other conditions must be met:

▸ The vector should preferably be endophilic. However, spraying may be effective to some extent against vectors which are partially exophilic, i.e. they rest indoors only for a few hours after biting and then spend most of the time digesting blood and developing eggs outdoors.

▸ The human habitations should have walls to which the insecticide can be applied.

▸ The required coverage must be achieved before the onset of the transmission season and maintained for the length of the season. This is particularly important in the control of epidemics. When an epidemic is recognized following an alarming increase of malaria cases, it is essential to ascertain whether transmission is likely to continue; IRS is not advisable when the epidemic is subsiding and transmission is coming to an end.

IRS should not be planned unless full capacity for implementation, monitoring and evaluation is in place at national, provincial and district level. Delays in implementation of IRS programmes can have disastrous public health consequences.

Definition of targets of IRS application

The targets to be sprayed should be clearly defined and a geographical reconnaissance of the area should be undertaken so that maps and guidelines can be prepared for the spraymen as follows:

Areas to be sprayed. The intervention units must be mapped or clearly marked so that they can be easily recognized by spraying squads. Maps and identifying criteria should be made available to guide those responsible for the spraying operations.

Structures. The types of structure to be sprayed should be defined and include all human habitations where vector-human contact is likely to occur. In many rural areas, for example, people may spend long periods of time in "farm huts" within their fields and these structures may be very important in maintaining transmission. Similarly, other structures, such as animal shelters, latrines, stores or outhouses, may be important resting places for blood-fed mosquitoes.

Sprayable surfaces. IRS requires a high degree of coverage of potential resting places, including all walls, ceilings and furniture. The spraying of window frames and both sides of doors is often necessary, since they may be temporary resting places for vectors entering or leaving a room.

Organizational and logistic requirements of IRS

IRS requires very high coverage in order to be effective. Spraying should be:

▸ total – all the dwellings are sprayed,

▸ complete – cover all sprayable surfaces,

▸ sufficient – uniform application of the required dose to all sprayable surfaces,

▸ regular – repeated at regular intervals to ensure an effective residue is present during the transmission season.

The need to cover all houses means that a complete knowledge of the geography of the area is necessary and that the spraymen must cover all outlying houses and scattered populations. A geographical reconnaissance is generally required to update local maps and census data. Meeting these standards requires a disciplined and competent organization with properly equipped and trained spraymen and efficient logistic support. Traditionally, IRS has been

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based on the operational model of the malaria eradication campaigns of the 1950s and 1960s, which called for a strong autonomous and centralized organization. This model no longer exists in most areas and the need for such centralization has been questioned, particularly where countries are embarking on a policy of decentralization. Special attention should be paid to:

▶ the logistics of operational support, supplies, supervision and monitoring,

▶ the planning required for the regular application of IRS and the technical guidance needed for decentralized operations,

▶ the responsibility of individuals and the community – decentralized operations will benefit from decisions and actions at the local level through sustained local capacity and participation of communities.

Selection of insecticides for IRS

Residual insecticides for spray application are formulated as:

▶ water-dispersible powder, a dry powder insecticide mixed with a surface-active agent that allows it to dissolve in water. Made ready for mixing with water to form a spray suspensions normally containing 1% – 5% of active ingredient.

▶ emulsifiable concentrate, a solvent + emulsifying agent in which the insecticide is dissolved. When mixed with water it forms an emulsion suitable for delicate surfaces and does not cause spots or stains, but this is more expensive.

▶ suspension concentrate, particles of insecticide with wetting agent and water, providing a water-based suspension. This is non-flammable, long-lasting, but less effective than water-dispersible powder on porous surfaces.

The choice of insecticide and its formulation should be based on: *(i)* the susceptibility of the local vectors; *(ii)* the characteristics of the various compounds; *(iii)* the type of wall/roof surface; *(iv)* the formulations of the available products (e.g. residual effect); and *(v)* cost. Information on the insecticides recommended by WHOPES for IRS is given in Table 5.8.

The choice of insecticide and its formulation depends on its effectiveness against the local vector species and its safety. Susceptibility testing should therefore be done first. Even if an insecticide is effective elsewhere, it may be necessary to conduct small field trials to determine its effectiveness and residual efficacy under local conditions.

Once an insecticide and its formulation are selected, it is essential to choose a quality product. Products that appear to be similar may not contain the same concentration of active ingredient. Even if the concentration is accurate, the product may be poorly formulated, may not suspend well, block sprayers, and give uneven coverage. It may also deteriorate rapidly during storage and produce toxic derivatives.

WHOPES can assist national vector control programmes in strengthening and/or establishment of the capacity for quality control of pesticides. WHOPES can also provide the specifications, criteria and guidelines for this purpose.[1] When necessary the procedures can be carried

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[1.] WHO (2000). *Guidelines for the purchase of public health pesticides.* Geneva, World Health Organization. Document WHO/CDS/WHOPES/2000.1.

out at WHO-designated collaborating centres on behalf of the programme. WHO country representatives can provide information on how to order insecticides using the WHO supply service.

Regulatory mechanisms, national policies and legislation for public health must be available in relation to the selection, importation, and use of insecticides. These will ensure the safety, quality, and efficacy of insecticides. In the long-term it will lead to vector resistance management. Insecticide registration must be based on adequate evaluation data (WHOPES, supplemented where possible with data from evaluations undertaken in the country itself).

Imported insecticides should conform to the WHO specifications for public health use. When procuring insecticides, reports of conformity of the selected insecticides to WHO specifications must be examined by an independent institution before the insecticides concerned leave the place or country of origin.

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Table 5.8   WHO recommended insecticides for indoor residual spraying (IRS) against malaria vectors

Insecticide compounds & formulations[a]	Class group[b]	Dosage (g a.i./m²)	Mode of action	Duration of effective action (months)
DDT WP	OC	1–2	Contact	> 6
Malathion WP	OP	2	Contact	2–3
Fenitrothion WP	OP	2	Contact & airborne	3–6
Pirimiphos-methyl WP & EC	OP	1–2	Contact & airborne	2–3
Bendiocarb WP	C	0.1–0.4	Contact & airborne	2–6
Propoxur WP	C	1–2	Contact & airborne	3–6
Alpha-cypermethrin WP, SC	PY	0.02–0.03	Contact	4–6
Bifenthrin WP	PY	0.025–0.050	Contact	3–6
Cyfluthrin WP	PY	0.02–0.05	Contact	3–6
Deltamethrin WP, WG	PY	0.020–0.025	Contact	3–6
Etofenprox WP	PY	0.1–0.3	Contact	3–6
Lambda-cyhalothrin WP, CS	PY	0.02–0.03	Contact	3–6

[a.] CS: capsule suspension; EC = emulsifiable concentrate; SC = suspension concentrate; WG = water dispersible granule; WP = wettable powder

[b.] OC = organochlorine; OP = organophosphate; C = carbamate; P = pyrethroid

Note: WHO recommendations on the use of pesticides in public health are valid ONLY if linked to WHO specifications for their quality control. WHO specifications for public health pesticides are available at http://www.who.int/whopes/quality/en/.

Acceptability. House spraying requires the coordinated coverage of all sprayable surfaces at regular intervals (spraying cycle). The aim is to have a high coverage of all potential vector resting places with the effective dose of insecticide during the entire period when transmission is to be controlled.

When residual spraying is used, a plan must ensure that the required coverage will be achieved for the specified period and that sufficient human and material resources will be available for this purpose.

Whether spraying is done by a specialized organization or by the community itself, indoor spraying requires the continued collaboration of the population, which may easily be eroded

if people are not made continuously aware of the need for vector control. This is particularly important if some of the early benefits of spraying, such as the control of nuisance insects, are lost with time. It is therefore essential to maintain active contact with the community through an effective IEC mechanism.

Dosage. Dosage is the amount of insecticide applied per unit area. It is normally expressed as grams or milligrams of active ingredient per square metre (g/m^2 or mg/m^2) of sprayable surface. Doses vary considerably for the different insecticides. Most of the pyrethroids are effective at doses of 10–50 mg/m^2, while DDT, organophosphate and carbamate insecticides generally require doses of 1–2 g/m^2.

Preparation of houses before spraying. Correct spraying requires the careful preparation of the rooms to be sprayed. In particular: all food, cooking utensils, bedding and clothes must be protected from the insecticide by taking them outside of the house before spraying starts; and all portable furniture and any pieces of furniture leaning against the walls should be removed so that the walls and all sides of all the pieces of furniture can be sprayed.

When to apply the insecticide. The repetition of spraying operations at regular intervals is termed the "spraying cycle". It is the interval between repetitions, e.g. a 6-month cycle. Each spraying of all sprayable houses in an area over a period of time is termed a "spraying round". The epidemiological requirements and the residual effect of the insecticide formulation on the main sprayable surfaces will determine the frequency of the spraying cycle.

In areas with seasonal transmission the insecticide selected for use should be effective during the period of time that transmission is likely to occur. Areas requiring continuous protection should be sprayed regularly. To maintain effective coverage during the entire transmission season, spraying of the whole area to be protected must be completed before the beginning of that season (often the rainy season). This requirement has operational implications that must be taken into account, particularly when the operations are conducted by decentralized health services, in order to ensure the timely reception of supplies and the training or retraining of spraymen.

Residual spraying techniques. The application of IRS has been standardized throughout the world. It is always necessary to check the working practices of spraymen in order to ensure that neither humans nor the environment are endangered. This is particularly important when insecticides with greater acute toxicity are to be used.

The application of a uniform dose of insecticide to all the sprayable surfaces can best be achieved by means of compression sprayers that meet WHO specifications.[1] WHO-approved compression sprayers (Fig. 5.7) are sturdy enough to maintain the pressure needed to produce a flat fan swath and to resist rough handling in the field. These sprayers should be fitted with nozzle tips producing the required swath and discharge rate, and with pressure gauges or control flow valves (CFV) graduated to deliver the required rate of application. Nozzle tips erode fairly quickly when insecticide suspensions are used at high pressure and should therefore be made of highly resistant materials (hardened steel, ceramics, etc.) and be checked frequently to avoid waste of insecticide or irregular dosage.

[1.] WHO 2013. *Indoor residual spraying: An operational manual for IRS for malaria transmission, control and elimination.* Geneva, World Health Organization. http://www.who.int/malaria/publications/atoz/9789241505123/en/index.html
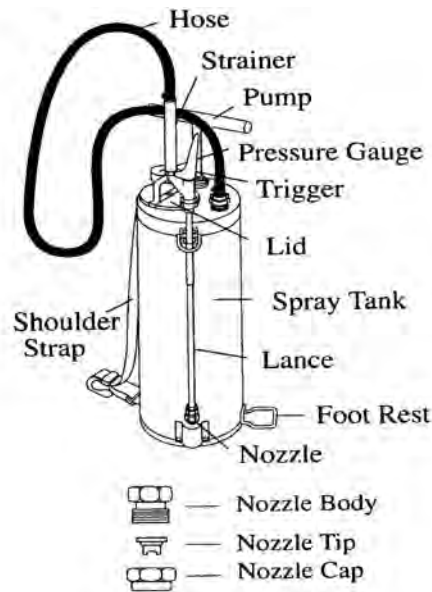
Figure 5.7   WHO-approved compression sprayer (Hudson X-pert)
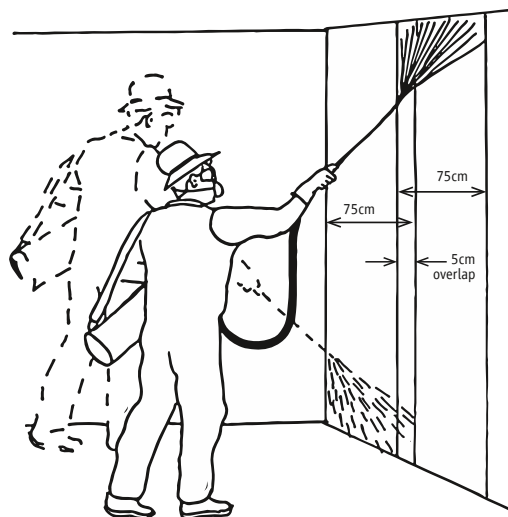
Figure 5.8   Spraying technique

The WHO manual on IRS describes procedures for the safe and effective use of insecticides for IRS and also covers the maintenance of equipment.[1] The spray is applied in swaths 75 cm wide. Swaths should overlap by 5 cm. Spraying is done from the roof to the floor, using a downward motion, to complete one swath. The sprayman steps sideways and sprays upwards from floor to roof (Fig. 5.8).

Safety measures. The safe use of insecticides for IRS requires a number of precautions. The removal or physical protection of all foodstuffs and cooking or eating utensils is imperative. Inhabitants should be advised not to enter a sprayed room until the spray is dry, and to sweep all floors before allowing free entry into the house. This is particularly important for families with small children or indoor domestic animals that may have greater contact with the floor.

[1.]   WHO 2013. *Indoor residual spraying: An operational manual for IRS for malaria transmission, control and elimination.* Geneva, World Health Organization. http://www.who.int/malaria/publications/atoz/9789241505123/en/index.html

The use of protective devices and safe working practices is essential to avoid or reduce insecticide contamination of spraymen, packers and mixers. In most spraying programmes in which insecticides of low acute toxicity (such as DDT) have been used, it is sufficient to wear overalls, broad-brimmed hats to cover the neck of the overalls, gloves and shoes or boots (the openings of which should be covered by the long trousers of the overalls). More toxic or more irritating insecticides require more elaborate protective devices such as light masks, goggles, visors and respirators.

Spray operators have a higher risk of contamination and should therefore use rubber gloves, masks or respirators and protect their eyes with a visor made of transparent plastic attached to the hat. The current trend is for insecticides to be delivered from the manufacturer in pre-packed pump charges, preferably in water-soluble sachets, which can be dropped directly into the pump tank, so that packers and mixers are not needed. Suspension concentrate (SC) and emulsifiable concentrate (EC) formulations in dosage-regulating containers and water-dispersible granular formulations are also available and limit exposure during spray tank preparation.

Squad leaders must enforce safe practices and the appropriate use of protective devices. They must be familiar with early signs of intoxication and monitor members of their squad for any sign of poisoning.

Basic precautions to prevent unnecessary contamination include:

▸ Hands and face should be washed after filling each pump charge.

▸ Eating, drinking and smoking should be forbidden, except after washing and before starting to spray.

▸ Spraymen should not be exposed to insecticide for more than six hours each day.

▸ Overalls and hats should be washed daily, especially if they have been heavily contaminated.

▸ Spraymen must take a shower at the end of each day's work, particularly when they have been working with organophosphate insecticides.

▸ If respirators are used, they must fit well around the nose and mouth. They must be washed, dried and the cartridge must be changed daily or whenever it becomes obstructed.

Empty insecticide containers must be collected by the team supervisors and brought to the central storage area for proper disposal by qualified staff, in accordance with the FAO/WHO/UNEP Guidelines.[1] It is also essential to follow the recommendations for the disposal of larger metal containers. Reuse of containers is always dangerous. If containers are to be reused they must be selected and cleaned by properly trained personnel.

The chart opposite shows facts about indoor residual spraying (IRS).

Advantages of IRS

▸ Very effective for species with endophilic and endophagic behaviour

▸ Greater impact in reducing vectorial capacity than other measures

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[1.] FAO (1999). *Guidelines for the management of small quantities of unwanted and obsolete pesticides*. Rome, FAO, Field document GCP/ INT/650/NET.

> **Indoor residual spraying (IRS)**
> • The main purpose of IRS is to reduce the survival of malaria vector(s) that enter houses
> • IRS is of little use for control of malaria vectors that rest outdoors, particularly if they also bite outdoors and do not enter the sprayed house
> • To ensure its expected impact (community protection), all potential resting surfaces of vector(s) should be sprayed with appropriate insecticide, at a dosage that is sufficient to remain effective throughout the transmission season
> • Understanding of malaria epidemiology and resting behaviour of the vector is crucial to proper targeting of insecticide application in time and space
> • The residual action of the insecticide depends on the insecticide compound and formulation, as well as the type of surface and climatic conditions

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Disadvantages of IRS

▸ High cost

▸ Requires well trained personnel

▸ In long term use, community participation may decrease

▸ Due consideration should be given to the safe disposal of excess insecticide

▸ Programme failure (i.e. missing or delaying a spray cycle) can have disastrous public health consequences

Monitoring and evaluation of IRS implementation

Table 5.9 lists the main important indicators for evaluating IRS.

Table 5.9   **Process (operational), output, outcome and impact (entomological) indicators for evaluation of indoor residual spraying (IRS)**

Indicators	Control Method: IRS
Process indicators	Insecticides provided for spraying (R)
	Spraymen trained (R)
	Status of spraying equipment (R)
	Appropriate timing of spraying (R)
Output indicators	Number of structures sprayed (R)
	Number of spraying cycles (R)
Outcome Indicators	Proportion of population at risk protected by IRS within 12 months (R)
	Proportion of house/structures sprayed with insecticide (R)
Impact (entomological) indicators	Human blood index (T)
	Insecticide susceptibility status (R)
	Human biting rate (T)
	Sporozoite rate (S)

R: Indicators may be monitored regularly; S: Selectively for specific purpose; T: For detecting trends

Estimating insecticide and human resource requirements for IRS

In a small community of 500–1000 people (100–200 houses) one or two operators equipped with compression sprayers can complete a cycle of residual spraying in 2–3 weeks working at the rate of 8–10 houses per day. The optimal timing of spraying recommended for the project by the central vector control service should be carefully respected. To this end, any elements must be assembled by the project officer a month before the spraying is to start. The frequency of treatment depends on the residual duration of the insecticide formulation at the dosage used, the type of surface sprayed, vector bionomics, the transmission season, and climatic conditions (wind, temperature and humidity). However, early re-treatment is required if insecticide deposits are removed from surfaces through re-plastering, whitewashing, re-roofing, or by smoke deposits.

In order to carry out a successful IRS programme it is important to *(i)* map the project area and number the houses, *(ii)* estimate the average area per house that needs to be sprayed, and *(iii)* determine the insecticides, personnel, spraying equipment and transport requirements.

Insecticide formulation in sufficient quantity for one year; spraying, mixing; and protection equipment and transport – example of estimates based on geographical reconnaissance data:

▸ Number of houses in the project area = 5000

▸ Average sprayable surface per house = 200 m²

▸ Insecticide dosage to be applied (Lambdacyhalothrin or deltamethrin) = 25 mg/m²

▸ The formulation to be used is Wettable Powder (WP) = 10% concentration

▸ Duration of spraying cycle is 2 months (2 x 20 days = 40 working days)

▸ Treatment cycle per year = 2 times

▸ Average output of sprayman per day =10 houses (10 x 200 = 2000 m²)

▸ Insecticide formulation required for one cycle will be calculated as follows:

    ▸ Technical insecticide per house = 200 m² x 25 mg/m² = 5000 mg

    ▸ Insecticide as 10% WP per house = 5000 x 100 / 10 = 50 000 mg = 50 g

    ▸ Insecticide formulation required for the project area per cycle = 5000 houses x 50 = 250 000 g = 250 kg

▸ Total insecticides required for 2 cycles = one year = 250 x 2 = 500 kg

▸ Reserve provision of insecticides =10% = 50 kg

▸ Total insecticides for one year = 550 kg

▸ Total area to be covered = 5000 houses x 200 m² = 1 000 000 m²

▸ Area covered/sprayman/cycle = 2000 m² x 40 days = 80 000 m²

▸ Number of spraymen required = 1 000 000 / 80 000 = 12.5 = 13

    Or:

▸ Number of houses covered per spraymen = 10 houses per day x 40 days = 400 houses

▸ Number of spraymen required for one cycle = 5000 / 400 =12.5 = 13

5.3.2 Space spraying

A space spray – technically a fog (sometimes referred to as an aerosol) – is a liquid insecticide dispersed into the air in the form of hundreds of millions of tiny droplets less than 50 μm in diameter. Space spraying is defined as the destruction of flying mosquitoes by contact with insecticides in the air. The main objective is to reduce vector density and increase vector mortality as soon as possible. It has limited use in malaria control and is used as a complementary method of vector control in specific situations. It is used mainly in conjunction with mass treatment of fevers. It has also been used with some reported success in the control of malaria epidemics or of highly exophilic vectors, such as *A. dirus* in refugee camps in Thailand and *A. nuneztovari* in the Bolivarian Republic of Venezuela. It has been used in the emergency control of epidemics (in the developing phase) and where there is sufficient evidence that the main determinant factor is an abnormal vector density.

Space spraying is designed for:

- ► rapid knock-down,
- ► rapid mortality, and
- ► rapid method of control in epidemic and emergency situation,

Space spraying can be applied as *(i)* Thermal fogging or *(ii)* Cold fogging (see Fig. 5.9).

5.3.2.1 Thermal fogging

The insecticide used in thermal fogs is diluted in a carrier liquid, which is usually oil-based. Hot gas is used to heat the pesticide spray, decreasing the viscosity of the oil carrier, and vaporizing it. When it leaves the nozzle the vapour hits colder air and condenses to form a dense white cloud of fog. The hot emission gas is obtained from engine exhaust, friction plate/engine exhaust or from a pulse jet engine.

Advantages of thermal fogging (versus cold fogging)

- ► Easily visible fog can be observed and monitored.
- ► Good public relations as people can see something being done about the problem.
- ► Low concentration of pesticides in the spray mixture and limited operator exposure.

Disadvantages of thermal fogging (versus cold fogging)

- ► Large volumes of organic solvents are used as diluents, which may have a bad odour and result in staining.
- ► High cost of diluent and spray application.
- ► Requires special and expensive equipment.
- ► Householders may object and obstruct penetration of fog into houses by closing windows and doors.
- ► Efficacy often depends on the meteorological conditions at the time of application, including wind direction, rain, and temperature.
- ► Fire risk from machinery operating at very high temperatures with flammable solvents.
- ► Can cause traffic hazards in urban areas due to reduced visibility

5.3.2.2   Cold fogging

With cold fogs the droplets are formed by the mechanical breaking up of the spray mixture, either by passing it through high-pressure nozzles or by passing a slow stream of the mixture through a high-velocity vortex of air. Some equipment is fitted with high-speed rotary nozzle(s). The spray droplets are generated without any external heat.

Advantages of cold fogging (versus thermal fogging)

▸ The amount of diluent is kept to a minimum, resulting in lower application cost and increased acceptability.

▸ Some formulations are ready to use, thereby reducing operator exposure.

▸ May use water-based and water-diluted formulations, which pose a low fire hazard and are relatively environmentally friendly.

▸ Because a lower volume of liquid is applied, application is more efficient.

▸ No traffic hazard as the spray cloud is almost invisible.

Disadvantages of cold fogging (versus thermal fogging)

▸ Dispersal of the spray cloud is difficult to observe.

▸ Higher technical skills and regular calibration are required for efficient operation of the equipment.

To have an impact on vector density the spray operation must be timed to coincide with peak activities of the vector. An important characteristic of space spraying is the size of the droplets dispersed, which governs the time they remain in suspension, and their ability to penetrate into spaces that are not fully open. Operational costs are high and the killing effect is transient. Space spraying must be considered only exceptionally, and for very limited periods of time.

Fogging may be considered in exceptional emergency situations, e.g. in refugee camps. In such situations, if the target mosquito species is exophilic, treatment should be applied outdoors on mosquito resting places. If the vector is endophilic, treatment should be applied indoors and outdoors, coinciding with local vector flying time.

5.3.2.3   Insecticides and formulations for space spraying

Table 5.10 shows the insecticides suitable for use as thermal fogs or cold aerosols for mosquito control (see Fig 5.9). Pyrethroids are becoming the predominant insecticides for use in space spraying, while organophosphates are becoming less acceptable because of their smell.

5.4   Larval control

Larval control is indicated as the sole method of vector control only if a high proportion of the anopheline breeding sites within the vector's flight range of the community to be protected are few, fixed, findable and manageable.[1] Larval control may be also undertaken to supplement the effects of the core vector control interventions (LLIN and IRS). Larval control affects only

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[1.]   WHO (2012). Interim position statement: the role of larviciding for malaria control in sub-Saharan Africa. Geneva, World Health organization, available at http://www.who.int/malaria/publications/atoz/larviciding_position_statement/en/

Table 5.10   Selected insecticides suitable for cold or thermal fogging for mosquito control

Compounds and formulations	Indoor (g AI/ 1000 m3)		Outdoor (g AI/ha)	
	Cold fog	Thermal fog	Cold fog	Thermal fog
Deltamethrin UL	0.5	0.05	0.5–1.0	0.5–1.0
Deltamethrin EW	—	0.05	1	
Lambda-cyhalothrin EC	—	—	1–2	2
Malathion UL	—	—	112–600	112–600
Permethrin (25 cis:75 trans; 10.35% w/w) + sbioallethrin (0.14 w/w) + piperonyl butoxide (9.85% w/w) EW	0.55 permethrin	0.73 permethrin		
d-d, trans-cyphenothrin EC	0.1–0.2	0.2	3.5–4.0	3.5–4.0

EC = Emulsifiable concentrate; EW = emulsion, oil in water; UL = ultra-low volume (ULV) liquid

Notes:
1. Reports of the WHOPES Working Group Meetings (available at http://www.who.int/whopes/recommendations/wgm/en/) and the WHOPES publication *Pesticides and their application for control of vectors and pests of public health importance* (available at http://whqlibdoc.who.int/hq/2006/WHO_CDS_NTD_WHOPES_GCDPP_2006.1_eng.pdf) should be consulted for guidance on use and recommendations;
2. WHO recommendations on the use of pesticides in public health are valid ONLY if linked to WHO specifications for their quality control (available at http://www.who.int/whopes/quality/newspecif/en/).

U5
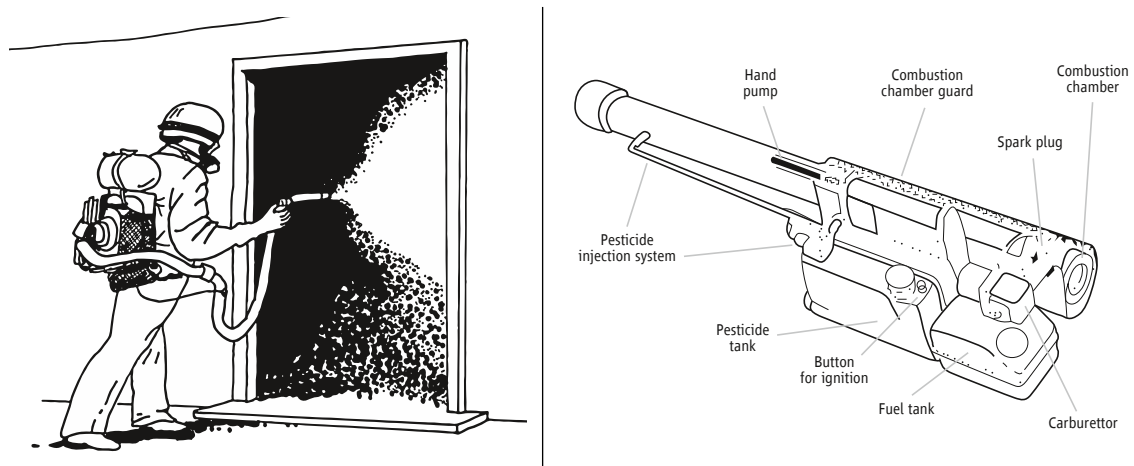
Figure 5.9   Space sparing by thermal (above) and cold fog (below)

the vector density and requires a high coverage to be effective. Only a proportional reduction in vectorial capacity through reduction in m is expected from larval control, whereas reducing adult survival p and m (by using residual spraying) produces a much greater reduction in vectorial capacity. The larval control methods include larviciding and larvivorous fish.

Larval control may be useful in the following settings:

- ▶ in densely populated areas with relatively few, fixed and findable breeding places, e.g. towns and cities

- ▶ in areas where breeding sites are easy to locate and not extensive, e.g. highland areas, desert fringe areas, during very dry periods in endemic areas when the breeding sites are very limited, definable and manageable

- ▶ in refugee camps

- ▶ in development areas such as small-scale irrigation schemes and construction sites

5.4.1 Larviciding

Larviciding includes the use of chemicals or biological agents to kill larvae and pupae. Larvicides are used in areas where the breeding sites are few, fixed (water body relatively long-standing duration that persists during or beyond the rainy season) and findable (Fig. 5.10).

Figure 5.10 Larviciding in breeding places

5.4.1.1 Indications

Larviciding is indicated only for vectors which tend to breed in semi-permanent water bodies that are few, findable, fixed and where the density of the human population to be protected is sufficiently high to justify the intervention. These prerequisites make larviciding suitable for areas of clearly defined habitats such as urban areas, desert fringe, highland areas, labour or refugee camps and development projects. In these situations, it is possible that larviciding programmes may be complementary to environmental measures aimed at controlling malaria and other mosquito-borne diseases or nuisance mosquitoes in integrated control programmes. In areas of high malaria transmission, larviciding should normally be used only as a supplement to the core interventions (ITNs or IRS); larviciding should never be seen as a substitute for ITNs or IRS in areas with significant malaria risk.

Because of limited indications for larviciding for malaria control and the high degree of coverage needed for it to be effective, it is very important to define precisely the area and the points where the intervention should be applied. Ground application of larvicides is not recommended in areas of extensive flooding such as large-scale irrigated rice production or the floodplains of large rivers.

5.4.1.2 Residual effect

The residual effect of larvicides varies considerably depending on the water quality and type of the breeding place, but is relatively short for most larvicides. It is normally necessary to repeat larviciding operations at weekly intervals during the rainy season, whatever the residual characteristics of the product used, because new breeding sites are always appearing, and eggs laid in new sites may reach adulthood in just 7–10 days.

5.4.1.3 Available larvicides

A variety of larvicides are or have been used for malaria control (Table 5.11), including chemicals and insecticides of biological origin; these vary in their modes of action, efficacy, safety, formulations, cost and availability. Larvicides of potential use are discussed below.

Oils

These are used for stagnant water bodies which are unsuitable for animal drinking and irrigation. Oils act mainly by forming a film on the water surface, thereby preventing larvae from breathing. The heavier the oil the less dispersible it will be and the more easily blocked by vegetation. The use of petroleum oil is not recommended due to environmental contamination.

Table 5.11   WHOPES recommended compounds and formulations for control of mosquito larvae

Insecticide compounds and formulation(s)[a]	Class group[b]	Dosage (active ingredient)	
		General (g/ha)	Container breeding mg/L
Bacillus thuringiensis israelensis, strain AM65-52, WG	BL	125–750[c]	1–5[c]
Diflubenzuron DT, GR, WP	BU	25–100	0.02–0.25
Novaluron EC	BU	10–100	0.01–0.05
Pyriproxyfen GR	JH	10–50	0.01
Fenthion EC	OP	22–112	–
Pirimiphos-methyl EC	OP	50–500	1
Temephos EC, GR	OP	56–112	1
Spinosad DT, EC, GR, SC	SP	20–500	0.1–0.5

[a] DT = tablet for direct application; GR = granule; EC = emulsifiable concentration; WG = water-dispersible granule; WP = wettable powder.
[b] BL = Bacterial Larvicide; BU = Benzoylureas; JH = Juvenile Hormone Mimics; OP = Organophosphates; SP = Spinosyns.
[c] Formulated product.

Notes:
1. Reports of the WHOPES Working Group Meetings (available at http://www.who.int/whopes/recommendations/wgm/en/) and the WHOPES publication *Pesticides and their application for control of vectors and pests of public health importance* (available at http://whqlibdoc.who.int/hq/2006/WHO_CDS_NTD_WHOPES_GCDPP_2006.1_eng.pdf) should be consulted for guidance on use and recommendations;
2. The WHO *Guidelines for drinking-water quality* (http://www.who.int/water_sanitation_health/dwq/gdwq3rev/en/) provides authoritative guidance and should be consulted for application of insecticides in potable water for mosquito larviciding; and
3. WHO recommendations on the use of pesticides in public health are valid ONLY if linked to WHO specifications for their quality control (available at http://www.who.int/whopes/quality/newspecif/en/).

Common chemical insecticides

Organophosphate insecticides (Table 5.11) are widely used despite increasing levels of resistance in some areas. Temephos, which has a very low mammalian toxicity, has been the most widely used mosquito larvicide worldwide. It may be applied to water used for the irrigation of food

crops, and has also been used for treating drinking water. It is, however, toxic to fish. Fenthion is also commonly used when there is no risk of contamination of drinking water and food.

Insect growth regulators

These are chemical compounds which are highly toxic to mosquito larvae by preventing their development into adults. Their use has generally been limited by their high cost. Insect growth regulators can be grouped as *(i)* juvenile hormone analogues, which prevent the development of larvae into viable pupae, or of pupae into adults (they do not kill larvae) and *(ii)* chitin synthesis inhibitors, which interfere with the molting process by killing the larvae when they molt.

Microbial larvicides

The bacterium *Bacillus thuringiensis israelensis* (*Bti*) produces toxins which are very effective in killing mosquito larvae after ingestion. It is harmless to other insects, fish, higher animals and humans at normal dosages and, at appropriate doses, may be suitable for use in water used for drinking (with due attention to potential microbial contaminants in the formulated product) or for the irrigation of food crops. It has the disadvantage that it breaks down quickly in the environment and must be reapplied periodically. Recent formulations have been developed to keep the bacteria high in the water column so that they can be eaten by anopheline larvae. Another bacterium, *B. sphaericus,* also produces a toxin. It has characteristics similar to those of *Bti* but is more effective in polluted water whereas *Bti* is more effective in clean water.

5.4.1.4 Monitoring and evaluation of larviciding

Table 5.12 shows the process and impact indicators for evaluating larviciding.

Table 5.12   Process (operational), output, outcome and impact (entomological) indicators for evaluation of larviciding

Indicators	Control method: Larviciding
Process indicators	Number of breeding sites identified (R)
Output indicators	Number of breeding sites treated with larvicides (R)
Outcome Indicators	Proportion of breeding sites treated among those targeted (R)
Impact (entomological) indicators	Presence and density of larvae (R)
	Adult mosquito density (R)
	Insecticide susceptibility status (R)

R: Indicators may be monitored regularly; S: Selectively for specific purpose; T: For detecting trends

5.4.2   Source reduction

The term source reduction refers to any measure that prevents the breeding of mosquitoes or eliminates their breeding sites. Source reduction is a component of environment management which aims to modify the environment in order to deprive the vector population of its requirements for survival (mainly breeding, resting and feeding), thus reducing human-vector contact and transmission risks.

If such measures bring about long-lasting or permanent changes on land, water or vegetation, they are referred to as environmental modification (e.g. filling, drainage, planting water loving trees such as eucalyptus trees in swampy areas, and closing or covering breeding sites).

When such measures have a temporary effect and need to be repeated, they are known as environmental manipulation (e.g. water-level fluctuation, intermittent irrigation, flushing, changing water salinity, clearing vegetation in streams, and irrigation canals).

Many development-linked activities (e.g. irrigation) lead to environmental changes and often inadvertently increase the risk of malaria transmission. Appropriate safeguards and mitigation actions are required in the planning, construction, and maintenance phases of development projects. Irrigation canals should be lined and the vegetation cleared to discourage breeding in the canal edges and allow free flow of water. Periodicity of water release can also be adjusted to allow flushing of larvae from the pooling canal beds.

Source reduction can provide permanent prevention of anopheline breeding and should be encouraged where possible. This may entail collaborating with other sectors such as agriculture and city planning departments. Whilst costs can be high, they may be met outside the health sector, e.g. in the case of city drainage.

5.4.2.1 Advantages of source reduction

- Relies on non-chemical methods.
- When implemented well, can lead to total elimination of mosquito breeding sites.
- Provides opportunity to collaborate with sectors other than vector control programmes for malaria, i.e. agriculture, public works and roads, environment and the community.

5.4.2.2 Disadavantages of source reduction

- Can be costly
- Is not applicable everywhere
- Requires periodic maintenance

5.4.3 Larvivorous fish

Larvivorous fish feed on mosquito larvae. Some of the most successful species introduced in different countries are the top minnow or mosquito fish (*Gambusia affinis*) and the guppy (*Poecilia reticulata*). *Gambusia* is most efficient in clean water, while *Poecilia* can be used successfully in organically polluted water. *Poecilia* tolerates higher temperatures than *Gambusia* and may therefore be more effective in rice fields in hot areas. However, unlike *Gambusia*, it cannot survive temperatures below 10 °C. The annual killifishes, *Cynolebias, Nothobranchius* and *Aphyosemion*, have drought-resistant eggs and can be used in breeding sites that temporarily dry out, such as borrow-pits and irrigated rice fields. In addition, locally collected fish have been evaluated for their efficacy in controlling mosquitoes and a number of species have proven useful. The use of local larvivorous fish is particularly important to avoid the risk of disturbing the ecological balance by introducing "exotic" fish species (Fig. 5.11). However, the role of fish in controlling malaria has been difficult to evaluate.

5.4.3.1 Potentially suitable settings for larval control using fish

- Mosquito breeding places are well defined
- Water conditions are suitable
- Chemical larviciding in not suitable

Figure 5.11 Different larvivorous fish

5.4.3.2 **Potential larvivorous fish should have the following characteristics:**
- ▸ High preference for mosquito larvae
- ▸ Surface feeder
- ▸ Terminal or superior mouth
- ▸ Small in size
- ▸ High fecundity
- ▸ Tolerant to transportation, stressful environmental conditions, temperature extremes, the presence of pollutants and high turbidity.

Larvivorous fish can be used in both natural and constructed habitats such as water tanks, lakes, fountains, pools, ponds, cattle troughs, swimming pools, water storage tanks, seepages, water storage sites, irrigation cisterns, canals, small dams, rice fields, slow moving small streams, swamps and temporary water collection sites.

5.5 Monitoring the impact of vector control interventions on malaria

Table 5.13 summarizes effect of the different vector control methods on vector population and behaviour. Any vector intervention for malaria control should set objectives in relation to its expected contribution to the overall goal of malaria control. Monitoring and evaluation are essential for the effective management of any vector control programme, starting with identification of clearly stated, realistic, measurable objectives, and the identification or development of an appropriate information system. Table 5.14 presents selected indicators for evaluating the impact of vector control on malaria.

Table 5.13   Effect of different methods of control on vector population and behaviour

Method	Adult density	Adult survival	Human biting
Larval control			
Source reduction	+	–	–
Larvivorous fish	+	–	–
Larviciding	+	–	–
Control of human-vector contact			
Insecticide-treated nets	+/–	+/–	+
Improving housing	–	–	+
Mosquito repellent	–	–	+
Adult mosquito control			
Indoor residual spraying	+	+	+
Space spraying	+	+/–	–

+: reduction expected; –: no effect; +/–: effect in some situation

Table 5.14   Selected indicators for evaluating impact (health) of vector control on Malaria

Vector control method	Target population	Impact indicator (disease aspect)
Indoor residual spraying	Number of persons in the area of spray operations	Confirmed malaria cases per 1000 persons per year
Insecticide-treated mosquito net	Number of persons in the area of ITNs/LLINs operations	In-patient malaria deaths per 1000 persons per year All-cause <5 mortality rate
Larviciding	Number of persons in the areas of larviciding operations	Parasite prevalence: proportion of children 6–59 months with malaria infection

Exercise 5.5

Working in small groups, participants will answer the following questions:

a) *Partial spraying may be counterproductive, e.g. by spraying of cattle sheds without fully spraying human residences. Why?*

b) *Apart from the high refusal rate by inhabitants, what other problems limit the effectiveness of IRS?*

c) *The aim of IRS is to reduce average life span of mosquitoes. True or false? Explain your answer.*

d) *The number of people protected equals the number of inhabitants of sprayed houses, e.g. if 50% of houses have been sprayed, 50% of the population have been protected. True or false? Explain your answer.*

e) *In cases of malaria epidemics, priority for IRS should be given to spraying structures with sprayable surfaces. True or false? Explain your answer.*

5.6   Persistent organic pollutants (POPs)

POPs are chemicals with the following characteristics:

▶ remain intact in the environment for a long time

▶ become widely distributed throughout the environment (via atmosphere, ocean, and other pathways)

▶ accumulate in fatty tissue of living organisms

▶ are toxic (carcinogenic) to humans and wildlife

▶ bio-accumulate in organisms and food chains

▶ evaporate and spread long distances through the air and water

There are three types of POPs:

▶ Pesticides (such as mirex and DDT)

▶ Industrial chemicals (such as PCB)

▶ Waste by-products (such as dioxin and furans) (see Table 5.15)

The UN signed the convention on POPs in 2001. Under the convention, countries commit to reduce and/or eliminate the production, use and/or release of POPs. The POPS used as pesticides and of greatest environmental concern are listed in Table 5.15.

Table 5.15   The Persistent Organic Pollutants

Persistent organic pollutants	Type
Aldrin	Pesticide
Chlordane	Pesticide
Dichlorodiphenyltrichloroethane (DDT)	Pesticide
Dieldrin	Pesticide
Endrin	Pesticide
Heptachlor	Pesticide
Hexachlorobenzene	Pesticide, industrial chemical, by-product
Mirex	Pesticide
Toxaphene	Pesticide
Polychlorinated biphenyls (PCBs)	Industrial chemical
Polychlorinated dibenzo-p-dioxins (dioxins)	By-product
Polychlorinated dibenzo-p-furans (furans)	By-product

DDT has several characteristics which are of particular relevance in malaria vector control. Among the 12 insecticides currently recommended for this intervention, DDT is the one with the longest residual efficacy when sprayed on walls and ceilings (6–12 months depending on dosage and nature of substrate). Depending on the duration of the transmission season, the use of DDT alternatives might require more than two spray cycles per year. DDT has a spatial repellency and an irritant effect on malaria vectors that strongly limit human-vector contact, thereby contributing to effective disease-transmission control. WHO recommends its use only for IRS, and conditional on full compliance with the guidelines and all recommendations of WHO and the Stockholm Convention.[1]

5.7   Geographical reconnaissance (GR)

Geographical reconnaissance is a management tool for field operations. Data are collected and mapped on the type, size, quality, and location of local housing, the habits of the local population, the location of breeding places of mosquitoes, presence of roads, paved and unpaved paths, and major natural characteristics, e.g. rivers and forests. The main objectives of GR are to:

► Determine the number and location of houses and the population at risk of malaria and therefore to be included in the vector control programme

► Provide a basis for planning the programme needs for equipment, supplies, transport and personnel

► Establish the nature and extent of the mosquito breeding sites in relation to population centres as a basis for planning mosquito control measures

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[1.]   WHO (2007). *The use of DDT in malaria vector control. WHO position statement.* Geneva, World health Organization. http://www.who.int/malaria/publications/atoz/who_htm_gmp_2011/en/

> ► Provide basic information for health education, case detection and treatment programmes

> ► Prepare a map for planning, conducting, supervision, monitoring and evaluation of malaria control activities.

Valuable information for developing the GR can be found from a variety of sources within each country.

Equipment required for GR

> ► Clip board or mapping board (30 x 40 cm)

> ► Ruler (30 cm)

> ► Simple pocket compass (fixed to the board)

> ► Drawing pins, or rubber bands

> ► Measuring tape

> ► Paint kit and brush (tacks, nails and hammer)

> ► Rucksack

> ► Glue

> ► Drawing sheet

> ► GR record

The GR method for constructing sketch maps was first employed during the malaria eradication era. Nowadays information obtained from satellites can be used to prepare maps of any area for the purpose of malaria control activities (for more information, see Annex 3).

5.7.1 Measuring the sprayable volume of the houses

For indoor residual spraying a sketch map produced by GR for estimation of "sprayable volumes" can be used. If all rooms are to be sprayed, the house volume can be estimated by pacing its width and length, and estimating its height by eye, from which:

House volume = width x length x height

For instance in a room: 4 x 3 x 3m, the volume is 36 m^3. The area, in m^2, is 1.5 times the number of m^3 calculated for the volume, i.e. 36 x 1.5 = 54 m^2.

5.8 Geographical Information System (GIS)

GIS is a computerized database for management and mapping technology that provides an excellent means to analyse epidemiological data, reveal trends, and interrelations that would have been more difficult to discover in tables. The GIS allows representation of villages and cities on a map, according to their geographical coordinates (longitude and latitude). Small electronic devices based on global positioning systems (GPS) are used for locating the position of any object and can be used for recording information about the object, e.g. the location of a house and details on its occupants. The GPS is a hand-held receiver that can obtain latitude and longitude details. Information required for every village (name, administrative division, coor-

dinates, population, availability and type of health infrastructure, number and types of schools, drinking water sources, latitude and longitude) can then be entered into a database compatible with the GIS system such as Excel, Access or Epi Info (see Fig. 5.12).

Figure 5.12 Arc view GIS programme

5.9   Integrated vector management (IVM)

The principles of vector control have changed since the mid-20th century when vector eradication was replaced by vector control as the main programme objective. Today's emphasis on integrated vector management (IVM) enhances control programmes and makes more efficient use of resources.

The IVM approach to vector control is a unified plan of control that selects the most appropriate methods of control, based on the environmental conditions and the population dynamics of the vector, which maintains the vector population at a level that does not cause a public health problem.

IVM is a rational decision-making process for the optimal use of resources in the management of vector populations, in order to reduce or interrupt transmission of vector-borne diseases.[1] The approach seeks to improve the efficacy, cost-effectiveness, ecological soundness and sustainability of disease-vector control.

5.9.1   Features of IVM

► Selection of proven vector control methods based on knowledge of local vector biology and ecology, disease transmission and morbidity

► Use of a range of vector control interventions, separately or in combination and often synergistically

► Collaboration within the health sector and with other public and private sectors that have an impact on vector breeding

► Engagement with local communities and other stakeholders

► Operates within a public health regulatory and legislative framework

► Rational use of insecticides

► Good management practices

1. WHO (2008). *WHO position statement on integrated vector management*. Geneva, World Health Organization. Available at http://www.who.int/malaria/publications/atoz/who_htm_ntd_vem_2008_2/en/index.html

5.9.2   IVM stakeholders

- ▶ WHO and the World Bank
- ▶ Industry
- ▶ Environment Protection Agency
- ▶ Ministries of Health, Agriculture, Foreign Affairs, Energy, and of Fisheries
- ▶ Municipality
- ▶ Meteorology Department
- ▶ Veterinary Department
- ▶ Research Centres and Universities
- ▶ NGOs
- ▶ Community and the private sector

Example

What should be done if the selected vector control method does not satisfy the criteria for success? For instance, if the health outcome to be achieved was the reduction of malaria by 35% in 5 years, but in year 3 it becomes apparent that only a 15% reduction will be achieved, it will be necessary either to accept the lower outcome or to improve the control programme.

If, after a thorough evaluation of the vector control programme to rule out operational deficit, the human-vector contact and adult densities are not decreasing as expected, a decrease in susceptibility of the vector to the insecticide would be suspected initially. However if the vector is found to be still susceptible to chemical control, what should be done? In this case it would be necessary to envisage using either another vector control method, or a complement to the original method selected, i.e. the vector control programme should be integrated and managed in a different way.

Exercise 5.6

Working in groups, the participants should answer the following questions.

1. *Can you list the three important stakeholders/collaborators for IVM in your country and state why they are important?*

2. *Do you agree that the use of different vector control interventions for malaria control is the only example of IVM?*

3. *If not already in use, would you like to see an integrated approach incorporated into the malaria vector control programme where you work? Would this be a realistic option? Explain why.*

4. *What are the advantages and limitations associated with the IVM concept?*

The results should be presented in plenary. The tutor will lead a discussion on vector control and the questions presented in this exercise.

Exercise 5.7

All the participant should do the IRS. Each participant should operate the spray pump and apply the required amount of insecticide in 19 m² in 60 minutes as shown in Fig. 5.8.

LEARNING UNIT **6**

Monitoring and management of insecticide resistance

Learning Objectives:
by the end, participants should be able to...

■ Recall the historical development of insecticides

■ Explain the mode of action of insecticides

■ Discuss the mechanism of insecticide resistance

■ Determine resistance to insecticides using the susceptibility test kit (adults)

■ Determine the susceptibility of mosquito larvae to insecticides

■ Carry out the standard bioassays and discuss their significance

■ Determine the residual efficacy of an insecticide-treated net at any particular time after distribution

■ Determine how to monitor insecticide resistance in a population

6.1   Historical development of insecticides

As a result of the actions of insect pests, human populations have always had to cope with disease, discomfort and great economic loss. Since the beginning of civilization people have endeavoured to improve their well-being, and this has included the use of chemical agents for the control of insects responsible for the transmission of disease and destruction of crops. Some of the methods for insect control date back many centuries.

Ancient peoples relied almost entirely on the use of natural products and preparations derived from these. Until the 1940s, the chemicals used for destroying insect pests were largely inorganic chemicals, such as compounds of lead and arsenic which are poisons for humans and animals. Some organic chemicals of plant origin, such as nicotine, pyrethrum and rotenone were also used for pest control.

The modern era of organic pesticides, called the "pesticide revolution", began in the 1940s when DDT was first used as an insecticide. DDT was first synthesized by Zeidler in 1874, but its insecticidal properties were discovered by Paul Muller in 1939. He received a Nobel Prize for medicine in 1948 for his discovery. DDT was commercially manufactured in 1943 and soon became the most extensively used insecticide. Following the discovery and application of DDT, other new groups of synthetic insecticides have been manufactured and used against medically important insects. With the appearance of DDT, it was thought that the 'magic bullet' for malaria control had been found. In 1955 the World Health Assembly adopted a resolution calling for the global eradication of malaria, excluding sub-Saharan Africa, following the success achieved using insecticides in reducing malaria cases in many parts of the world. However, the optimism regarding insecticide use was short-lived due to the development of insecticide resistance by the vectors, and from 1976 the WHO strategy for malaria was changed from eradication to control.

6.2   The mode of action of insecticides

Pyrethroids and DDT act by interfering with the sodium channels of both the peripheral and central nervous system. They open sodium channels, leading to continuous nerve excitation, paralysis and death of the vector; they also have an irritant effect, causing an excito-repellency response, resulting in hyperactivity, rapid knock-down, feeding inhibition, shorter landing times and undirected flight, all of which reduce the ability of vectors to bite. Organophosphates and carbamates act by inhibiting cholinesterase, preventing breakdown of the neurotransmitter acetylcholine, resulting in neuromuscular overstimulation and death of the vector. Understanding the mode of action of insecticides is important for several reasons:

▸ understanding the health hazards of these chemicals to people and other non-target organisms

▸ enabling additional chemicals with a similar mode of action to be designed

▸ providing insight into the mechanisms of development of insecticide resistance, particularly those involving target insensitivity, so that countermeasures to avoid resistance or reverse the development of resistance can be devised

▸ providing valuable basic information on the nature of the target systems (e.g. the weakness of sensitive insects) in terms of physiological, biochemical and biophysical knowledge of vital biological systems.

6.3 Mechanisms of insecticide resistance in arthropods

Insecticide resistance is the term used to describe the situation in which the vectors are no longer killed by the standard dose of insecticide or manage to avoid coming into contact with the insecticide. Several years of intensive use of organic insecticides to control arthropod pests and disease vectors led to the selection of insecticide resistance in some species. Factors that induce resistance are numerous and the mechanism adopted by an organism depends on the prevailing pressure and on the mode of action of the insecticide in use. Intoxication of an insect by an insecticide encompasses different levels of pharmacokinetic interaction: penetration of barrier tissue, distribution, storage, metabolism in internal tissue, and molecular interaction with the specific target site. Many chemicals are in use against arthropods and there are hundreds of examples of resistance, with a number of resistance mechanisms identified as described below.[1]

6.3.1 Metabolic resistance

In metabolic resistance, the metabolic pathways of the insect become modified in ways that detoxify the insecticide, or prevent metabolism of the applied compound into its toxic form. Metabolic resistance to insecticides is mediated by qualitative and quantitative changes in proteins that can often be difficult to define precisely at the biochemical level. Three broad enzyme classes are involved in insecticide detoxication glutathione-S-transferases (GST); the mixed function oxidases (MFO); and esterases. Their involvement in resistance is commonly identified by increases in the characteristic metabolites they produce. All three classes exist in multiple forms within each species and it is often not known whether increased activity arises from qualitative or quantitative changes in these enzyme complexes. For pyrethroids, MFOs are of most importance, followed by esterases. For DDT, GSTs are of most importance, followed by MFOs. For organophosphates and carbamates, esterases and MFOs are of most importance. Increased synthesis of these enzymes seems to result from gene amplification

Glutathione-S-transferases (GST)

This group of enzymes catalyses the conjugation of glutathione with compounds having a reactive electrophilic centre, leading to the formation of a water-soluble, less reactive product. Although there are many examples of increased metabolism of insecticide or model substrates by the GSTs of resistant insects, few are characterized at the molecular level. Metabolism mediated by these enzymes has been implicated in DDT and organophosphate resistance. Increased levels of DDT-dehydrochlorinase have been reported in different species resistant to DDT. The natural function of DDTase is not known, although it is present in different insect organs including probably the peripheral nervous system where DDT appears to exert its major effect. Characterization of partially purified GSTs from DDT susceptible and resistant strains demonstrated both quantitative and qualitative differences for the enzymes.

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[1.] WHO (2012). *Global plan for insecticide resistance management in malaria vectors (GPIRM)*. Geneva, World health Organization. Available at http://www.who.int/malaria/publications/atoz/gpirm/en/index.html

Mixed Function Oxidases (MFO)

MFO enzymes are of a great significance both in mammals and arthropods in providing protection against a variety of insecticides, particularly to some chlorinated hydrocarbons, to many organophosphates, carbamates and some pyrethroids. The mono-oxygenases, or mixed function oxidases (MFO), complex involves a reductase and one or more cytochrome P-450s. An increase in MFO activity is one of the most versatile mechanisms of resistance in insects. Insect P-450 enzymes also activate certain types of insecticides, for instance the conversion of organophosphates to their active insecticidal form. Several factors including species, strain, sex, development stage, age and nutrition affect mono-oxygenase activity. The MFO system has been shown to occur in the fat body, Malpighian tubules, and midgut. By far the most intensively studied MFO system is that of the house fly. MFO's are thought to be important in recently-discovered cases of pyrethroid resistance in *A. gambiae.*

Esterases and hydrolysis

Esterases are the most significant enzymes for insecticide detoxication in insects. Organo-phosphate, carbamate and pyrethroids contain carboxylester and phosphotriester bonds that are subject to attack by esterase enzymes. These esterases can often be separated into isozymes with different substrate specificities. Insect esterases are very diverse and can include monomers, dimers and multimers, which means that their relative molecular mass can cover a wide range. Polymorphism is a notable characteristic of insect esterases. Multiple forms of esterases are present in the soluble, cytosolic fraction of insect. Of the multiple forms of esterase isozymes that exist in insects, few participate in insecticide metabolism. Each isozyme probably has a particular range of substrates.

6.3.2   Target-site resistance

Many insecticides are toxic to the insect's nervous system, acting either on the synapse or the axon. Different parts of nervous system channels such as voltage-dependent sodium channels, potassium channels, ATPase, GABA-gated chloride channel, and acetylcholinesterase are the primary target sites for several classes of insecticides. Target-site resistance occurs when the site of action that an insecticide acts on is modified in resistant strains such that the insecticide no longer binds effectively and the insect is therefore unaffected, or less affected, by the insecticide. This mechanism of insecticide resistance can take place in the sodium channel, which may result in: different types of sodium channel; structural alteration of the sodium channel; modified sodium channels; change in nerve membrane phospholipids; reduction in sodium channel density; and reduced affinity of the sodium channel. Resistant mutations, known as knock-down resistance (kdr) mutations, can affect acetylcholinesterase, which is the molecular target of organophosphates, and carbamates, or voltage-gated sodium channels (for pyrethroids and DDT).

6.3.3   Behavioural resistance

Behavioural resistance is any modification in insect behavior that helps it to avoid the lethal effects of insecticides. The irritant property of some insecticides can cause a proportion of insects to leave sprayed surfaces before acquiring a lethal dose, so that repeated contact is required before mortality occurs. The increased activity of mosquitoes caused by the insecticides is termed

"irritability". The disturbance of resting mosquitoes is the most obvious result of irritation; the term repellency (more often excito-repellency) is sometimes applied to this phenomenon. Repellency is the stimulation by a chemical of oriented movements away from the source, or the prevention of the insect from approaching the insecticide. Both definitions imply that repellency is not only brought about by the contact of mosquitoes with an insecticide, but also by the fumigant action of the insecticide. This irritability would produce heightened activity in the landing mosquito and it would only remain on the treated surface for a short period of time.

Africa has been a continuing source of reports of behavioural avoidance of DDT residues. In some cases, this behavioural response of vectors was thought to have a negative impact on control efforts.

Although classifying resistance into biochemical, physiological and behavioural mechanisms provides a convenient way of viewing resistance, in reality these three groups represent an interrelated spectrum of biological responses. For example, a physiological response such as altered nerve insensitivity is the sum total of a series of biochemical events potentially involving changes in nerve structure. Similarly, a behavioural response is the sum total of a series of physiological and biochemical changes. However, in some cases a shift in vector population may be mistaken for the development of behavioural resistance.

Insecticide repellency could prevent vectors from entering human habitations treated with insecticide. Such behavioural responses may greatly reduce human-vector contact, which may be accompanied by sustained reduction in malaria transmission. Careful monitoring of both physiological and behavioural responses to pyrethroids will be essential in evaluating the merits of initiating or continuing large-scale use of pyrethroids.

6.3.4 Reduced penetration (cuticle resistance)

The composition of the insect's exoskeleton may become modified in ways that inhibit insecticide penetration. Decreased penetration of insecticides would allow ample time for detoxifying enzymes to metabolize the chemical which would therefore be less effective. Decreased cuticular penetration, possibly caused by a similar gene to *pen* was also found in a permethrin-selected strain of the house fly.

6.3.5 Excretion

Increased excretion is one of the mechanisms of resistance developed by insects. Larvae of resistant strains of *Aedes aegypti* respond to DDT by excreting the insecticide into the perithrophic membrane. This behaviour was more evident in some resistant strains than others and occurred to a much lesser extent in larvae of susceptible strains. It appeared to constitute a resistance mechanism for removing DDT from the alimentary canal and preventing access to the body.

6.3.6 Resistance mechanisms by major malaria vector species

Both metabolic and target site resistance mechanisms are found globally; however, different resistance mechanisms can be found in different species. For example, only metabolic resistance has currently been reported in *An. funestus s.s*, whereas both metabolic and target site resistance mechanisms have been found in *An. gambiae s.s.* Table 6.1 summarizes resistance mechanisms in major malaria vector species.

Table 6.1   Resistance mechanisms in major malaria vector species[1]

Vector Species	Pyrethroids Metabolic	Pyrethroids Target site	DDT Metabolic	DDT Target site	Organophosphates Metabolic	Organophosphates Target site	Carbamates Metabolic	Carbamates Target site
An. gambiae s.s	✓	✓	✓	✓		✓	✓	✓
An. funestus s.s			✓				✓	
An. arabiensis		✓	✓	✓		✓		✓
An. Culicifacies (C)		✓		✓				
An. Culicifacies (B)		✓	✓	✓	✓			
An. stephensi	✓	✓	✓	✓	✓			
An. dirus			✓					
An. sacharovi			✓		✓	✓	✓	✓
An. albimanus	✓		✓		✓	✓		✓

6.4   Knock-down and kill

The term "knock-down" as applied in entomology denotes paralysis in insects, whether reversible or not. Non-recovery from knock-down is given a separate name, "kill". The term "knock-down" was originally introduced to describe the rather quick induction of paralytic symptoms produced by pyrethrins, but its usage was eventually extended to all cases of insect paralysis by chemicals. It is most often used in connection with the time required for the onset of paralytic symptoms in a certain fraction of the test population (e.g. KD_{50}, KD_{90}, etc.). Recovery from knock-down has been observed in a number of insect species. It is not known whether recovery from knock-down is merely a matter of metabolism of the insecticide to a non-lethal level, or whether the poisoning processes that cause knock-down are the same as those that eventually lead to kill. Speed and duration of knock-down, and the possibility and extent of recovery from it, are important considerations in pest control.

6.5   Methods of detecting resistance

Different approaches to detect the emergence of resistance are now available.[2] Detection of resistance is the basis for the application of resistance management techniques to counteract resistance. The process involves obtaining base-line susceptibility data and regularly monitoring for resistance using the most sensitive methods availableas described in the sections below.

6.5.1   Susceptibility tests

The susceptibility tests (bioassays) are used to measure the percentage mortality after exposure of vectors to a standard diagnostic dose of insecticide, which allows comparison of independent studies. Either WHO standard paper or CDC bottle bioassays can be used.[2] However, the results obtained with the two methods are not comparable. Therefore, the same

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[1.]   WHO (2012). *Global plan for insecticide resistance management in malaria vectors (GPIRM)*. Geneva, World health Organization. Available at http://www.who.int/malaria/publications/atoz/gpirm/en/index.html

[2.]   WHO (2013). *Test procedures for insecticide resistance monitoring in malaria vector mosquitoes*. Geneva, World Health Organization. Available at http://www.who.int/malaria/publications/atoz/9789241505154/en/index.html

method must be used consistently over time for observing longitudinal or temporal patterns in resistance. WHO paper bioassays are the basic standard method but CDC bottle assays offer an alternative that can provide complementary information. Susceptibility tests can also be used to determine the appropriate dosage required to kill 50% or 90% of the insect populations, thereby providing more detailed information about the extent of resistance in a population. Bioassays identify the existence of resistance once it is at a detectable level but do not establish the resistance mechanism involved. They may also not identify resistance if the frequency is too low. However, these bioassays remain extremely important for resistance monitoring because they detect resistance regardless of the physiological mechanism involved. Some of the assays described below, although more sensitive, are only suitable for detection of one particular type of resistance.

6.5.2   Biochemical and immunological assays

There are two major causes of resistance: *(i)* changes in the target site that reduces insecticide binding (target-site resistance) and *(ii)* increases in the rate at which the insecticide is metabolised (metabolic resistance).

Three enzyme families are responsible for insecticide metabolism: carboxylesterases, cytochrome P-450s and glutathione-S-transferases. Biochemical assays detect the level of activity of these enzymes. These assays use model substrates that produce a colour change visible to the naked eye. The intensity of the colour is proportional to the enzyme activity. Although they do not directly measure the level of insecticide metabolism, they can provide an indication of the mechanisms of resistance in a population and provide clues to possible patterns of cross resistance.

The advantage of biochemical tests include the ability to carry out multiple assays on a single insect, enabling multiple resistance mechanisms to be detected. Disadvantages include difficulties to use it in the field as it requires sophisticated equipment, and interpretation of the results requires strong technical skills. When using these assays, it is important to test a susceptible strain of the same species alongside the field population to allow comparison of the levels of the detoxifying enzyme. Protocols for these assays are available.[1]

6.5.3   Molecular test

DNA-based diagnostics are now available to detect target-site resistance to all the major insecticide classes for many vector species. These assays detect the actual mutation responsible for resistance. They are extremely sensitive and can detect resistance as soon as it arises in a population, giving early warning of potential control failure. However, given that molecular assays are currently only available for target-site resistance, they cannot be used as a substitute for bioassays. A negative result from a molecular assay does not indicate that the specimen is not resistant to a particular insecticide, only that it does not contain the particular resistance mechanism being assayed.

A number of different platforms have been developed for these molecular assays. The simplest methods use allele-specific PCR, or PCR followed by restriction enzyme digestion. The

[1.]   WHO (1998). *Techniques to detect insecticide resistance mechanisms (field and laboratory manual)*. Geneva, World Health Organization. WHO/CDS/CPC/MAL/98.6.

products are resolved on an agarose gel and only a PCR machine and a UV light box are needed to carry out these tests. Other molecular assays require more sophisticated equipment but have the advantage of greater sensitivity and specificity and greater throughput. Some of the alternative assays for detecting target-site resistance to DDT and pyrethroids (the kdr allele) are compared in Bass et al, 2009.[1]

Very few molecular assays are currently available to detect metabolic resistance in insects and, as yet, there are no field-based assays for detection of the genes responsible for metabolic resistance in the major mosquito vectors. The presence of metabolic resistance can be indicated via the use of synergists in bioassays. In addition, several microarray platforms have also been developed specifically to study metabolic resistance in mosquito vectors. However, these experiments are still expensive to run and require specialized equipment.

6.6   Monitoring of insecticide resistance

Appropriate monitoring of vector susceptibility to insecticides is an integral component of planning and evaluation of insecticide usage in malaria control programmes.[2] The increasing problem of resistance in disease vectors has steadily reduced the choice of alternative insecticides and there is no single, simple solution to the problem. It is important to recognize that only a limited number of insecticides are available for use in public health programmes and they should be treated as a valuable resource. Existing insecticide "resources" need to be used in the most effective way possible. The rational use of insecticides largely depends on a broad knowledge of the possible or probable resistance mechanisms. Based on this knowledge, all necessary precautions to prevent the occurrence of resistance can be taken and a plan prepared in advance to deal with the development resistance in its early stages in the field. The potential development of insecticide resistance is a common threat to all programmes that relies on the continuous or repeated use of insecticides. It is therefore important to monitor vector susceptibility periodically during programme operations.

Resistance often results from using the same or a related insecticide that is used in agriculture. The selection of the insecticide to be used for vector control should be based not only on the susceptibility of the vector population, but on the general use of insecticides in the area. It is also desirable to study the history of resistance in neighbouring areas and for the same vector species in other areas.

Similar considerations apply with respect to the avoidance of contact with the sprayed surfaces by the vector. It will be necessary to monitor possible changes in vector behaviour by means of exit traps and the observation of human-biting behaviour.

One of the most important reasons to sample vector populations is to determine their susceptibility to insecticides. When insecticides are used in malaria control, it is important to monitor changes in the susceptibility level of the target vectors from time to time. The residual efficacy of the insecticide used should also be determined at intervals after its application or use. In this unit, participants will learn the skills required to carry out these activities.

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[1]   Bass et al (2009). Detection of knockdown resistance (kdr) mutations in Anopheles gambiae: a comparison of two new high-throughput assays with existing methods. *Malaria Journal*; 6:111.

[2]   WHO (2012). *Global plan for insecticide resistance management in malaria vectors (GPIRM)*. Geneva, World health Organization. Available at http://www.who.int/malaria/vector_control/ivm/gpirm/en/index.html

6.6.1 Discriminating concentrations of insecticides

Discriminating concentrations (or dosages) of insecticides are routinely used to detect and monitor insecticide resistance in mosquitoes. These concentrations were established under standardised laboratory conditions, using known "susceptible" strains or populations of a range of mosquito vector species.[1] The discriminating concentrations of insecticides commonly used for adult mosquitoes as well as for larvae are provided in Annex 4 and 5, respectively.

6.6.2 Susceptibility tests on adult mosquitoes

Susceptibility tests are carried out to determine the proportion of the vector population that is physiologically resistant to a particular insecticide.

Physiological resistance to insecticides has been defined as the "ability of a population of insects to tolerate doses of an insecticide which would prove lethal to the majority of individuals in a normal population of the same species".

The efficacy of IRS and ITNs depends, among other things, on the proportion of vectors resting on the sprayed surface and on the susceptibility of the vectors to the insecticide used. It is therefore important to monitor the development and extent of insecticide resistance in a given vector population.

Susceptibility test using standard WHO bioassay

The standardized WHO method involves checking the mortality of several female *Anopheles* of a known species exposed in special tubes to filter papers impregnated with a lethal concentration (known as discriminating dose) of a given insecticide dissolved in oil. For detailed description of the procedures of the WHO insecticide susceptibility test refer to Annex 4.

Susceptibility test using CDC Bottle Bioassay

The CDC bottle bioassay determines if a particular insecticide is able to kill a vector, such as a mosquito, at a specific location at a given time. The objective of the bioassay is to measure the time it takes for a given insecticide to kill the adult mosquito. The CDC bottle bioassay can be tested on mosquito populations reared in an insectary from larval field collections or on those collected from the field.

The CDC bottle bioassay is being used increasingly for routine, day-to-day monitoring of mosquito populations. The advantages of the bottle bioassay over the WHO susceptibility tests are: 1) no need to source impregnated papers from the WHO collaborating centre in Malaysia; 2) no 24-hour holding period, 3) all available insecticides can be evaluated at one time, 4) using lower discriminating dosages resistance may be detected earlier. Disadvantages include the lack of quality assurance in preparing the bottles between different laboratories/people, transport of bottles in the field, particularly over extended periods when no access to labs is possible, and extracting live mosquitoes after the required exposure period for storage for species identification. Results from the bottle bioassay are not directly comparable with WHO test tube results. The bottle bioassay is, however, suitable for synergist assays, research purposes

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1. WHO (2013). *Test procedures for insecticide resistance monitoring in malaria vector mosquitoes*. Geneva, World Health Organization, 2013. Available at http://www.who.int/malaria/publications/en/

and for establishing diagnostic concentrations of new insecticides or for new mosquito species. The detailed methodology, *"Guideline for evaluating insecticide resistance in vectors using the CDC Bottle Bioassay"*, can be found on the US Centres for Disease Control and Prevention (visit http://www.cdc.gov). An on-line version of the guidelines, complete with animation is available at http://www.cdc.gov/ncidod/wbt/resistance/assay/bottle/index.htm.

6.6.3   Susceptibility tests on larvae

The purpose of the susceptibility test on larvae is to detect the presence of resistance in a mosquito larval population as early as possible so that alternative control plans can be made in time to deal with the situation when the insecticide in question is no longer having the desired effect. For detailed description of the procedures of the susceptibility tests for larvae refer to Annex 5.

6.6.4   Bioassay methods

Residual efficacy of insecticide on treated walls

The residual efficacy of an insecticide on a sprayed surface is determined by bioassay testing. This is done by checking mortality of the target mosquito vector exposed to the sprayed surface at intervals of weeks or months after the spraying. This technique can be also used to evaluate the quality of a residual spraying operation. Detailed description of the procedure of this method is presented in Annex 6.

Residual efficacy of insecticide on treated nets

Bioassay method is also used to determine residual efficacy of an insecticide on treated nets, which can be used to decide when to re-treat nets in the case of ITNs or replace in case of LLINs and also to assess the quality of treatment. The bioassay procedure for insecticide-treated nets is similar to the procedure used for sprayed walls, except that the cone is attached to the fabric with a rubber band, and the mosquitoes are usually exposed only for three minutes. Detailed description of the procedure of this method is described in Annex 7.

Most programmes do not have insectary facilities to raise mosquitoes of known age and susceptibility status for the bioassay tests. New methods of monitoring insecticide residues on ITNs/LLINs and surfaces are in development. An example of such method is Insecticide Quantification Kit (IQK) by the Innovative Vector Control Consortium (IVCC). The IQK is simple to be used in the field.

6.7   Managing insecticide resistance

Insecticide resistance management (IRM) strategies are intended to maintain the effectiveness of vector control, despite the threat of resistance. Following are IRM strategies which have been used or proposed for managing resistance to insecticides for vector control:[1]

 ► *Rotation of insecticides.* Two or more insecticides with different modes of action are rotated from one year to the next;

[1.]   WHO (2012). *Global plan for insecticide resistance management in malaria vectors (GPIRM)*. Geneva, World health Organization 2012 available at http://www.who.int/malaria/vector_control/ivm/gpirm/en/index.html

- *Combination of interventions.* Two or more insecticide based vector control interventions are used in a house (e.g. pyrethroids on nets and an insecticide of a different class on the walls), so that the same insect is likely, but not guaranteed, to come into contact with the second insecticide if it survives exposure to the first;

- *Mosaic spraying.* One compound is used in one geographic area and a different compound in neighbouring areas, the two being in different insecticide classes; further research is required on the use of mosaics;

- *Mixtures.* Two or more compounds of different insecticide classes are mixed to make a single product or formulation, so that the mosquito is guaranteed to come into contact with the two classes at the same time. Mixtures are not currently available for malaria vector control.

All malaria vector control programmes must have an insecticide resistance management strategy. Resistance management activities and policies must be introduced from the outset, and cannot be delayed until resistance has appeared. Such resistance management activities include the following:

- For IRS, the minimum resistance management policy is to alternate between insecticide classes in rotation system; this means rotating between insecticides with different modes of action (changing from one pyrethroid to another is not considered a rotation).

- A pyrethroid may be used as one element of the rotation, except where there is high LLIN Coverage in the same geographic area.

- As already noted, pyrethroids should not be used for IRS in areas with high LLIN coverage; conversely, the combination of LLINs with non-pyrethroid-IRS is a recommended resistance management strategy.

- In the process of approving insecticide procurement requests, funding agencies should check that recent and relevant data on insecticide resistance in and near the target area was available and taken into account in the decision to choose a particular insecticide.

- Resistance monitoring must be conducted at least once a year from several locations that are targeted with vector control activities. Wherever possible, resistance should be tracked not only with conventional bioassays but also with molecular genotyping methods. It is recommended that funds for these activities must be made available.

- WHO recommendations on resistance testing methods, and on the collation, analysis and interpretation of such data have been updated to guide countries on how to ensure that pre-emptive action is taken and resistance is managed timely.[1]

- The impact of resistance on the effectiveness of vector control is also a key question: where possible, monitoring schemes should attempt to assess whether vector control operations tend to have less impact in areas with relatively high levels of resistance.

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[1.] WHO (2013). *Test procedures for insecticide resistance monitoring in malaria vector mosquitoes.* Geneva, World Health Organization. Available at http://www.who.int/malaria/publications/en/

6.7.1 Important considerations in judicious use of insecticides

▸ **What** insecticide (compound and formulation) to apply? The product which is most appropriate, taking into consideration its safety, efficacy, acceptability, cost and availability.

▸ **Where** to apply the insecticide? This requires identification of priority geographical areas and specific locations for best targeting and coverage requirements.

▸ **When** to apply? This may refer to the time of year or to the time of day and the epidemiological requirements, taking into consideration duration of effect and time required for covering the target area.

▸ **How** to apply? What skills and equipment are required to ensure effective and safe application?

Figure 6.1 illustrates the factors involved in the judicious use of insecticides are presented.

WHAT to apply
Consider:
safety, efficacy, cost-effectiveness, acceptability and availability of quality products

HOW to apply
Consider:
staff skills and training, equipment and safety

WHERE to apply
Consider:
coverage requirements and best targetting

WHEN to apply
Consider:
time required for covering target area, duration of effect and epidemiological requirement

Figure 6.1 Different factors for using insecticides judiciously

6.8 Current situation of insecticide resistance

Since 2009, when there was increased focus on monitoring of insecticide resistance, more and more cases of resistance have been reported, particularly in Africa. Most countries in which susceptibility tests were conducted had at least one case of resistance. Resistance has been identified in 64 countries with ongoing malaria transmission, mainly to pyrethroids (Fig. 6.2). Resistance to DDT is also prevalent, and there are increasing reports of resistance to organophosphates and carbamates. Below is an overview of the known status of the insecticide resistance.

Africa Regions. Countries in West and Central Africa have long been reporting high frequencies of resistance, particularly Benin, Burkina Faso, Cameroon, Cote d'Ivoire and Ghana. These countries have widespread resistance to pyrethroids and DDT; Cote d'Ivoire has also reported resistance to carbamates and organophosphates. Ethiopia has reported resistance to all four classes of insecticide, including widespread resistance to DDT and an increasing

Figure 6.2 Countries with ongoing malaria transmission where insecticide resistance has been identified in at least one of their major vectors[1]

Countries with ongoing malaria transmission and resistance to at least one insecticide

Countries with ongoing malaria transmission and no reports of insecticide resistance
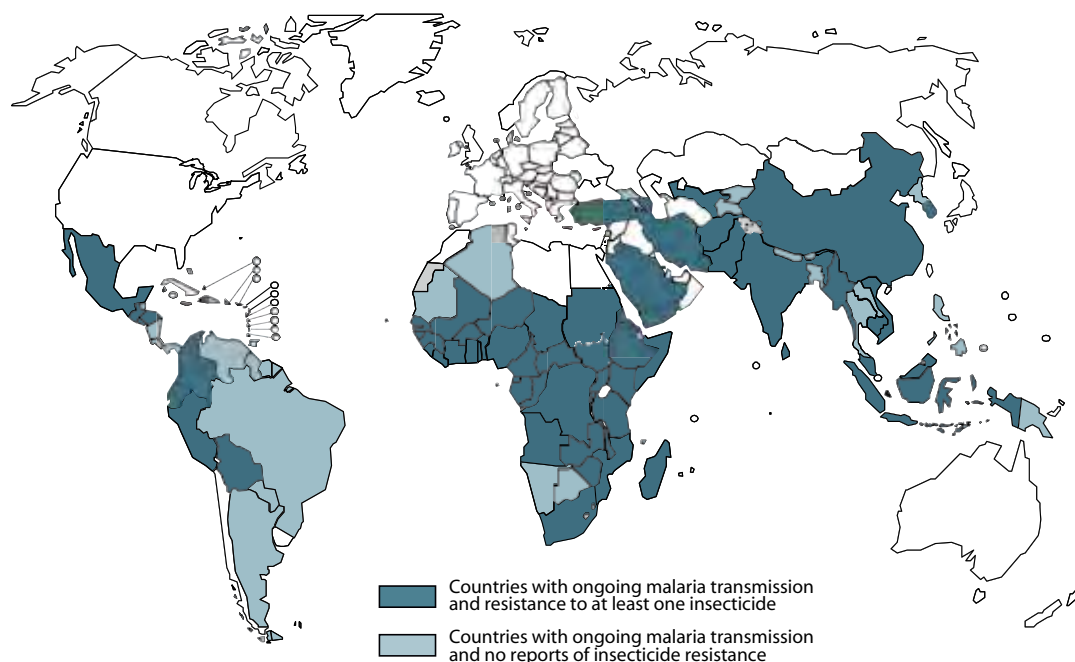
From WHO regional entomologists in WHO Regional Offices, completed by literature review by the Global Malaria Programme.

IR, insecticide resistance

1. Includes countries with confirmed susceptibility to all insecticides used and countries where susceptibility testing is not currently conducted or results are not available

2. The map provides no indication of how widespread resistance is within a country; therefore, a single report of resistance would be sufficient to mark a country as having resistance.

frequency of resistance to pyrethroids. Other places in East Africa with widespread pyrethroid and DDT resistance are Uganda and its borders with Kenya and the United Republic of Tanzania. In southern Africa, Mozambique and South Africa have reported a broad spectrum of resistance over the past decade. A high frequency of metabolic resistance to pyrethroids has now also been reported in Malawi and Zambia.

South-East Asia Region. There is widespread DDT resistance and patches of pyrethroid and organophosphate (malathion) in India. Indonesia and Myanmar are also reporting resistance to pyrethroids; in Myanmar there is also confirmed resistance to DDT and organophosphates.

Region of the Americas. Resistance to pyrethroids, carbamates and organophosphates has been reported in this region. In Colombia, widespread resistance in the mid-2000s was reversed in many localities by changing the insecticide and thus removing the selection pressure. Resistance, however, persists in other localities. Resistance has also been reported in Bolivia (pyrethroids and carbamates), Ecuador (pyrethroids and organophosphates), Honduras (pyrethroids) and Peru (pyrethroids, carbamates and organophosphates).

Western Pacific Region. Pyrethroid and DDT resistance has been reported in malaria vectors of local importance in coastal regions of Vietnam. In addition there is resistance to pyrethroids in China and DDT resistance in Cambodia and Malaysia.

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1. WHO (2012). *Global plan for insecticide resistance management in malaria vectors (GPIRM)*. Geneva, World health Organization. Available at http://www.who.int/malaria/vector_control/ivm/gpirm/en/index.html

Eastern Mediterranean Region. Resistance to pyrethroids has been reported in several countries in this region, notably Afghanistan, the Islamic Republic of Iran and Oman. In addition, there is DDT resistance in Yemen. These data need to be confirmed. Somalia and Sudan have reported resistance to all four classes of insecticide, including widespread resistance to DDT and an increasing frequency of resistance to pyrethroids.

European Region. Resistance has been reported to all four classes of insecticides in Turkey; to DDT in Azerbaijan; and to carbamates and organophosphates in Uzbekistan.

Exercise 6.1

Susceptibility tests on adults. Working in pairs, participants will carry out susceptibility test on adult mosquito as described in Annex 4.

Exercise 6.2

Susceptibility tests on larvae. Participant will carry out larval test as described in Annex 5.

Exercise 6.3

Bioassay on LLINs. Working in pairs, participants will test the efficacy of pyrethroids on treated nets as described in Annex 7.

LEARNING UNIT **7**

Vector control in different malaria epidemiological strata

Learning Objectives:
by the end, participants should be able to...

■ Describe the concept of stratification

■ Describe malaria strata based on transmission intensity

■ Describe the characteristics of the major malaria eco-epidemiological strata

■ Select effective vector control strategies for the malaria eco-epidemiological strata

Introduction

Malaria is a focal disease; its distribution varies considerably from one area to another, even within relatively small boundaries such as districts. Consequently, the malaria situation varies throughout a national territory due to administrative, operational, technical, and financial factors, and to the heterogeneity of malaria epidemiology in different areas. This variability can be studied in relation to the potential effectiveness and sustainability of available interventions, which permits the recognition of a limited number of types of malaria situation. These situations are characterized by predominant demographic, parasitological, entomological, ecological, social, and politico-administrative factors. The geographical distribution of these types of malaria situations is the basis of stratification, on which appropriate malaria interventions are selected.

7.1 The concept of stratification

Stratification is the process of uniting areas, populations or situations that exhibit a relative resemblance of a set of specified relevant characteristics, known as variables, thereby distinguishing them from other areas, populations or situations dissimilar by the same set of characteristics. The process of stratification defines different strata that share similar epidemiological, geographical, socioeconomic, and ecological characteristics. The variables that define different strata can be grouped in to the following categories, or criteria:

▸ Macro-ecological and social (socioeconomic): population, geographical data (climate, latitude), settlement pattern and population movement, ethnic groups, major economic and developmental activities, cultural and socio-political aspects.

▸ Epidemiological: parasite species, vector species and behaviour, human-vector contact host, level of endemicity, morbidity and mortality data, risk of epidemics, parasite resistance to drugs, vector resistance to insecticides.

▸ Micro-ecological: presence and location of permanent and temporary vector breeding sites, agricultural production that provide favorable conditions for the vector.

▸ Anthropological: G6PD deficiency, sickle-cell trait.

▸ Health services organization: coverage of general health services, primary health care and malaria-related care, structure of logistic and administrative support.

▸ Specific antimalarial activities: current and past antimalarial interventions, outcome and impact of interventions, reasons for success and failure, cost of interventions, plans for the improvement and expansion of intervention operations.

7.2 Stratification of malaria by risk

Malaria risk can be stratified into three broad categories based on the degree of transmission as follows:

▸ malaria-free: There is no malaria transmission

▸ unstable (epidemic): Malaria transmission is variable, being subject to marked seasonal and annual fluctuations. Consequently, the collective population immunity is low.

▶ stable (endemic). Transmission is generally high and not subject to annual fluctuations, and the resulting population immunity is high.

It is not always easy to distinguish between stable and non-stable malaria. Within each stratum it is possible to further classify malaria. For example, in areas of unstable (epidemic) malaria it is possible to classify two distinct types of transmission:

▶ highly seasonal but intensive transmission with a more or less predictable pattern each year associated with explosive epidemics at 5–10 year intervals;

▶ highly seasonal with very little, or no, transmission for several years. These areas are also affected at times by dramatic and devastating epidemics, which result from environmental or meteorological changes.

With stable malaria, some areas may show a marked seasonal variation in intensity of transmission, while others have a more uniform pattern of transmission throughout the year.

7.3 Stratification based on spleen and parasite rates

Endemicity of malaria has traditionally been classified into four categories of transmission intensity, defined mainly by the prevalence at one point in time of parasitaemia and palpable enlarged spleen in the population, usually children aged 2–9 years (Table 7.1).

Table 7.1 Classification of malaria endemicity levels

Criterion	Hypoendemicity	Mesoendemicity	Hyperendemicity	Holoendemicity
Spleen rate 2–9 years[1]	0–10%	11–50%	Constantly > 50–75%*	Constantly > 75%**
Parasite rate 2–9 years[1]	0–10%	11–50%	Constantly > 50%	Constantly > 75% in infants aged 0–11 months
Stability[2]	Unstable	Unstable	Stable	Stable
Entomological inoculation rate[3]	< 0.25	0.25–10	11–140	> 140

* Also high in adults (>25%); ** But low in adults (<25%)

The four categories of malaria transmission intensities are:

▶ Hypoendemic areas: very little transmission and low risk of infection to the population;

▶ Mesoendemic areas: typically rural villages mainly in subtropical regions with varying intensity of transmission and often prone to malaria epidemics;

▶ Hyperendemic areas: Seasonally intense malaria transmission with disease in all age groups;

▶ Holoendemic areas: high level year round transmission with a high degree of immunity among the population, particularly adults.

[1.] WHO (1963). *Terminology of malaria and of malaria eradication: report of a drafting committee.* World Health Organization, Geneva. http://whqlibdoc.who.int/publications/9241540141.pdf.

[2.] WHO (2006). *Systems for the early detection of malaria epidemics in Africa – An analysis of current practices and future priorities.* WHO/HTM/MAL/2006.1115.WHO, Geneva.

[3.] Beier JC, Killeen GF & Githure JI (1999) Short report:entomologic inoculation rates and *Plasmodium falciparum* malaria prevalence in Africa. *American Journal of Tropical Medicine and Hygiene* 61, 109–113

Entomological inoculation rates and vectorial capacity are also useful in expressing the risk of malaria infection and distinguishing between different endemicity levels (Table 7.1).

7.4   Stratification by eco-epidemiological types

It is essential to identify which vector control measures might work best under which circumstances. The process is guided by the recognition of eco-epidemiological and socioeconomic factors indicative of particular vector bionomics, human/vector contact patterns and the operational feasibility of certain control measures. Malaria epidemiology is now divided into various ecotypes for better understanding of the transmission dynamics of malaria, the parasites and vectors, and human ecology.

Currently there are six main eco-epidemiological types occurring in steady state ecosystems.[1] These ecotypes do not necessarily represent all possible variants of malaria epidemiology worldwide. On the contrary, in some situations, the most relevant stratification may require distinguishing between the sub-divisions of these ecotypes. Moreover, those ecotypes are not mutually exclusive and mixed situations are frequently found (e.g. forest and altitude fringes, dry savannah and river forest corridors). The proposed eco-epidemiological types that will be used to describe the vector bionomics, transmission status and appropriate vector control measures are described in the following sections.

7.4.1   Tropical African Savanah

This ecotype extends from the Sahara desert to the humid equatorial African tropics and is further stratified in to three main agroclimatic types:

▶ Sahel "pastoral zone" grading southwards to dry savannah vegetation;

▶ western and central African belts progressively more humid grading into equatorial rainforest;

▶ extensive savannahs covering much of eastern and southern Africa, between the equatorial rain-forest and temperate highlands, and subtropical south.

Malaria vectors and transmission: *An. Funestus* and *An. gambiae* complex, two of the world's most efficient vectors, are found throughout the savannahs. *An. funestus* breeds in grassy swamps while members of the *An. gambiae* complex breed in freshwater temporary pools wherever they occur with rainfall, irrigation, borrow pits, footprints and road ruts. The two most important members of the *An. gambiae* complex are *An. arabiensis*, with females blood-feeding on livestock or humans plentifully indoors or outdoors, and *An. gambiae* s.s. with females more likely to bite humans indoors. Other species that may be locally important vectors include *An. nili*, *An. coustani* and *An. pharoensis*.

Malaria transmission is typically intense, regular, long, perennial or seasonal, according to the rainfall pattern and presence of water bodies around human settlements.

Vector control measures: ITNs/LLINs is the vector control measure of choice for tropical Africa. IRS can be combined with ITNs/LLINs to accelerate and maximize transmission reduction.

[1.]   WHO, 2006. *Malaria vector control and personal protection. Report of a WHO study group (12–14 March 2004).* Geneva, World health Organization. http://www.who.int/malaria/publications/atoz/who_trs_936/en/index.html

7.4.2 Plains and valleys outside Africa

This ecotype is found in South Asia, Central and South America and China.

Malaria vectors and transmission: the main malaria vectors in South Asia are *An. culicifacies* (in plains); *An. fluviatilis* and *An. minimus* (in river valleys); and *An. stephensi* (in arid, semi-arid plains, and cities). *An. annularis, An. philippinensis* and *An. aconitus* (in eastern coastal areas) are considered secondary vectors. *An. sinensis* and *An. anthropophagous* are vectors of malaria in China. *An. darlingi* and *An. pseudopunctipennis* are main malaria vectors in Central and South America.

Malaria transmission is very intense on the foothills and medium altitude valleys. Transmission in plains and river valleys is often seasonal or, in humid tropical areas, perennial with marked seasonal peaks associated with agricultural cycles, and determined by availability of surface water, temperature, humidity, climatic variations and other physical perturbations. Transmission occurs mainly as either mesoendemic or hypoendemic form. *P. vivax* is the predominant parasite though *P. falciparum* transmission often occurs.

Vector control measures: in plains and valleys with mesoendemic to hypoendemic transmission, use of ITNs/LLINs may be targeted for high risk groups/communities. In Central and South America, ITNs/LLINs would be a rational choice in large areas where endophagic populations of late night-biting mosquitoes such as *An. darlingi, An. nuneztovari* and *An. marajoara* are involved in malaria transmission. Malaria epidemics are likely to occur in arid areas, in the altitude fringes or to occur following massive arrival of displaced populations or refugees from areas of lower endemicity. Functioning early warning or early detection systems for epidemics are required.

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7.4.3 Forest and forest fringes

This is found in South-West Pacific and South-East Asia, South America and Africa. Malaria risk is often associated with forest areas.

Malaria vectors and transmission: forests and forest fringes harbour very efficient malaria vectors, particularly *An. gambiae* s.s., *An. funestus* and *An. moucheti* in tropical Africa; *An. darlingi,* and *An albitasis, An. marajoara* and *An. nuneztovari* in South America, and *An. dirus, An. fluviatilis, An. minimus* in South-East Asia. These vectors are preferentially attracted to bite humans in their shelters, but are exophilic, thus avoiding the effect of any residual insecticides that may have been sprayed on the walls or roofs of those shelters. Malaria transmission is, therefore, more intense and more difficult to control in temporary or newly established forest settlements than in neighbouring savanna farmlands. *P. falciparum* is the predominant species.

Human settlements and activities vary greatly in these area, leading to a wide range of epidemiological situations which may need different approaches of malaria control. This may need sub-stratification depending on the type of human settlements and activities as follows:

- ▶ *Native forest populations of hunters and food gatherers*
- ▶ *Agricultural activities in forest areas*
- ▶ *Collectors of forest products*
- ▶ *Gold and gem mining*

> ▶ *Police and army posts in forest areas*

> ▶ *Illegal activities and rebel groups*

> ▶ *Workers in economic development projects*

> ▶ *International border posts*

Vector control measures: ITNs/LLINs can be used in relatively stable camps. IRS is relatively ineffective because of the resting habits of the vectors (exophilic), the incomplete temporary shelters that frequently do not have walls that can be sprayed, and the mobility of settlements, which remain unreported and inaccessible.

7.4.4   Highland and desert fringes

This ecotype represents the areas lying between the highly endemic areas and the deserts or high altitude areas with complete absence of transmission. The highland fringe areas are found in Africa, South-East Asia, South-West Pacific, and South America while the desert fringes are found in Sahel, southern Africa, and in West Asia.

Malaria vectors and transmission: The vectors in the highland fringes include *An. fluviatilis* and *An. minimus* in South and South-East Asia and *An. pseudopunctipennis* in Central and South America. Desert fringe areas share vectos with neighbouring savannah or plains, such as *An. arabiensis,* which are more adaptable to dry conditions. Malaria vectors in foothills and medium altitude valleys are more efficient than in the plains in South and South-East Asia (e.g. *An. fluviatilis* and *An. minimus*), while the converse is the case in Central and South America (*An. pseudopunctipennis*). In highland and desert fringes in Africa, *P. falciparum* is the dominant species, while outside Africa, *P. vivax* generally dominates.

In highland fringes, temperature is the main determinant for transmission potential while in desert fringe areas availability of surface water and relative humidity play the major roles. Malaria transmission is thus reduced in these areas to a short season (decreasing with altitude or aridity), with areas where transmission only occurs in years with exceptionally long warm periods or high rainfall. These areas are, therefore, epidemic-prone.

Vector control measures: Malaria control in many of these areas still depends to a great extent on routine use of IRS. The use of ITNs may be promoted in these areas targeting the population at risk. Larviciding, may be effective if the breeding sites of vectors are few, fixed and findable. If resources allow and when transmission is likely to continue for a few months, selective and targeted vector control measures such as IRS can be carried out.

7.4.5   Coastal areas and wetland

Coastal areas offer particularly favorable conditions for malaria transmission. Efficient vector species thrive in brackish water or wetland habitats, and coastal areas are often attractive to a variety of human activities including tourism and development projects.

Malaria vectors and transmission: The vectors of this ecotype include *An. subpictus* complex and the *An. sundaicus* complex in South-East Asia and the *An. punctulatus* complex of the Australasian region. In tropical Africa, the coastal species *An. melas* in West Africa and *An. merus* in East Africa are lesser vectors than the freshwater species *An. arabiensis* and

An. gambiae across the tropical African Savanah. In Central and South America, some efficient malaria vectors are associated with coastal brackish water habitats, notably *An. aquasalis* and *An. albimanus* in South America and *An. albimanus* in some areas in Central America.

Malaria transmission in coastal areas and wetland is influenced by population migration from the malaria endemic rural areas to the cities and low-lying coastal areas. The risk of malaria transmission in coastal areas can be greatly increased by changes in land use or human disturbance of the environment which result in the accumulation of stagnant and brackish water and consequently increased vector population. Similarly, human activities, including siting of residential areas in close proximity to extensive tropical wetlands or brackish lagoons, creeks etc., influence malaria incidence in coastal areas and wetlands.

Vector control measures: the vector control of choice include ITNs/LLINs, limited IRS and larval density reduction by larviciding or physical methods. Understanding the behavior of the vectors is critical as some vectors are often early-biting and exophilic, reducing the effectiveness of both IRS and ITNs

7.4.6   Urban and peri-urban areas

Urban malaria occurs in Africa, South Asia, South America and also superimposed on any of the other ecotypes.

Malaria vectors and transmission: Urban malaria can result from any of the following situations.

▶ existence, either within or in the periphery of the city, of areas which retain the ecological characteristics of rural areas appropriate for malaria transmission. This is an extension of surrounding rural malaria transmitted by same vectors as in neighbouring rural areas.

▶ situations where urban settings create favourable conditions for the establishment of an efficient malaria vector.

▶ use of any unoccupied plot in the cities for the cultivation of vegetables or eventually rice, generally with informal irrigation and no proper drainage, thus creating favourable breeding places.

▶ existence of urban malaria sensu stricto that is transmitted in the city by vectors specially adapted to the urban environment such as (i) *An. stephensi* in many Indian cities; (ii) *An. claviger* in cities of Israel, Lebanon and the Syrian Arab Republic; and (iii) *An. arabiensis* in cities of Mauritius and La Réunion islands.

Urban malaria in Africa is generally associated with lower risk than the savannah malaria and often the centers of cities are free from malaria transmission. In contrast, urban malaria in the Indian subcontinent is associated with higher risk than malaria in most adjacent rural areas.

Vector control measures: ITNs/LLINs and focal IRS can be used. Larviciding is particularly indicated in urban areas, where breeding sites are few, fixed and findable. Other measures include environtmental management, e.g. improved drainage, screening of overhead water tanks against *An. stephensi*, as well as improvement of houses to make them more mosquito-proof.

In addition to the six ecotypes in steady state ecosystems, there are two other eco-epidemiological types that occur in situations of rapid development change as described below.

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7.4.7   Agricultural development projects

This ecotype is related to established irrigated agricultural systems which seem to generate high disease burdens through the interplay of bio-ecological, socioeconomic and political factors. These factors include high vector densities, resistant strains of parasites and vectors, human aggregation and migration, poverty, ignorance, inadequate physical and trained human resource infrastructure to provide adequate preventive and curative care, and violent political unrest.

Malaria vectors in development projects are normally the same as in neighbouring areas. However in some cases differences in species distribution are observed, and malaria transmission may differ considerably due to the local environmental modifications and the changes in population distribution introduced by the project. The major vector control measures used are IRS and ITNs/LLINs.

7.4.8   Socio-political disturbances (humanitarian emergencies)

Context-specific factors giving rise to high malaria burdens in complex emergencies include breakdown of health services, concentration of non-immune refugees in malaria risk areas, malnourishment, siting of refugee camps on marginal land prone to flooding or vector breeding, and problems in gaining access or supplying medicine to the displaced population. As conflict progresses, complex emergencies usually evolve from acute emergency to post-emergency phases. The acute phase is characterized by sudden population displacement from the areas of disturbance and high mortality rates, and may last only a few weeks or months. During the post-acute phase, as refugees re-settle, the situation stabilizes, the health situation is generally brought under control and basic needs are met.

Malaria vectors in complex emergencies are often the same as in neighboring areas. The choice of vector control interventions will depend on local factors such as the type of shelter available, human and vector behaviours. In complex emergencies, priorities are prompt diagnosis and effective treatment. In acute-phase, this can be supplemented, where feasible, with ITNs/LLINs aiming to cover all population at risk. IRS is not suitable in settings where shelters are temporary and without surfaces that can be readily sprayed. Both LLINs and IRS has a role to play in post-acute phase where the situation stabilizes and people have shelters with sprayable surfaces. Although there is no formal WHO recommendation, insecticide-treated plastic sheeting has also been used in the acute-phase where use of LLINs and application of IRS are not practical.

Exercise 7.1

Working in small groups, participants will answer the following questions:

1. *What are the impacts of the entomological and environmental factors on the prevalence of parasite, immunity of the population, age distribution of infection, illness and death due to (i) unstable malaria and (ii) stable malaria? Compare and contrast the differences.*

2. *Explain why it is difficult to reduce transmission in areas of stable malaria compared to areas of unstable malaria.*

3. *What are the factors that affect vector density in urban settings?*

Meet in plenary to discuss the conclusions with the rest of the class.

Exercise 7.2

Working in small groups, participants will determine which vector control options are most appropriate for each of the following strata:

1. unstable malaria

2. stable malaria

3. urban setting

4. agricultural development projects

5. socio-political disturbances (humanitarian emergencies).

List the results and the justification for this selection on a flip chart. Present the results in plenary.

Discussion. The tutor will lead a final discussion on vector control options as they relate to epidemiological strata.

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ANNEX 1

Protocol for mosquito rearing

A1.1 Introduction

It is important for entomological laboratory work to rear and maintain mosquitoes under insectary conditions. In the following description of the specific requirements and methods for mosquito rearing, it is assumed that the insectary has thermostatically controlled temperature (heating/air conditioning as needed) and humidity control.

A1.2 Equipment for handling mosquito adults

A1.2.1 Cages

A wide variety of rearing cages have been developed for adult mosquitoes. The most important factor is size in relation to number of mosquitoes caged, and particularly the area of available resting space per mosquito. This density affects mating, feeding and longevity. A vertical resting surface of 1.8 cm^2 per mosquito seems best for many mosquitoes. The other size-related factor is the ease with which mosquitoes can mate. Many species will not mate in cages less than 30 x 30 x 30 cm. Indeed some species freshly colonized from the field will only mate successfully in cages of 1 x 1 x 1 m or larger. Species that will not mate at all in laboratory cages may have to be maintained by artificial mating procedures.

Aedes, Culex, and *Anopheles* mosquitoes can be colonized in cages 30 x 30 x 30 cm. *Toxorhynchites* and some *Anopheles* species are usually colonized in cages 1 x 1 x 1 m, and the same size of cage is occasionally used for maintaining large stock colonies of other genera.

Cages usually have a frame of wood or metal covered with netting of cotton, synthetic fabric, or aluminium. The gauge of mesh should be such that the holes are not more than 1.1 mm across; laboratory-bred mosquitoes may be small due to overcrowding or underfeeding in the larval stages and males reared in such conditions may squeeze through mesh any larger than this. The front of the cage should have a fabric sleeve wide enough to allow the introduction of small bowls (12 cm diameter) for egg laying. As cotton readily rots, synthetic netting is generally preferable. The base and back of the cage can be of plastic laminate material; a solid base is preferable to one of netting as it must support bowls containing pupae or water for egg laying (Fig. An1.1).

Figure An1.1   Different size and types of cages

A1.2.2 Adult food

Water for adults may be provided in small bowls of 12 cm diameter or in the form of water-soaked cotton wool pads placed on top of the cage. Sugar may be provided to prolong the life of males and to sustain females between blood meals. A 10% solution of household sucrose is a convenient form of carbohydrate food. Cotton wool balls soaked in this solution are squeezed to remove excess liquid and placed on top of the cage. Alternatively cotton wool wicks in small pots of 10% sucrose solution may be placed within the cages. Other sources of sugar include sliced apples, raisins or sultanas placed on the cage tops, but these are likely to make the netting dirty.

Blood-meals are necessary for egg production in all but the few autogenous species. Colonies are usually fed blood twice a week. Membrane feeding systems are preferable and they can be used for routine colony maintenance (Fig. An1.2). When establishing an insectary or when mosquitoes do not feed readily on membrane feeders, placing an anesthetized animal on the cage should be considered. Sources of blood include guinea pigs, mice, rats, chicken or rabbits. Mammals are usually shaved with hair clippers on the flank prior to feeding (Fig. An1.2). Difficult species of mosquito may have to be fed on the arm of the experimenter. Figure An1.3 shows apparatus for artificial feeding of mosquito.

Figure An1.2   Blood feeding of mosquitoes on guinea pigs

Figure An1.3   Apparatus for artificial feeding of mosquitoes

For field use, smaller numbers of mosquitoes are often housed in cylinders of plastic or cardboard cartons, each with netting over the end (Fig. An1.4).

Adult mosquitoes are transferred between cages by means of an aspirator or suction device (Fig. An1.5). With mouth-operated aspirators there is a risk of inhalation of mosquito scales which causes irritation. Mechanical aspirators based on portable vacuum cleaners avoid this problem.

Figure An1.4   Plastic or cardboard ice-cream cartons with netting tops
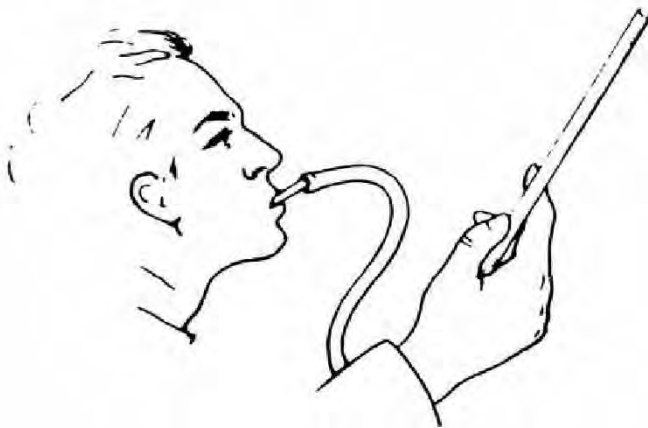
Figure An1.5   Mosquito aspirator used for collection and transferring mosquitoes

A1.2.3   Egg collection

The eggs of *Anopheles* mosquitoes are rarely drought-resistant and are laid individually on the water surface, usually fresh, in a bowl. Sometimes folded filter paper is placed inside the bowl to prevent eggs sticking to the sides or to facilitate transfer of eggs. The eggs may be transported on damp filter paper although at the temperature in the insectary they will hatch within a couple of days (chilling may sometimes be used to delay hatching).

A1.2.4   Larval rearing procedures

Larvae are kept in trays or bowls. A wide variety of sizes and shapes have been used. For maximum holding capacities on benches or shelves rectangular shapes are better than round ones. White enamel trays have been widely used, as have those made of polyethylene or polycarbonate. Trays about 30 x 25 x 5 cm are suitable for holding water to a depth of around 4 cm with 300 larvae per tray. Larvae are handled and transferred using glass pipettes fitted with rubber teats or bulbs.

An extraordinary variety of materials have been used as larval food, ranging from guinea pig faeces to well-defined chemical diets. Commercial dog biscuit has been widely adopted,

as have yeast tablets, liver powder, dried blood, cereal baby food (e.g. Farex), fish food flakes, and mixtures of two or more of these materials are commonly used. It is often necessary to experiment with different diets for different strains or species. If too much food is added to the larval tray there may be a problem with scum formation on the water surface causing the larvae to suffocate and die. Small quantities of grass, turf or other plants are sometimes added to the rearing water for *Anopheles* spp.

If larvae are overcrowded in trays, pupation is delayed and the resultant adults are smaller and weaker. This may have important consequences for experimental studies, e.g. such mosquitoes take smaller blood-meals.

A1.2.5   Handling of pupae

Pupae must be removed from the larval trays shortly after they have formed otherwise the adults will emerge and escape into the rearing room. Pupae picking is usually carried out daily, the pupae being transferred to fresh water before transfer to adult holding cages. Pupae may be picked with teat-ended glass pipettes of the type used to handle larvae, or with small nets.

Large numbers of pupae may sometimes be separated from larvae by sieving the contents of a rearing tray and placing them into ice water. The larvae sink immediately while pupae float at the surface and can be skimmed off. A variety of mechanical devices have been described which can be used to separate pupae from larvae.

Where mosquitoes are to be used for crossing experiments it will be necessary to sex the pupae, usually by size (sometimes male pupae are smaller), shape or details of the terminalia. To check that the sexes have been adequately separated, the pupae can be placed, four to a tube, in flat-bottomed glass vials 75 x 25 mm, the neck of the vials being secured with cotton wool plugs (see Fig. An1. 6).

Figure An1.6   Equipment for maintaining eggs, larvae and pupae

A1.2.6   Insectary design

The three essential requirements for an insectary are the ability to control: *(i)* temperature, *(ii)* humidity, and *(iii)* lighting.

The degree of sophistication of rearing techniques will depend on both the facilities and the requirements of the worker. For example, in the tropics mosquito larvae may be reared in covered trays in a simple unheated room and kept with adult mosquitoes in the same room. High humidity is essential for rearing adults and it can be maintained by placing a saturated wad of cotton wool in an open Petri dish on top of the cage and polythene sheeting placed over the cage. When it is necessary to maintain mosquitoes in a field station, this sort of improvisation may be essential.

In some situations, particularly where small numbers of insects are to be maintained, or where particularly careful control of environmental conditions is necessary, the insects can be reared in an environmental cabinet kept in an ordinary laboratory.

A1.2.7   Building

Because of the need to control temperature and light, a windowless room is necessary, preferably with no external walls. In temperate climates insectaries with external walls usually suffer from excessive condensation, while in the tropics they may absorb too much heat from sunlight.

As far as possible each insect rearing room should have an ante-room which can serve as a buffer zone to prevent undue disturbance of temperature and humidity on opening the door. The ante-room also serves to limit the escape of adult mosquitoes. The walls of the room should be smooth and painted white or a very light colour. The paint must not be insecticidal. Totally waterproof epoxy paint is often recommended but in fact a slight permeability of the wall surface to water vapour can help to limit condensation. Totally impervious terrazzo wall surfaces suffer from excessive condensation. A large sink with hot and cold running water is essential in any rearing room and particularly where insects with aquatic stages are being reared. It is an advantage to have a separate room for cleaning of cages and rearing trays or other equipment. Readily movable tables, benches, and racks are preferable for flexibility of use and to facilitate cleaning.

A1.2.8   Temperature control

Temperature control may be part of an elaborate air-conditioning system or a simple thermostatically controlled bar heater. If there is no fan-assisted air movement in the room, the temperature may be stratified between floor (coldest) and ceiling (warmest). Thermostatic controls are prone to fail after a relatively short period in a humid atmosphere and it is desirable to have a thermostat probe in the room with the switching device mounted externally. A modular system of readily replaceable thermostat and heater is an alternative. In tropical insectaries air-conditioners will usually be needed to keep temperatures within the desired control range.

Regular temperature records should be made in the insectary. It is often convenient to have a maximum-minimum thermometer and/or recording thermohydrograph for this purpose. Insectaries used for mosquitoes are usually maintained within the range 26 °C–28 °C.

A1.2.9   Humidity control

Humidity regulation is usually provided by small commercially available mist/aerosol producing devices. These have flat discs turned at high speed by an electric motor, resulting in a mist of fine water droplets being forced out through a top nozzle. Humidity control is maintained by a humidistat. As these have to be mounted in the room, the micro-switch eventually fails due to moisture and it is best to have both humidifier and humidistat replaced regularly. Humidity should be measured regularly, either continuously with a recording thermohygrograph or daily using a whirling hygrometer.

A1.2.10 Lighting control

Both photoperiod and light intensity affect the development of various stages in the life-cycle, e.g. mating, feeding, egg-laying and time of pupation. Lighting periods are readily controlled by time clocks and switches built into the lighting supply. Standard daylight fluorescent lights are the most suitable source of lighting. Separate red light incandescent lamps may be provided for

work during periods of darkness. A photoperiod of 12 hours of light and 12 hours of darkness is often chosen, although some workers may prefer 14 hours light and 10 hours darkness.

Species which take the blood-meal at night can be kept in a room where the light cycle is offset. Light off at midday and light on at midnight allows routine work in the morning and blood-feeding of adult female mosquitoes in the afternoon. Some species may require a crepuscular period to swarm and mate, which may necessitate controls for automatic gentle dimming of lights to provide a dust effect and gradual increase in brightness to simulate dawn (see Fig. An1.7).

Figure An1.7   An insectary with racks of trays for raising mosquito larvae

A1.2.11 Safety

Special measures must be taken in the maintenance of colonies of insect vectors of diseases to isolate the insects from the surrounding environment and to prevent escape. In the case of malaria, mosquitoes escaping from a laboratory may seriously jeopardize control/elimination efforts. Escape of insecticide-resistant mosquitoes into a susceptible native mosquito population could have serious consequences for control measures in the area.

Correct rearing procedures and sets of double doors are basic measures to limit escape from insectaries. White painted walls allow escaped mosquitoes to be detected more readily. A white painted ante-room is desirable and this can be fitted with light-traps to further prevent escaped mosquitoes from reaching the outside. Drains may require screening, including the drains from sinks. This may be of great importance in an insectary in the tropics where aquatic stages of mosquitoes from a rearing facility could be released into the surrounding drains and become established. Oviposition traps in and around insectaries may help. It may be necessary to place oviposition traps outdoors adjacent to insectary facilities in areas of potential colonization of species such as *Aedes aegypti*. Insectaries should not be sited in close proximity to animal rooms.

A1.2.12 Mosquitoes infected with human pathogens

Particularly strict precautions are necessary for the rearing and maintnance of mosquitoes which are infected with human pathogens. The measures required will vary according to the

magnitude of the risk involved.[1] Microorganisms producing a potentially fatal infection or infection which is difficult to cure are assigned to a higher risk group than those which are not fatal or for which effective chemoprophylaxis and/or chemotherapy is readily available. Mosquitoes experimentally infected with *Plasmodium falciparum* in a non-endemic area carry a potentially lethal infection for non-immune workers and the risk may extend to people who are not associated with the research programme.

National regulations may be in force concerning the conditions under which pathogens may be kept, and compliance with such regulations is essential.

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[1]   Further information on risk groups for human pathogens and laboratory containment of infected arthropods can be accessed in the WHO *Laboratory Biosafety Manual,* 3rd edition, 2004. www.who.int/csr/resources/publications/biosafety/Biosafety7/pdf

ANNEX 2

Treatment of insecticide treated nets

A2.1   Introduction

From a programmatic perspective, treatment and retreatment of nets will be needed until coverage with LLINs is more widespread and replace all the conventional ITNs already delivered and distributed. Furthermore, certain communities in malaria endemic settings might continue to use nets that need treatment and re-treatment.

The conventional ITNs need re-treatment every 6 to 12 months depending on the length of the transmission and washing frequencies. Nets are retreated by simply dipping them in a mixture of water and insecticide and allowing them to dry in a shady place. Nets can be treated through:

▶ *Home treatment: nets are treated by* any adult in the home using treatment kits (self-do-kit) which are available through shops or provided by health centres.

▶ *Mass treatment: nets are treated by (i)* trained personnel working from a dipping centre, a fixed place where people bring their nets for re-treatment; *(ii)* community campaigns that offer re- treatment once or twice a year, preferably before the transmission season; or *(iii)* mobile teams that provide re-treatment at many sites including health centres, markets and homes."

A2.2   Treatment procedure

Following are 10 steps to treat and re-treat nets:

Step 1: Collect the necessary supplies/materials

The necessary materials for net treatment consist of mosquito nets, insecticide, water, basin, measuring container, rubber gloves and protective clothing and soap. Make sure the net is washed/cleaned before treatment. The nets should preferably be treated outdoors in the shade. If treatment is to be carried out indoors, a room with open windows should be used. Use a basin and gloves that are not used for any other purpose.

Step 2: Put on protective gloves before treating nets

Step 3: Measure the correct amount of water

The amount of water needed depends on the net material. Regardless of the size and shape of net, the amount of water required for one synthetic net (polyester) is ½ litre (if the net is very large, more water may be needed). If a measuring container comes with insecticide, use it to measure water. Otherwise, use any measuring container that is not used for food, drinks or medicines.

Step 4: Measure the correct amount of insecticide

The amount of insecticide needed to treat a net depends on the type of insecticide used. Follow instructions on the container/sachet/packet. Generally, 10–15 ml of insecticide is required to

treat one single net. The leftover insecticide should be stored in its original container, in the dark and away from children.

Step 5: Mix the water and insecticide thoroughly by gloved hands in basin

Step 6: Treat the nets

▶ Always impregnate one net at a time.

▶ Put the net in the basin containing water and insecticide.

▶ Soak the net long enough to ensure that all parts of the nets are impregnated.

▶ Take out the nets and allow excess liquid to drip back by squeezing it gently, but do not wring it.

Step 7: Drying the nets

▶ Let the net dry flat in the shade on plastic sheets.

▶ Later, the net can be hung up to finish drying in the shade.

Step 8: Disposal of leftover mixture of water and insecticide and insecticide containers/sachets

▶ Following the treatment of all available nets, the leftover mixture of water and insecticide, if any, may be used to treat curtains.

▶ Otherwise, dispose the liquid in the toilet or a hole away from habitation, animal shelters, drinking water sources, ponds, rivers and streams.

▶ Destroy empty insecticide containers, sachets and packets and/or bury in a hole away from habitation, animal shelters, drinking water sources, ponds, rivers and streams.

Step 9: Washing and cleaning of hands and equipments

▶ Wash equipments (basin, measuring container) with lots of water while wearing protective gloves.

▶ Wash gloves (if non-disposable ones are used) with soap and lots of water, or dispose with insecticide containers.

▶ Wash hands with soap and lots of water.

Step 10: Washing and re-treatment of nets

▶ Washing removes the insecticide from the net. So, wash the nets as seldom as possible and gently with soap and cold water and dry flat on plastic sheet in shade.

▶ Do not wash/rinse treated net in or near drinking water sources, ponds, lakes, rivers, streams.

▶ Dispose of water for washing/rinsing in the toilet or in a hole away from habitation, animal shelters, drinking water sources, ponds, rivers and streams.

▶ Nets must be re-treated again after it has been washed three times; or, at least once a year even if it is not washed, preferably just before the rainy season. Nets may be treated twice a year in areas that have a lot of mosquitoes all year long.

Table An2.1 WHO recommended insecticide products, dosages and amount for net treatment

Insecticide	Formulation[a]	Dosage[b]	Dosage per mosquito net
Alpha-cypermethrin	SC 10%	20-40	6 ml
Cyfluthrin	EW 5%	50	15 ml
Deltamethrin	SC 1%	15–25	40 ml
Deltamethrin	WT 25%	15–25	One tablet
Etofenprox	EW 10%	200	30 ml
Lambda-cyhalothrin	CS 2.5%	10–15	10 ml
Permethrin	EC 10%	200–500	75 ml

[a] SC = aqueous suspension concentrate; EW = emulsion, oil in water; WT = water dispersible tablet;
    CS = capsule suspension; EC = emulsifiable concentrate.
[b] Milligrams of active ingredient per square metre of netting.

Note: WHO recommendations on the use of pesticides in public health are valid ONLY if linked to WHO specifications for their quality control. WHO specifications for public health pesticides are available at http://www.who.int/whopes/quality/en/.

A2.3   Insecticides and formulations for treatment

Table An2.1 lists the insecticide products and dosage per mosquito net recommended by WHOPES (WHO Pesticide Evaluation Scheme) for the treatment of mosquito nets.

A2.4   Safety issues

While field use of pyrethroids for the treatment of mosquito nets at the recommended dose poses little or no hazard to those treating the nets, the supply of insecticide "over the counter" (OTC) for the treatment of nets by householders raises particular safety concerns. It is strongly recommended, therefore, that insecticides for the home treatment of mosquito nets be marketed only in single-unit doses. Moreover, if presented as a liquid formulation in bottles, the use of child-proof caps is essential. The OTC supply of high- concentration permethrin (e.g. 50% EC) should be avoided. Only trained staff should use such high concentrations of permethrin.

Acute toxicity or irritation may occur as a result of the handling of insecticides when applying them to mosquito nets. People directly involved in dipping large numbers of nets are at greater risk than people who occasionally treat their own nets. The use of rubber gloves is essential; mouth and nose masks should be worn when dipping large numbers of nets, especially with emulsifiable concentrate formulations.

A2.5   When to apply the insecticide

Special attention must be paid to net distribution systems and to periodic re-treatment of nets with insecticide. The activities of the malaria control programme need to be adapted to the method of distribution when this vector control option is adopted.

A key issue is the establishment of functional periodic re-treatment cycles based on the epidemiological needs, the residual effect of the formulations on different materials, and the habits of the population in washing their nets.

Maximum protection is required during the transmission season or its peak, where transmission is perennial. When control programmes play an active role in the distribution of nets, whether free or subsidized, re-treatment is usually carried out at special events, such as Nation-

al Anti- Malaria Week (or day) or Health Day. Distribution and re-treatment should be timed, if possible, to ensure the maximum coverage with freshly treated nets during the transmission season. When distribution is carried out by private entities, official events to promote and demonstrate the use of ITNs should be organized just before the start of the transmission season.

The periodicity of re-treatment should be based on investigations that determine the actual residual effect of the insecticide under the conditions of use in the area concerned (climate, exposure to direct sun when used outdoors, washing habits, etc.) and on the seasonality of transmission. These studies should determine the best method of washing the nets, taking into account effects of local soaps, use of hot water, drying conditions, frequency of washing etc., which should be promoted by information, education and communication (IEC) and during treatment or promotional events.

If nets are sold commercially and individuals are responsible for treatment, the users should be informed that if they wash their nets more often than recommended, they should also re-treat them more frequently. Wherever possible, insecticide treatment should be provided free of charge especially to the poor and vulnerable groups.

When the risk of an imminent epidemic is detected, or at an early stage of an epidemic, it is advisable to organize a re-treatment event in areas where coverage with treated nets is high, provided that this will not interfere with the implementation of emergency control measures.

ANNEX 3

Geographical reconnaissance

A3.1 Definition, objectives and scope of geographical reconnaissance

Geographical reconnaissance (GR) may be defined as a field operation involving census, mapping and sampling procedures. It may require recording the quantity, types, size, quality, location, accessibility and other pertinent information on housing, the habit and customs of the local population in relation to the needs of the programme, the presence and location of actual or potential mosquito breeding sites, the habits of mosquitoes, and any other information considered necessary for planning and conducting a mosquito vector control programme. The collection of such data on: population, housing, communication, maps, and entomological and epidemiological information, should precede control activities.

The objectives of geographical reconnaissance are:

► to determine the number and location of houses and population at risk from the disease and thus to be included in the vector control programme;

► to provide a basis for planning the programme needs in terms of equipment, supplies, transport and personal; and

► to establish the nature and extent of the mosquito breeding sites in relation to population centres as a basis for planning mosquito control measures such as larviciding and source reduction.

In brief, the primary objective of GR is to provide the basis for planning and conducting a mosquito control programme to include residual spraying of houses, space spraying, larviciding, and source reduction, as appropriate for the individual situation.

A secondary objective can be health education. This is an excellent opportunity to conduct a public relations and education campaign to ensure the understanding and cooperation of the people. If properly carried out GR can be very useful for most other health programmes. The scope of GR will vary from country to country and programme to programme, depending on the nature of the problem and available resources. For malaria control/elimination programmes, the primary need is for information on houses to facilitate the residual spray programme, and information on people to facilitate the case detection and treatment programme. These needs apply to malaria control programmes and to control programmes for other vector-borne diseases. However, with the current approach to vector control, i.e., comprehensive vector control utilizing all available methods in an integrated programme, the scope of GR required is much broader.

To adequately meet the needs of such a programme, GR should include:

▶ Gathering and analysing pertinent epidemiological and entomological data in order to establish the area at risk from the disease.

▶ Preparation of maps showing location of houses and means of access (roads and paths).

▶ Numbering houses and attachment of a house card to be initialed by all those making official visits to the house for the purposes of supervision. Although this system has been abandoned by many countries in the process of converting from malaria eradication to control, it is still the best system for supervision so far developed and is appropriate for any health programme involving repeated contact with villagers in their homes.

▶ Identification and mapping of mosquito sources in relation to villages.

The result of the GR should be available at the headquarters where the planning and administrative management of the mosquito control activities takes place. These results should also be available at the periphery for the supervisors of field operations. Sufficient copies of the maps should be made for use by the operators as well as the supervisors. GR, and particularly the maps, should be updated at regular intervals, preferably annually.

Much valuable information for developing the GR is available from a variety of sources within each country. Suitable maps may be available from many of these sources. Such sources of information include:

▶ Department of Census and Statistics
▶ Ministry of Health
▶ Ministry of Education
▶ Home or Interior Ministry
▶ Post and Telegraph Department or Companies
▶ Department of Local Self-Government or Municipal Affairs
▶ Department of Housing
▶ Surveyor-General
▶ Public Works Department
▶ Irrigation Department
▶ Food or Agricultural Ministry
▶ Labour Ministry
▶ Commerce Ministry
▶ Armed Forces
▶ Department of Information and Publicity
▶ Ministry of Planning or Industry
▶ Ministry of Communications
▶ Water Resources or Utility Department
▶ Chamber of Commerce
▶ Private business concerns – bottled drinks, fuel, etc.
▶ Atlases and almanacs
▶ Geographical societies
▶ International organizations
▶ Public libraries
▶ Private individuals

A3.1.1   Maps for mosquito control

A map is a spatial representation of an area. It shows natural and man-made features through symbols, lines, colours, and codes. Maps are essential for planning, conducting, supervising and evaluating vector-borne disease control programmes.

Before starting to prepare maps for vector control purposes, an attempt should be made to gather whatever maps and aerial photographs that are already available from other government agencies, international organizations and commercial sources, such as Google maps.

A3.1.2   Types of maps needed in a vector-borne disease control programme

a. **The project map** should cover the entire area in which vector control activities are planned. For small countries this may be a single map. Larger countries may require a separate project map for each state, province, district or even sub-district. In former malaria eradication programmes, zone, sector, unit or sub-unit boundaries were established in response to the malaria problem and often ignored political boundaries. With the conversion to a control strategy many countries have adjusted their operational units to be consistent with political realities. Future planning of vector-borne disease control programmes in coordination with primary health care, should utilize district or other appropriate political subdivisions for establishing areas of operational activity.

Maps suitable for conversion to project maps of appropriate scale should be readily available from other government sources, but may need to be traced or re-drawn to eliminate unnecessary detail. In addition to the normal geographic features, these maps should show malaria or other vector-borne disease problem areas subject to various control activities, areas of supervisory responsibility for disease control and for health services. Epidemiological and entomological information may be included as well as any other pertinent information to facilitate understanding of the problem and the control situation.

In order to prepare a project map, the person in charge should first try to obtain a reasonably accurate, detailed map of the community and surroundings. Such a map should be enlarged to a convenient scale so that important features that are needed can be copied. For instance, the official 1:25 000 topographic map of the district, where the project is located, shows the village streets, wooded and cultivated areas, a few of the buildings and the rivers or streams. An irrigation scheme built since the map was printed is not shown, but can be a landmark. Good maps are simple, clear, and show the principal features of the locality approximately in correct relative position to each other. The main features needed in a mosquito control project map are water courses (with direction) and the names of swamps or marshes, forest and fields, hills and ridges, streets and paths. Also included should be constructed landmarks, such as railway lines, canals, power lines, water tanks and buildings of all kinds, classified according to their use.

b. **The operational maps** required for mosquito vector control are of two types:

▶ *A village map* which shows the entire population (not just the village as a political entity) in a given area such as a district or sub-district. In addition to the village, the map should show all roads used to access the villages, streams, lakes, ponds, swamps, location of all

Table An3.1 Scale of maps

"A" Small scale

Scale	1 cm equals	1 inch equals	1 mile equals	1 km equals
1:500 000	5 km	7.89 mi	0.127 in	0.2 cm
1:100 000	10 km	15.78 mi	0.063 in	0.1 cm

"B" Medium scale

Scale	1 cm equals	1 inch equals	1 mile equals	1 km equals
1:50 000	0.5 km	0.789 mi	1.27 in	2.0 cm
1:63 360	0.634 km	1.0 mi	1.00 in	1.58 cm
1:75 000	0.75 km	1.18 mi	0.845 in	1.33 cm
1:100 000	1.0 km	1.58 mi	0.834 in	1.00 cm
1:250 000	2.5 km	3.95 mi	0.253 in	0.4 cm

"C" Large scale

Scale	1 cm equals	1 inch equals	1 mile equals	1 km equals
1:5000	5 m	13.9 yds	10.56 ft	2 cm
1:1000	10 m	27.8 yds	63.36 in	100 cm
1:2000	20 m	55.6 yds	31.88 in	50 cm
1:3.168	31.7 m	0.05 mi	20.0 in	31.7 cm
1:5000	50 m	139 yds	12.67 in	20 cm
1:10 000	100 m	0.158 mi	6.54 in	10 cm
1:20 000	0.2 km	0.316 mi	3.17 in	5 cm
1:25 000	0.25 km	0.395 mi	3.64 in	4 cm
1:31 680	0.317 km	0.5 mi	2.0 in	3.17 cm

Note: 1 mi = 63,360 in and 0.05 mi = 105.6 yds

health facilities and population figures for the smallest political subdivision possible. This map is for the use of the supervisor at the district or sub-district level to enable him/her to do the necessary planning, carry out the supervision of field operations and evaluate the status of the programme. District village maps may be to scales of 1:25 000 to 1:50 000 or even greater depending on the size of the area to be covered.

▶ *A locality sketch map* which shows the location of individual houses in a given locality in relation to roads and mosquito breeding sites. This map provides the basis for establishing itineraries for the field workers distributing bednets or spray squads doing residual house spraying, space spraying or larviciding, and together with the district map it can be used for planning source reduction projects.

The locality sketch map would normally vary in scale from 1:500 to 1:5000 depending on the distribution of houses, but a precise scale is not essential. However, the location and numbering of the houses must be clearly identifiable and the roads and mosquito sources must be accurate enough to be readily located. Several copies of these maps should be prepared so that one is always available to the supervisors, one for the squad chief, and, when necessary, one for the individual workers. These maps should be updated continuously as new houses are built, old houses are abandoned and as mosquito sources change.

A3.1.3 Equipment required for a geographical reconnaissance agent:

- Clip board, or mapping board (40 x 30 cm)
- Ruler (mm division) 30 cm long
- Simple pocket compass (preferably fixed to the board)
- Drawing pins or rubber bands (to hold the drawing sheet to the board)
- House measurement stick, wooden (1.5 or 2 m long), or a measuring tape
- Paint kit and brush (or tacks, nails and hammer)
- Haversack
- Glue, for attaching house card
- Drawing sheets (20 x 30 cm)
- GR records

The procedure to make a sketch map is as follows:

1. Extensive tours must be made in the area in order to obtain general knowledge of the local topography which serves as a guide for planning. Attention is paid to finding easily-identifiable boundaries and recognizable landmarks.

2. To determine the scale, the size of the area to be mapped must first be known, both its maximum length in the north-south as well as east-west directions. This is found out by pacing the two dimensions either within the area, or if this is not possible, outside the area. These two dimensions may be called L and B, and the paper used length "l" and breadth "b" l/L and b/B are calculated and the smaller scale of the two used to decode the map scale.

3. The mapper's pace length should be calibrated. This is done by pacing a measured distance, e.g. 100 m on level ground, and calculating the average pace length. A simple pocket compass should be fixed on the map board and the north line drawn in one corner of the sketch, parallel to the shorter side of the paper if the locality has the shorter dimension in the north-south direction, or parallel to the longer side of the paper if the locality has the larger dimension in north-south direction. Alternatively a Global Positioning System (GPS) can be used for determining the geographical coordinates for any point on the map or used to trace the position of a line, such as the banks of a river or a road.

A framework is first made on which to hang the details. The area boundaries may be roads, rivers, railway lines, canals, or other features. Within the boundaries the area may be criss-crossed by footpaths, trails, streets, cultivation boundaries, streams, etc. All these features will be plotted as lines in the map. The important features must be selected which bound as well as criss-cross the area and these drawn first to give a skeleton framework. The advantage of drawing the framework is that since these lines are drawn in soft pencil with no other confusing details, the errors in mapping them can be corrected, before any filling in of details. So the rule is: OUTLINE first, DETAIL afterwards.

A3.1.4 Reproduction

Where a great deal of reproduction is done the provision of computer equipment with printer is well justified. Copies of pencil or ink tracing can be made by a photocopying machine,

if available. Original tracings must be carefully stored in a map case. Small maps can be reproduced by photocopier.

A3.1.5 Locating and recording mosquito habitats

The various mosquito species which infest a community have typical breeding, flying and resting places, and preferred seasons and times of day for their activities. Knowledge of these places and times forms the starting point for the project officer (and staff and volunteers) in locating the actual habitats of mosquito larvae and adults, and in monitoring changes which occur during the course of the project.

At the beginning of the project and at different times of the year an attempt should be made to locate and number all the outdoor breeding places, and to estimate the corresponding water surfaces in m^2 or hectares which may have to be treated with larvicide, and finally to mark them with distinctive signs and symbols both on the ground (if possible) and on the project map. Geographical reconnaissance of outdoor breeding sites should be conducted at different times of the year. The breeding place number on the sign and on the map should be painted a distinctive colour (e.g. red) and enclosed in a distinctive symbol (e.g. a red circle). A wooden rod 1.5 or 2 m long, painted alternately black and white to show half metre lengths, can be used in estimating small surfaces and channel widths; large surfaces can be measured by pacing the length and width.

When new breeding places are found in the course of the project, they should be numbered consecutively on the ground (if possible) and on the map. All larviciding and larval searches should be identified in project reports by breeding places numbers.

Places where adult mosquitoes are active outdoors, as determined by landing rates or observations, should be identified at different times of day and at different seasons as well as for different species. These outdoor sites should be identified and numbered on the ground and on a separate project map by distinctive signs and symbols, such as a red number in an inverted red triangle. The record should show the date, time, species, and density. New sites discovered as the project proceeds should be numbered consecutively and on the map. All outdoor space spraying and adult catches should be identified in project reports by the numbers at field sites.

Mosquito resting places should be identified ideally before any insecticidal measures are undertaken, or at least before the beginning of residual adulticiding operations. Searches should be made by hand catching or flitting in a representative sample of inhabited houses on walls, beams, ceiling, under furniture and in animal shelters. Searches should also be made if possible in all uninhabited buildings such as schools, offices, shops, and outlying temporary shelters. Searches should be made for resting mosquitoes under culverts and bridges in the project area, and in a representative sample of wells, cistern, cesspits, septic tanks, drainage pipes and catch basins. All positive mosquito resting places outside the houses and buildings should be identified and numbered on the ground and on a map by distinctive signs and symbols. The record of resting places should show the outdoor resting place number or house number, the date, time, species and density, the type of surface (i.e. stone, cement, plaster, mud, wood, straw) and the estimated sprayable surface of the testing places in m^2. New outdoor resting places should be serially numbered and marked on the ground on the map. Particular attention should

be paid to changes in resting places occurring in sprayed houses and other sprayed structures in order to ascertain whether adults rest on surfaces which they previously avoided.

Separate maps may be used for recording: *(i)* breeding places and larviciding, *(ii)* flying places and outdoor space adulticiding, and *(iii)* resting places and residual adulticiding. Tables An3.2, An3.3 and An3.4 are examples of forms which may be used for recording breeding places, flying places and resting places, and the corresponding estimated surfaces. Such maps and records are useful in the planning stage and for the checking and evaluation of control measures. Seasonal maps will show how work is proceding.

Recording of too much information on a single map may lead to confusion. Separate maps for mosquito sources, residual spraying, larviciding and spaces spraying may be indicated. A better solution would be to prepare a single basic map with a series of transparent overlay sheets for each of the separate categories.

A3.1.6 Locating and recording human habitats

Places used by people of the community for living and working should be numbered and located on the sketch map for eventual adulticiding operations. Measurements of sprayable surfaces, and information about materials used for house construction, need to be obtained on a sample basis.

A3.1.7 Numbering of houses

A good house numbering system is one which leads from one house to the next with minimum effort. House numbers should start from 1 in each locality and run serially. Numbering may begin from the main entrance to the locality and proceed in a given direction. The public should be informed regarding the purpose of numbering and asked for their consent to ensure cooperation. It is entirely up to the individual to decide, and no pressure should be exerted to persuade the occupant to agree to be numbered.

For closely grouped houses in irregular blocks the locality should be reconnoitred with the sketch board first, and all the blocks plotted, then the houses are numbered following the most convenient system.

For scattered houses, before numbering, the streets or paths in the locality must sketched. When locating the houses, they can be numbered at the same time following a logical itinerary.

The number given to the house should be clear on the main entrance. A "house visit card" or "mosquito control card" should be posted or given to the householders at the time of the reconnaissance (see Table An3.5). The card is recommended both to facilitate supervision of all operations and also as an insurance against loss or removal of the number.

New houses built after the initial mapping may be given hyphened numbers, as annexes to the nearest house. A note on the sketch map will indicate the total number of houses. Houses destroyed, or without occupants, or where the occupants refused to have their homes sprayed, should be shown on the locality sketch map during the spraying cycle by crossing out the number and marking "D" in pencil, for the office to make corrections. A separate record form for such houses (as well as new houses) will also be completed.

A3.1.8 Measurement of sprayable surfaces of houses

The examination of a representative sample of houses for mosquito resting places will determine what surfaces are to be included in case residual adulticiding is undertaken. Based on those decisions, total sprayable surfaces for the community must be estimated on a sample basis. The total sprayable surfaces are the basis for the operational planning including the amount of insecticide required, number of spraymen and supervisors, number of sprayers, mixing and protective equipment, and transport required.

The sprayable surfaces in houses chosen for measurement are measured room by room and recorded in forms (Table An3.6). Usually the field worker is provided with a measuring tape to determine room dimensions. An alternative measuring stick made of light, string wooden rod 1.5 or 3 m long can be marked at half meter lengths, and the end half meter portion being divided at 10 cm points by red and white paint markings. The work is most conveniently done in teams of two people, one doing the measurement and the other recording in the form and in a notebook. Sprayable surfaces are composed of geometric shapes like rectangles, triangles, sectors of circles, cones and spherical; surfaces, all of which can be accurately calculated from well-known formulae.

The usual procedure in measuring the sprayable surface of a room is first to measure the main room dimensions and estimate the area of wall and ceiling as if there were no irregularities; second to measure open doorways, open windows, alcoves and closets and calculate their + or – areas; and third to measure sprayable furniture and estimate its area. In cases where basic room areas account for most of the sprayable surface the remaining sprayable surfaces may be estimated as a percentage of the basic room area. The sprayable area of each room is to be recorded to the nearest 0.5 cm on the form. The sprayable areas for all rooms in the house are then added up and recorded on the form and on the house mosquito control card together with the date.

In some situations special information may be obtained on the existence of high ceilings requiring the use of extension lances by spray teams.

A3.1.9 Measurement of sprayable volumes of houses

In projects where indoor space adulticiding will be undertaken and where the project will provide insecticide (rather than the householders themselves), an estimate of "sprayable volumes" will be required. In case all rooms are to be sprayed, the house volume can be estimated by pacing its width and depth and estimating its height by eye, from which

House volume = width x length x height

If only the living and sleeping rooms are to be sprayed, they must be measured separately. It should be noted that in projects where rooms have already been measured for residual adulticiding of total walls and ceilings, the room volumes in m^3 closely approximate two-thirds of the sprayable surfaces in m^2. For example, in a room 4 x 3 and 2.5 high, the walls and ceiling have a surface of 47 m^2 and the room volume is 30 m^3 (2 / 3) 47 = 31.3.

A3.1.10 Population census

This information may already be available. If not, it should be recorded when the houses are being registered. Other information may be recorded also, in a separate form regarding the use of bednets, repellents, space spray, habits of sleeping outdoors, and the frequency of painting the house.

A3.1.11 Establishing the project area

The ultimate objective of GR is to determine the area to be included in a mosquito vector control programme and to provide a basis for developing an annual plan of action which gives details of which mosquito control methods are to be used where, when and by whom.

The project area may be subject to arbitrary limitations if it is planned in connection with a water development project, a military cantonment, a specific community or group of communities, a specific urban area, or agricultural areas or plantations. It is relatively easy to establish boundaries for such projects partly on the basis of self-interest. These entities may be as concerned with pest mosquitoes as they are with the threat of mosquito-borne disease.

However, in most countries the control of vector-borne disease is the concern of the government and is carried out by local health services with technical guidance in some cases, assistance from the state, provincial or central government. Even in those countries with long established vertical or semi-autonomous malaria or vector control programmes, there is a strong movement towards integrating with general health services or at least developing a close coordination of filed and laboratory work. There is also a tendency to share the services of volunteer workers at the village level particularly in the development of primary health care through community participation.

Since the ministry of health and local health services are concerned with the health of all the people within their jurisdiction, the establishment of boundaries for mosquito control activities must be based on the population at risk from the mosquito-borne disease of concern. In the case of malaria, the malaria control programmes have had considerable experience in malaria control. Any areas where indigenous transmission of malaria can be demonstrated are included. Under a malaria control strategy, this is feasible. Due to budget limitation the authorities must first make a decision as to what level of malaria incidence is acceptable. Epidemiological and entomological surveys are then used to determine the nature and extent of the risk to the various segments of the population in all of the areas of concern. The results of geographical reconnaissance are then used in developing the long-range plan of operations and the annual plan of action which should be revised every year based on updating of the geographical reconnaissance.

A3.1.12 Revising the project action plan

The technical, financial and action plans of the project are usually based upon preliminary estimation of the extent of mosquito infestation of the community and upon general information, hopefully confirmed by entomological and epidemiological surveys in the project area. When the geographical reconnaissance has been completed and maps are studied in collaboration with central vector control staff, some changes may appear to be desirable in the original plan. If these changes involve the need for additional resources, they will need to be approved by the

community and the approval of cooperating governmental services. The project officer should submit the completed geographical reconnaissance together with recommendations for action plan changes to the community, with a copy to the central vector control service. Once the recommended changes have been approved, the project must take action in rapidly obtaining needed supplies, and arranging for the necessary training of operators and volunteers.

GR does not cease as control measures commence, because maps and data need to be constantly revised. New constructions, destroyed houses, additions or alterations, changes in location of summer huts or tents of nomads, new water sources, new roads, new governmental or other facilities all need to be recorded both in the reporting forms and maps. Also, there may be some change observed in the habits of mosquitoes, in insecticide resistance, etc., and therefore the periodic revision of the project action plan and updating of the operational maps is essential in order to achieve effective chemical control.

Table An3.2 GR record of mosquito breeding places

Date	Hour	Breeding place number	Description (type of water surface)	Size m^2 or ha	Positive for species	Density

Table An3.3 GR record of mosquito flying places

Date	Hour	Flying place number	Description	Size m^2 or ha	Positive for species	Density

Table An3.4 GR record of mosquito resting places

Date	Hour	House or outdoor resting place number	Description	Positive for species	Density

Table An3.5 House mosquito control card

Commune .... House number.............................

Sprayable surface............................. m^2

Date	Activity	Signature

Table An3.6 Record of house number and house measurements

House No.	Number of occupants	Name householder	Areas of room (m^2)										Total sprayable
			1	2	3	4	5	6	7	8	9	10	

ANNEX 4

Protocol for determining the susceptibility of adult mosquitoes to insecticides: WHO bioassay test

A4.1 Introduction

Insecticides play an important role in vector borne disease control. In malaria for example, they are used for the treatment of mosquito nets and other materials, and for indoor residual spraying (IRS). Four classes of insecticides are recommended by the World Health Organization for this use in public health programmes: pyrethroids, organochlorines, organophosphates and carbamates.

Continuous and long-term vector control programmes based on repeated application of insecticides will select resistance gene(s) in the population of malaria vectors. An essential criterion in the choice of an insecticide is the susceptibility of the malaria vectors in the area to insecticides. The purpose of the susceptibility test is to detect the presence of resistance in the population as early as possible for a pre-emptive action by national control programmes to manage insecticide resistance.

A4.2 Discriminating concentrations of insecticides against adult mosquitoes

Discriminating concentrations or diagnostic concentrations (or dosages) of insecticides are routinely used to detect and monitor insecticide resistance in mosquitoes. These concentrations were established under standardised laboratory conditions for all insecticides currently used in malaria control programmes, using known "susceptible" strains or populations of a range of mosquito vector species. The anopheline species used were *Anopheles aconites, An. albimanus, An. arabiensis, An. dirus, An. freeborni, An. gambiae s.s, An. maculatus, An. minimus* and *An. stephensi.*[1] Papers already impregnated with insecticide at the appropriate discriminating concentrations are provided as part of the test kits supplied by WHO. In order to be certain that all susceptible mosquitoes are killed the discriminating concentration for a given insecticide and a mosquito species was defined in one of two different ways, that is, as either:

▸ twice the lowest concentration that gave systematically 100% mortality after 60 minutes exposure and a holding period of 24 hours on a susceptible strain or a susceptible population; or

▸ twice the $LC_{99.9}$ determined by the baseline susceptibility of a susceptible strain or a susceptible population. The baseline susceptibility is obtained by exposing batches of mosquitoes to different concentrations for 60 minutes and a holding period of 24 hours, and by adjusting the results to a log-probit model.

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[1] Test procedures for insecticide resistance monitoring in malaria vector mosquitoes. Geneva, World Health Organization, 2013. Available at http://www.who.int/malaria/publications/en/2.

Table An4.1 summarizes discriminating concentrations of insecticides commonly used in vector control and/or for research purposes (e.g. dieldrin) for adult mosquitoes.[1]

Table An4.1 Discriminating concentrations of insecticides for adult anopheline mosquitoes[2]

Insecticide Classes	Insecticides	Discriminating concentrations (1-hour exposure period)
Organochlorines	DDT	4%
	Dieldrin[a]	0.4% 4%
Organophosphates	Malathion	5%
	Fenitrothion[b]	1%
	Primiphos-methyl[c,d]	0.25%
Carbamates	Propoxur	0.1%
	Bendiocarb	0.1%
	Carbosulfan[c,e]	0.4%
Pyrethroids	Permethrin	0.75%
	Deltamethrin	0.05%
	Lambdacyhalothrin	0.05%
	Cyfluthrin	0.15%
	Etofenprox	0.5%
	Alpha-cypermethrin	0.05%
	Bifentrin	0.2
Pyrroles	Chlorfenapyr[c,f]	5%
Phenyl pyrazoles	Fipronil[c,g]	2%

[a] Exposure to dieldrin at 0.4% kills susceptible (ss) individuals but not resistant heterozygotes (Rs), while exposures to dieldrin at 4% kills heterozygotes (Rs) but not homozygous resistant (RR) individuals.
[b] Two-hour exposure.
[c] Tentative; to be confirmed by WHOPES.
[d] Based on unpublished industry data, 2006.
[e] Based on data published by N'Guessan et al. (2003)[3] and Ahoua Alou et al. (2010).[4]
[f] Based on data published by Raghavendra et al. (2011).[5]
[g] Based on data published by Kolaczinski & Curtis (2001)[6] and Brooke et al. (2000).[7]

A4.3   Equipment and supplies

A4.3.1   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:

[1] WHO 1998. Discriminating concentrations for adult mosquitoes (one hour exposure – WHO/CDS/CPC/MAL/98.12 and WHO Technical Report Series 818) – http://www.who.int/whopes/resistance/en/Diagnostic_concentrations

[2] *Test procedures for insecticide resistance monitoring in malaria vector mosquitoes.* Geneva, World Health Organization. http://www.who.int/malaria/publications/atoz/9789241505154/en/index.html

[3] N'Guessan R et al. Resistance to carbosulfan in *Anopheles gambiae* from Ivory Coast, based on reduced sensitivity of acetylcholinesterase, *Medical and Veterinary Entomology,* 2003, 17(1):19–25.

[4] Ahoua Alou LP et al. Distribution of ace-1R and resistance to carbamates and organophosphates in *Anopheles gambiae* s.s. populations from Côte d'Ivoire. *Malaria Journal,* 2010, 9:167.

[5] Raghavendra K et al. Chlorfenapyr: a new insecticide with novel mode of action can control pyrethroid resistant malaria vectors. *Malaria Journal,* 2011, 10(1):16.

[6] Kolaczinski J and Curtis C. Laboratory evaluation of fipronil, a phenylpyrazole insecticide, against adult Anopheles (Diptera: Culicidae) and investigation of its possible cross-resistance with dieldrin in *Anopheles stephensi. Pest Management Science,* 2001, 57(1):41–45.

[7] Brooke BD, Hunt RH, Coetzee M. Resistance to dieldrin + fipronil assorts with chromosome inversion 2La in the malaria vector *Anopheles gambiae. Medical and Veterinary Entomology,* 2000, 14(2):190–194.

▶ Four marked with a **red dot** for use as exposure tubes, i.e. for exposing mosquitoes to the insecticide-impregnated papers;

▶ Two marked with a **green dot** for use as control tubes, for exposure of mosquitoes to the oil-treated control papers, i.e. without insecticides;

▶ Six marked with a **green dot** for use as holding tubes, for pre-test sorting and post-exposure observation

▶ 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) for lining the holding tubes;

▶ 12 spring wire clips, 6 steel and 6 copper, to hold the papers in position against the walls of the tubes; the 6 steel clips are to be used with the green-dotted holding tubes and 6 copper clips are to be used with the 4 red dotted exposure and the two yello-dotted control tubes;

▶ 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;

▶ 3 sheets of log-probit papers for plotting the regression line for calculation of LT_{50} using variable times with constant concentration;

▶ A counter, to count the mosquitoes while releasing in the tubes;

▶ The insecticide-impregnated papers with the discriminating concentration of a give insecticide are packed in plastic boxes; each box contains 8 papers (see Fig An4.1)

Figure An4.1   Equipment for susceptibility testing with a box of insecticide impregnated paper

A4.3.2   Insecticide-impregnated papers

Test kits and impregnated papers are made at the University Sains Malaysia (Penang, Malaysia) on behalf of WHO. The procedures and conditions for procuring these items are specified in the WHO document on *Supplies for monitoring insecticide resistance in disease vectors. Procedures and conditions.*[1]

[1.] WHO, 2001. *Supplies for monitoring insecticide resistance in disease vectors. Procedures and conditions.* WHO/CDS/CPE/PVC/2001.2

For routine monitoring of insecticide susceptibility, only papers impregnated at the discriminating concentrations are used. The impregnated papers for each insecticide at the discriminating concentration 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. Lower temperature may cause crystallization of the insecticide. Prolonged storage at higher temperature should be avoided. Papers should not be used after the expiry dates shown on the box. The expiry date is valid only if the packages are kept sealed at all the times.

For establishing the baseline susceptibility of a mosquito species, impregnated papers at serial concentrations can be obtained from the University Sains Malaysia upon request.

A4.4   Test procedure and conditions

A4.4.1   General conditions for mosquitoes to be tested

The age, physiological status and gender of mosquitoes as well as the temperature at which insecticide exposure occurs can influence the results of the susceptibility tests. Therefore the following should be considered:

▸ Ideally non-blood fed adult females of known age (3–5 days post emergence) should be tested. Female mosquitoes of known age can be obtained:

  ▸ through larval collections from a number of breeding sites in order to avoid sampling individuals from single egg batches. Types of breeding sites should be specified. Larvae from the same place and the same type of breeding sites can be pooled before testing. All types of breeding sites should be sampled.

  ▸ from the F1 progeny from wild caught females. Since the genotypic variability of the progeny of one female is limited, the number of wild caught females has to be representative of the population (at least 30 egg batches, more if there is a mixture of species).

▸ 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 carefully noted.

▸ The use of males is not recommended for resistance monitoring since they are usually smaller, more fragile and therefore more susceptible than females.

▸ In situations where different mosquito species coexist, it is recommended that samples collected from the field be identified to the species level wherever possible.

A4.4.2   General conditions for tests procedures

▸ Around 100 adult mosquitoes should be tested for any insecticide at the diagnostic concentration, with 4–5 replicates of 20–25 mosquitoes per test tube. When it is not possible to collect this number of mosquitoes on a single occasion/day, multiple tests over a few days should be undertaken to achieve this number. In addition to the test mosquitoes, a minimum of two control (50 mosquitoes) should be included in the test.

▸ 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.

▶ For mosquito species that are not routinely monitored and/or basic data are not available, it is necessary to establish the baseline susceptibility.

▶ Since the efficacy of impregnated papers decline with the number of uses and the number of mosquitoes tested, the papers should not be used more than 6 times corresponding to the exposure of 150 mosquitoes.

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.

A4.4.3   Test procedure

1.   Into each of the holding tubes, insert a piece of clean white paper (12x15 cm). The paper should be rolled into a cylinder to line the wall and fastened in position with a spring-wire clip (silver). Attach the slides to the tubes.

2.   Aspirate gently adult females from the mosquito cage. Non-blood fed adult females, 24–48 hours post emergence, should be used (see Fig. An4.2). In each aspiration not more than 5 mosquitoes should be collected. Gently release the mosquitoes into the holding tube through the filling-hole (see Fig. An4.3). Insert a cotton pad into the filling-hole. Fill one
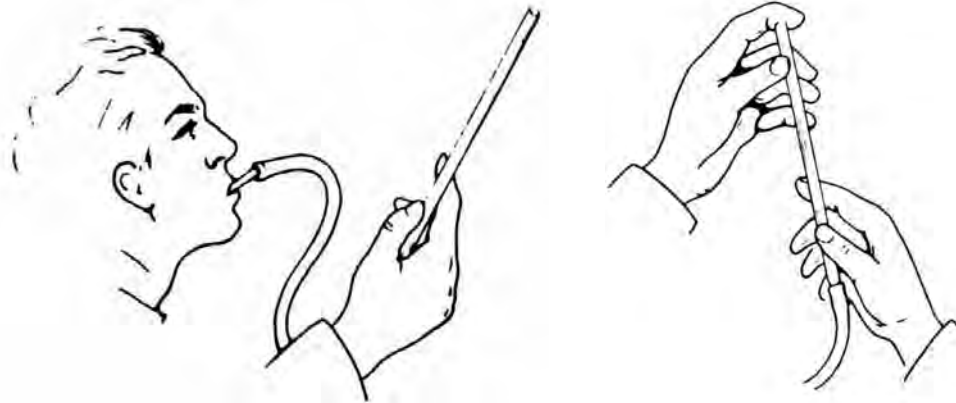

Figure An4.2   Aspirating mosquitoes from the cage
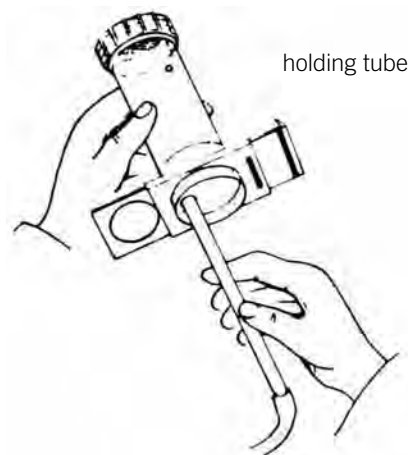
Figure An4.3   Releasing of mosquito to the holding tube

holding tube with 15–25 mosquitoes. After filling, remove the cotton pad and then close the slide unit. At least 4–5 replicates of 20–25 mosquitoes per test tube, representing at least 100 female mosquitoes, should be prepared.

3. Set the holding tubes upright for one hour. At the end of this time remove all the damaged insects.

4. Into each of the exposure tubes introduce a sheet of impregnated paper, rolled into a cylinder to line the wall and fastened into position with an appropriate copper spring-wire clip.

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 down into the exposure tube. Close the slide. Detach the holding tube and set it aside (see Fig. An4.4).

holding tube
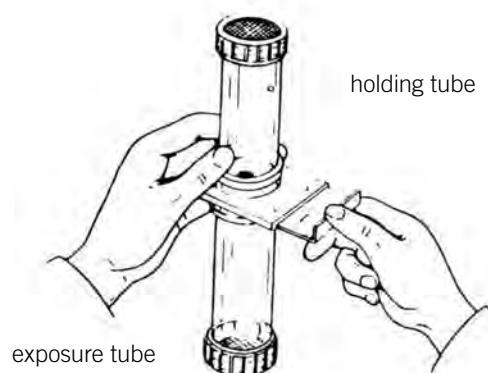
exposure tube

Figure An4.4   Introducing the mosquito into the exposure tube

6. During exposure period (60 minutes) for all insecticides, all the exposure tubes should be held at vertical position (see Fig. An4.5). To limit the contact of mosquitoes, which are attracted by light, with the mesh screen at top of the tubes, a piece of cardboard can be laid on the tubes.

Figure An4.5   Exposure tubes held at vertical position

7. At the end of the exposure period, transfer the mosquitoes to the holding tube by reversing the procedure. 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.

8. Keep all the holding tubes for 24 hours in a shaded place, preferably in an insectary. Store all the tubes under conditions of moderate, diffuse illumination and adequate humidity. All the tubes can be stored in a container and covered by a damp towel. Temperature and humidity should be recorded during the recovery period.

9. Mortality measured 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 (in the wild such mosquitoes are likely to be caught by predators and ants). The results should be recorded on the form provided.

10. For controls, oil-treated papers should be used at four replicates. The oils used for this purpose are: mineral oil for organochlorine; olive oil for organophosphate and carbamate; and silicon oil for pyrethroid insecticides.

A4.4.4 Recording and analysis of susceptibility testing data

Figure An4.6 shows a format for recording the susceptibility tests data including information on the study area, mosquito sample, collection method, physiological stages of the mosquito, test insecticide and test condition.

A4.4.5 Calculation of knock-down and mortality rates
Knock down rate

The assessment of knock-down is made within 60 minutes post-exposure. A mosquito is considered knocked down if it is unable to stand or fly in a coordinated way. The holding container may be tapped a few times before a final determination is made.

When knock-down resistance (kdr) is involved, the rate of knock down (KD) has been shown to be a sensitive indicator for early detection of resistance.

Recording the knock-down (KD) rates of mosquitoes during exposure is a simple procedure. After 10 minutes' exposure, the tube is gently handled in order to count the number of KD individuals at the bottom of the tube. An initial KD count can be made and thereafter at 10, 15, 20, 30, 40, 50 and 60 minutes (just before transfer to the observation tube). 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. In very susceptible populations, the recording of knock down should be done more frequently, every 3 minutes.

The knock-down rates according to time of exposure can be adjusted to a log time-probit model to obtain the KD time for 50% (or 95%) of mosquitoes.

Mortality rate

Usually, mortality is measured at 24 hours post-exposure. A mosquito is classified as dead if it is immobile or unable to stand or fly in a coordinated way. With some insecticides such as

Figure An4.6   Form for recording susceptibility test data in the field

a. Susceptibility testing information

Village Code ☐ ☐ ☐   Test Number ☐ ☐ ☐ ☐   Date (dd-mm-yy) ☐ ☐ ☐ ☐ ☐ ☐

Investigator name: ....   Code investigator ☐ ☐ ☐

Area information

Country: ....   Province: ....

District: ....   Commune : ....   Village: ….....................…..…………

GPS position **UTM_X** ☐ ☐ ☐.☐ ☐ ☐   **UTM_Y** ☐ ☐ ☐.☐ ☐ ☐

Sample information

Species tested: ....   Species control: ....

Sex: ....   Age (days): .... (only if known: colony & F1)

Collection method

Human Landing Indoor	☐	Resting night Indoor	☐	Resting morning Indoor	☐
Cattle Collect	☐	Human Landing Outdoor	☐	Resting night Outdoor	☐
Other: specify		Larval collection	☐	Progeny F1	☐
Colony	☐	Name of colony strain:			

Physiological stage

Non-blood fed ☐   Blood fed ☐   Semi-gravid ☐   Gravid ☐

Test insecticide information

Insecticide tested: ....   Date of expiry: ☐ ☐/ ☐ ☐ /☐ ☐

Impregnated papers prepared by: ....   Date box first open: ☐ ☐/ ☐ ☐ /☐ ☐

Concentration: ....   Number of times this paper is used: ☐ ☐

Storage conditions: Room temperature ☐   Refrigerated ☐

Test conditions

	Exposure period: Start	**after 12 hours**	**End test**
Temperature °C	☐ ☐.☐	☐ ☐.☐	☐ ☐.☐
Relative humidity (%)	☐ ☐	☐ ☐	☐ ☐

b. Test results: period of exposure (minutes)............................

No. exposed	Replicate 1		Replicate 2		Replicate 3		Replicate 4		Control 1		Control 2	
Number of knocked down (KD) mosquitoes after exposure for minutes												
	Replicate 1		Replicate 2		Replicate 3		Replicate 4		Control 1		Control 2	
	No. tested	No. KD	No. tested	No. KD	No. tested	No. KD	No. tested	No. KD	No. tested	No. KD	No. tested	No. KD
START		■		■		■		■		■		■
10 min												
15 min												
20 min												
30 min												
40 min												
50 min												
60 min												

c. Number of dead/alive mosquitoes at the End of holding period (24 hours)

	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Control
No. dead						
No. alive						

pyrethroids, mosquitoes tend to lose their legs some hours after insecticide exposure. However, adults are considered to be alive if they are able to fly regardless of the number of legs remaining.

Percentage mortality after the 24-hour recovery period should be recorded on the report form. The mortality of test sample is calculated by summing the number of dead mosquitoes across all four exposure replicates and expressing this as a percentage of the total number of exposed mosquitoes:

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

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 mortality in the control is below 5%, it can be ignored and no correction is necessary. When reporting mortality counts, the sample size should always be given, and preferably an estimate of the 95% confidence intervals.

Interpretation of the susceptibility test results

▶ 98–100% mortality indicates susceptibility.

▶ <98% mortality suggest 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. The confirmation of resistance may be obtained by performing additional bioassay tests with the same insecticide on the same population or on the progeny of any surviving mosquitoes (reared under insectary conditions) and/or by conducting molecular assays for known resistance mechanisms. If at least two additional tests consistently show mortality below 98%, then resistance is confirmed.

▸ If mortality is less than 90%, confirmation of the existence of resistant genes in the test population with additional bioassays may not be necessary, as long as a minimum of 100 mosquitoes of EACH species was tested. However, further investigation of the mechanisms and distribution of resistance should be undertaken.

▸ When resistance is confirmed, pre-emptive action MUST be taken to manage insecticide resistance and to ensure that the effectiveness of insecticides used for malaria vector control is preserved.

Establishing the baseline susceptibility

In establishing the baseline susceptibility for a mosquito population, for all insecticides, batches of mosquitoes are exposed to the diagnostic dose of insecticide at doubling time intervals, e.g. 2, 4, 8, 16, 32, and 64 minutes. The time intervals should be chosen so that at least one time gives 100% mortality, some give 50–90% mortalities, and at least two of the times give mortalities of 5–50%. For plotting the regression line the mortality at the exposure times should give 5–99% mortality. Table An4.3 shows an example for plotting the regression line and measuring LT_{50}.

Table An4.3 Mortality of *Anopheles sp* to DDT 4% at different time intervals

Exposure time (minutes)	Mosquitoes		% Mortality	Corrected mortality[b]
	No. tested	No. dead[a]		
4	100	5	5%	3%
8	100	30	30%	24%
16	100	52	52%	48%
32	100	78	78%	76%
64	100	99	99%	99%
Control (64)	100	8	8%	—

[a] after 24 hours post-exposure
[b] Abbott's correction

Table An4.4 shows the main parameters of the regression line of a species exposed to the discriminating concentration of an insecticide at different time intervals.

Table An4.4 Parameters of probit regression line of *Anopheles sp* exposed to DDT 4%

| A	B ± SE	LT50, 95% C.I.	LT90, 95% C.I.	χ2 (df)	P value
-3.5	2.9 ± 0.2	14	37	5.9 (3)	<0.05
		16	44		
		17	54		

A = Intercept
B ± SE = Slope ± standard error
LT_{50}, 95% C.I. = Lethal time causing 95% mortality, confidence interval
LT_{90}, 95% C.I. = Lethal time cause 90 % mortality, confidence interval
χ^2 (df) = heterogeneity about the regression line with degree of freedom

For plotting the regression line, standard log-probit paper can be used. In the X axis the interval times (4, 8, 16, 32, 64 minutes) are indicated, and in Y axis the mortality data transformed to the probit scale is plotted (50% mortality = 5 probit). From the regression line the LT_{50} can be calculated (see Fig. An4.7). The regression line can also be plotted using computer programmes such as HG3 or Excel.
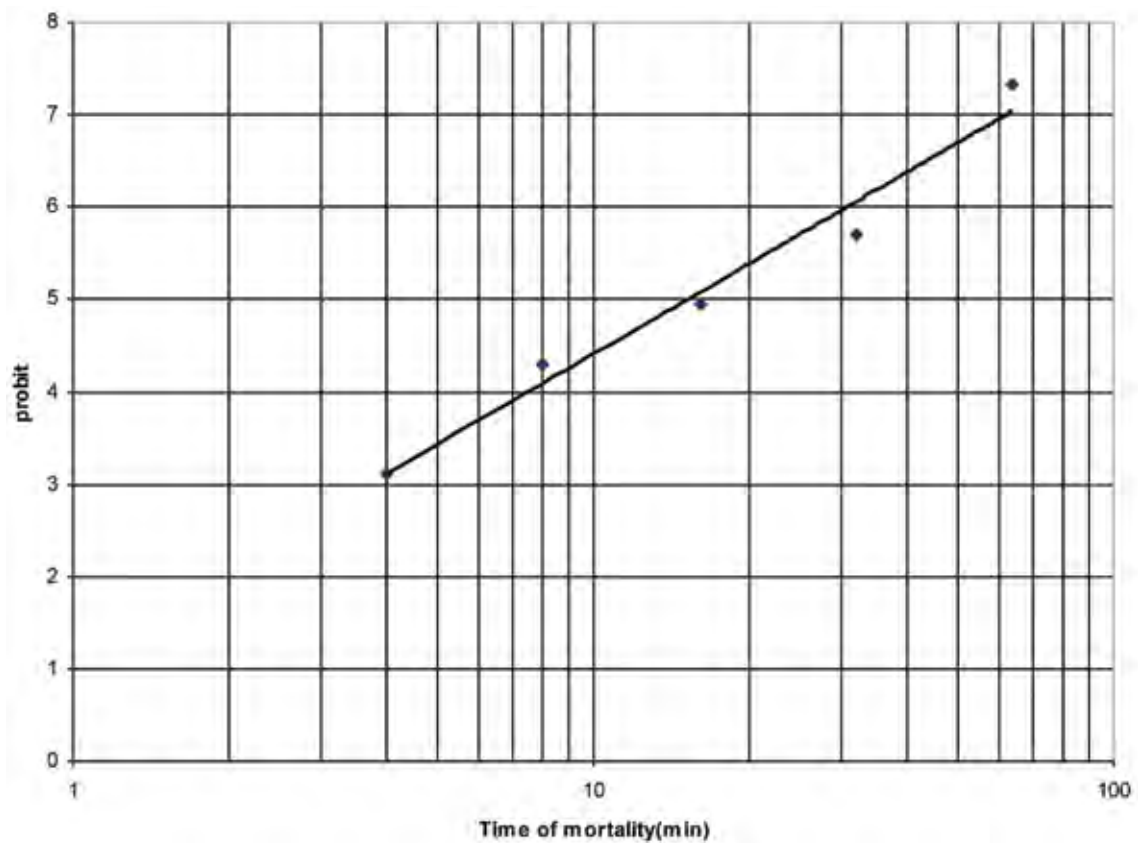
Figure An4.7   Probit regression line of *Anopheles sp* exposed to DDT 4%

ANNEX 5

Protocol for determining the susceptibility of mosquito larvae to insecticides

A5.1 Introduction

Larviciding can be a useful method for malaria control programmes, particularly in areas where breeding sites are few, fixed and findable. In sub-Sharan Africa, such criteria are often met in urban areas, where larviciding may be used to complement the other vector control interventions. The WHO recommended insecticides for larviciding are listed in Table 5.11 of Learning Unit 5 in the Guide for Participants.

The purpose of a susceptibility test is to allow control programmes to take a pre-emptive acyion to manage resistance through rotational use of insecticides thus maintaining the effectiveness of available vector control interventions/tools. There are two methods for determining the susceptibility status of mosquito larvae to insecticides. The first requires routine checks with a diagnostic (discriminating) concentration of larvicide, a method which is easy for technicians to carry out. The second requires establishing the baseline and calculating the LC_{50} from the regression line over time; this method is less straightforward and requires well-trained meticulous staff.

A5.2 Discriminating concentrations for larvicides

Table An5.1 shows the discriminating concentrations used for malaria mosquito larvae. For other larvicides shown in Table An5.1 the diagnostic dosages should be calculated for each local species.

Table An5.1 Tentative diagnostic dosages for anopheline mosquito larvae (mg/l=ppm)

	Discriminating concentrations
Malathion	3.125
Fenitrothion	0.125
Fenthion	0.05
Temephos	0.25
Chlorpyrifos	0.025

A5.3 Composition of test kit

► 24 beakers, 400 ml

► 24 beakers, 30 ml

► One 1 ml micropipette

► Sufficient micropipette tips

► 5 droppers with rubber suction tubes

▸ 5 strainers (2 wire loops, 1 piece of nylon netting (30 cm^2) and 1 tube of glue

▸ Tally counter

▸ Different solutions of larvicide (in 20ml bottles)

▸ Pesticide solvent (normally ethanol)

▸ Instruction sheet

▸ Log-probit mortality papers

▸ Water thermometer

▸ Larval tray

The composition of the test kit is shown in Fig. An5.1.

Figure An5.1   **Composition of the test kit for larval susceptibility testing**

A5.4   Test procedure

▸ Collect larvae of the same species of *Anopheles* from the field in sufficient number using a dipper or net (Fig. An5.2).

▸ Pour the larvae carefully into a container for transportation to the laboratory. Make sure that the larvae are treated gently, that they have plenty of air and do not become too hot.

▸ In the laboratory, transfer larvae into a tray.

▸ Maintain the larvae in a room at a temperature of 25 °C.

Figure An5.2   **Collecting mosquito larvae from breeding places**

▸ Identify the composition and species of the larvae.

▸ Select healthy larvae of the desired species, e.g. the main malaria vector in the region.

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

▸ Only 3rd instar or early 4th instar larvae should be chosen for the test.

▶ Release 20–25 larvae into a small beaker (30 ml) by means of a strainer or a dropper.

▶ Make up the volume of water to 25 ml.

▶ 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). Certain species such as salt-marsh or tree-hole mosquitoes should be transferred into water collected from the breeding places and which is free from insecticides and filtered to exclude organic detritus.

▶ Take 1 ml of stock solution of insecticide (for example 1000 ppm) using a micropipette.

▶ Add 1 ml of insecticide solution (1000 ppm) using a micropipette and add it to a large beaker (the total volume will be 225 ml). The final solution of insecticide to which larvae will be exposed is 4 ppm (1000/250).

▶ Wait for 15–30 minutes after preparing the test solution.

▶ 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.

▶ Add 25 ml of water containing 25 larvae into a large beaker (the total volume will be 250 ml).

▶ Leave the larvae for 24 hours.

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

▶ Record the number of dead and moribund larvae after the 24-hour exposure period for each replicate. Prior to counting the mortality, blow slowly on the surface of water and wait for 2–3 minutes. Dead larvae can not 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 discolouration, unnatural positions, tremors, lack of coordination or rigour.

▶ For each diagnostic concentration at least 4 replicates representing 100 mosquito larvae should be tested.

▶ At least two replicates of controls should be tested.

A5.4.1 Establishing baseline susceptibility

When establishing the baseline susceptibility for a mosquito larvae population, for all insecticides, batches of mosquito larvae are exposed to different concentration of insecticides using a series of doubling dosages, e.g. 2, 4, 8, 16, 32, 64 mg/l (ppm) (Fig. An5.3 and An5.4).

The interval concentrations should be chosen such that at least one time period gives 100%

Figure An5.3 Exposure of mosquito larvae to different interval concentrations of insecticides
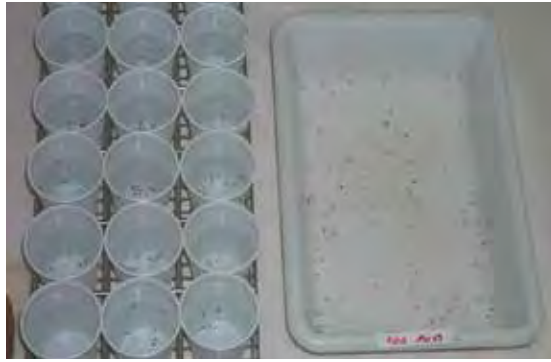
Figure An5.4 Exposure of mosquito larvae to different interval concentrations of insecticides (for some insecticides such as biopesticides and IGRs plastic cups can be used)

mortality, some give 50–90% mortalities, and at least two of the times give mortalities in the range 5–50%. For plotting the regression line the mortality in interval concentrations should give 5–99% mortality (Table An5.3).

Table An5.3 Mortality of larvae of *Anopheles sp* to a larvicide at different interval concentrations

Exposure time (minutes)	Mosquitoes larvae		% Mortality	Corrected mortality (Abbott's correction)
	No. tested[a]	No. dead[b]		
4	100	5	5%	3%
8	100	30	30%	24%
16	100	52	52%	48%
32	100	78	78%	76%
64	100	99	99%	99%
Control (64)	100	8	8%	—

[a] Number of larvae tested
[b] Number of dead larvae after 24 hours

For plotting regression line, a computer programme, other softwares such as SPSS, or probit paper can be used. Table An5.4 shows the main results of a probit analysis where larvae were exposed to different concentrations of insecticide.

Table An5.4 Probit analysis for Anopheles larvae exposed to a to different concentrations of larvicide

A	B ± SE	LT$_{50}$, 95% C.I.	LT$_{90}$, 95% C.I.	χ^2 (df)	P value
-3.5	2.9 ± 0.2	14	37	5.9 (3)	<0.05
		16	44		
		17	54		

A = Intercept
B ± SE =Slope ± standard error
LC$_{50}$, 95% C.I. = Lethal concentration cause 50% mortality, confidence interval 95%; LC$_{90}$, 95% C.I. = Lethal concentration cause 90% mortality, confidence interval 95%; χ^2 (df) = heterogeneity about the regression line with degree of freedom

For plotting the regression line standard log-probit paper can be used. Plot the interval concentrations (4, 8, 16, 32, 64 mg/l) along the X axis and mortality on the probit scale used

for the Y axis. Here 50% mortality equals probit 5. From the regression line the LC50 can be calculated (Fig. An5.5). Alternatively, the regression line can be plotted using HG3, Excel, SPSS or another statistical programme.
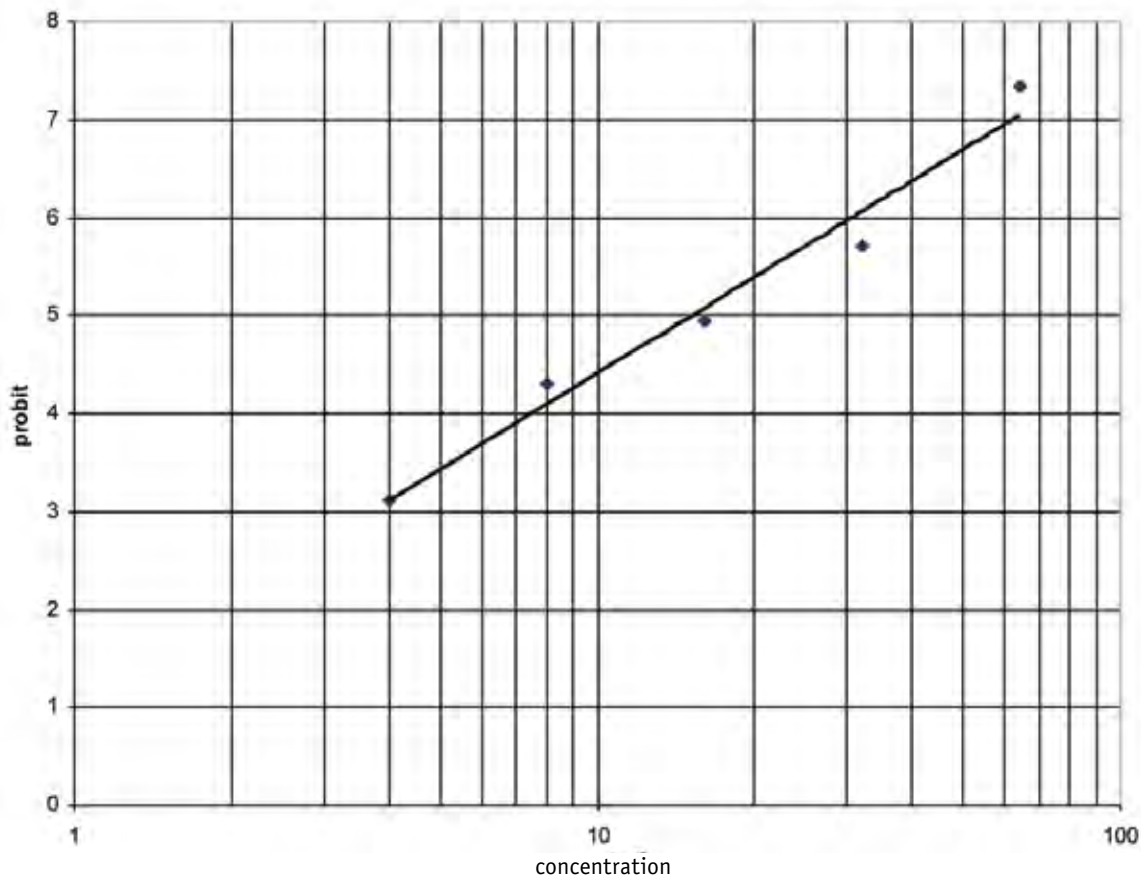

Figure An5.5   Probit regression line of *Anopheles sp* **exposed to different interval concentrations of a larvicide**

A5.4.2   Analysis of the susceptibility test results

Percentage mortality after the 24-hour recovery period should be recorded on the report form. The mortality of test sample is calculated by summing the number of dead mosquitoes across all four exposure replicates and expressing this as a percentage of the total number of exposed mosquito larvae:

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

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 mortality in the control is below 5%, it can be ignored and no correction is necessary.. When reporting mortality counts, the sample size should always be given, and preferably an estimate of the 95% confidence intervals.

A5.4.3   Interpretation of the susceptibility test result

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

▶ 98–100% mortality indicates susceptibility.

▶ <98% mortality suggest 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%, confirmation of the existence of resistant genes in the test

▶ population with additional bioassays may not be necessary, as long as a minimum of 100 mosquitoes of EACH species was tested. However, further investigation of the mechanisms and distribution of resistance should be undertaken.

▶ When resistance is confirmed, pre-emptive action MUST be taken to manage insecticide resistance and to ensure that the effectiveness of insecticides used for malaria vector control is preserved.

A5.4.4   Details of pesticide used

▶ Name of larvicide

▶ Year introduced

▶ Formulation

▶ Dosage

▶ Frequency

▶ Year terminated

▶ Total rounds

▶ Name of investigator

▶ Country

▶ Area

▶ Locality

▶ Date of test

▶ Species

▶ Location of larval collection

▶ Temperature

▶ Name of insecticide

▶ Concentration of insecticide

ANNEX 6

Test procedures for determining the residual effect of insecticides on wall surfaces

A6.1 Introduction

The effectiveness of an indoor residual spray (IRS) depends on a complex set of factors. These include the properties of the insecticide (intrinsic toxicity, mode of action, stability and volatility), and its effect on the vector. The effect also depends on the nature of the surfaces on which the insecticide is applied, the type of house construction, the number of domestic animals and the types of animal shelters, the local ecology, as well as the behaviour of the vectors and the human population. The WHO recommended insecticides for indoor residual spray as are listed in Table 5.8 of Learning Unit 5 in the *Guide for Participants*.

The main objective of the bioassay is to asses the potency of an insecticide deposit against adult mosquitoes at various times after application on different surfaces and thereby detect the onset of a definite decline in the toxic effect of the deposit due to ageing, sorption (absorption and adsorption), or other factors. The test is designed to provide information that may assist in:

► comparing the residual action of different insecticides or insecticide formulations

► ascertaining whether the spraying has been carried out satisfactorily.

The method will not measure the amount of insecticide remaining on the wall. It does not measure the overall rate of mortality of the vector achieved during the campaign, which can be only assessed by other entomological measurements in the area.

It is recommended that where possible supplementary methods, such as window-trap collections and survival tests, should be used in conjunction with the bioassay. This is particularly advisable where the bioassay is used to determine the time at which an insecticidal deposit on a given surface has lost its potency, or to decide on the appropriate insecticide dosage and spacing of spraying cycles for effective vector control. The method is not suitable for measuring the susceptibility or resistance of population. This is the reason the test uses laboratory-reared mosquitoes of known age and susceptibility.

A6.2 Equipment and test procedure

The first test for the evaluation of any given insecticide should be undertaken within a few days after its application or as soon as the deposit is completely dry. Where a previously sprayed area is due for another application of insecticide and no bioassay data are available, it is advisable to do the bioassay beforehand to ascertain the potency of the deposit present. The test should be carried out on an adequate scale and at regular intervals. It is necessary to test and to evaluate separately the potency of the insecticide deposit on each main type of resting surface on a given type of surface. 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. At least 2 controls should be used for every 10 tests.

The control runs are carried out at a suitable distance from the sprayed premises (over 100 m) and on an unsprayed surface. For this purpose, the investigator may carry a stock of index cards or similar unsprayed material to be discarded after each test.

When the main objective is to determine the rate of loss of potency, there are advantages in using the same points throughout the series of tests. The points should therefore be marked carefully at the time of the first tests, taking care to avoid rubbing or in any way impairing the deposit at these points during the performance of the tests. If different points are selected for subsequent tests, more information will be gained on the overall potency of the insecticide deposit on the types of surface examined.

Following the initial bioassay, subsequent tests should be made at least at monthly intervals. For comparability of the data, it is preferable to carry out successive tests at the same time of day. A strain of known susceptibility and age should be used in the bioassay. To acieve this, wild-caught females should be allowed to lay eggs and emerging F1 tested for resistance. If confirmed susceptible, they should be used to maintain a laboratory/insectary colony. This is the colony of mosquitoes to be used for wall surface bioassay.

A6.3   Composition of test kits

▸ 24 conical chambers of transparent plastic, 8.5 cm in diameter at the base and 5.5 cm high

▸ 10 hard glass aspirator tubes, 1 cm in outside diameter (with one end bent so as to facilitate removal of mosquitoes from the exposure chamber) together with 60 cm flexible rubber or plastic tubing. These tubes are suitable for handling mosquitoes of ordinary size. For very large species, tubes with an inside diameter of 12 mm should be used. These tubes can be made of glass or transparent plastic.

▸ 10 straight glass aspirators. The exposure chambers are best loaded with a straight glass or plastic tube of appropriate diameter.

▸ Rolls of thick and thin adhesive plastic sponge tape

▸ 1 box of upholstery tacks with large heads

▸ 1 box of small needles

▸ Instruction sheet

A6.2   Test procedure

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 room surface) (Fig. An6.1).

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

Figure An6.1   Exposure chambers fastened on different parts of wall surfaces

the surface. It is often advantageous to fasten a strip of plastic tape to the flange of each test chamber and leave it there permanently. Needles can be used to fix the cones on hard surfaces. Care should be taken not to slide the chamber on the surface while it is being attached or removed.

3. Release 10 female blood-fed mosquitoes with a straight loading tube into each chamber by blowing gently. Great care should be taken to ensure that the end of the tube does not touch the test surface (Fig. An6.2).

Figure An6.2 Releasing mosquitoes, using a straight aspirator, into the exposure chamber

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 left 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. Leave the chambers undisturbed for 30 minutes.

6. At the end of exposure period, the mosquitoes are collected carefully by means of the bent aspirator (Fig. An6.3).

7. Transfer the mosquitoes immediately into the container. Paper or plastic cups may be used (Fig. An6.4)

8. Room temperature and relative humidity (RH) are recorded at the beginning and end of each day's testing, and at hourly intervals during the work period.

9. Keep the recovery box tubes for 24 hours in a secluded and shaded place, where the temperature does not exceed 30 °C. It

Figure An6.3 Collection of mosquitoes using a bent aspirator at the end of the exposure period

Figure An6.4 Containers used for mosquito collections during the 24-hour recovery period

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is recommended to keep the box tubes in an insectary where possible; alternatively the humidity may be kept high using a damp towel.

10. 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.

11. After 24 hours, complete the form provided, recording the numbers of live and dead mosquitoes. Observed mortality (%) should be recorded for each individual test.

A6.4.1   Results and interpretation

After 24 hours, the dead and live mosquitoes are counted. 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 within the range 5%–20%, the average observed mortality is corrected by applying Abbott's formula:

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

Average mortality with standard deviation (SD) should be calculated for each part of the wall. Wide differences in mortality rates from one point to another may reflect either the unevenness of spraying or a differential in the rate of loss of potency, due to the particular composition of the surface, soot that may be deposited on the surface by domestic fires, the microclimate, or other local variables.

Testing should be continued until a marked decline is observed in the mortality of mosquitoes to 70%. The durability of insecticide on the sprayed surfaces can be determined. In this situation a fresh application of insecticide may be necessary.

A6.4.2   Information needed

Name of insecticide ....

Dosage of insecticide ....

Date of spraying ....

Location of spraying ....

Province .... County .... City .... Village ....

Date of test ....

Type of surface:

- ▸ ☐ Mud

- ▸ ☐ Wood

- ▸ ☐ Plaster

- ► ☐ Cement
- ► ☐ Palm leaves
- ► ☐ Thatch
- ► ☐ Others

Temperature ....

Humidity ....

Chamber number	Location of fastened chamber	No. of mosquitoes	No. of dead mosquitoes	Mortality (%)	Corrected mortality
1	Top				
2	Top				
3	Top				
4	Top				
5	Middle				
6	Middle				
7	Middle				
8	Middle				
9	Low				
10	Low				
11	Low				
12	Low				

A6.4.3 Determination of insecticide content applied per unit area

To determine the insecticide content per unit area, a 100 cm² area on a sprayed mud-wall should be marked with the aid of a 10 x 10 cm metal frame and a clean, sharp-pointed instrument. Samples of mud are collected from the inscribed 100 cm² area to a uniform depth of 1 mm with the aid of a clean chisel. A household dustpan held firmly against the wall immediately below the marked out area may be used for the collection of the samples. Similar 100 cm² samples can be cut from the thatch and other surfaces. For those surfaces from which scraping cannot be taken, Whatman No 1 filter papers may be attached to the wall prior to spraying, to sample what is sprayed. However, as operators often spray higher doses on such papers, careful supervision is needed.

The samples should be transferred to glass test tubes or to thick aluminium foil, and securely wrapped. Each sample should then be placed in a plastic bag which is firmly sealed. Each sample should be marked with the name of village and the household, date of insecticide application (if available), date of collection, location of the scraping of the wall (e.g. top, middle, lower part) and the surface area sampled. The latter information is particularly important to relate the results of chemical analysis to the surface area. Samples may be sent to a relevant reference institute for analysis.

This procedure is essential in order to check the proper application of an insecticide on the treated surface. The information can also be related to, and support the interpretation of, the bio-efficacy based on bioassay data.

ANNEX 7

Test procedures for determining the residual efficacy of insecticides on treated mosquito nets

A7.1 Introduction

Pyrethroids are the only insecticides currently recommended for the treatment of mosquito nets. This is due to the rapid knock-down effects and high insecticidal potency of pyrethroids at low dosages combined with relative safety for human contact and domestic handling. This annex includes the protocols for biossay methods to test the efficacy and stability of pyrethroids on treated nets.

A7.2 Bioassay methods

There are three methods for the determination of biological efficacy of pyrethroids on treated mosquito nets:

▸ Test using WHO holding and exposure tubes as used for adult susceptibility test

▸ Test using WHO cones

▸ Test using the netting apparatus with wire frame sphere

A7.2.1 Test using WHO holding and exposure tubes

Test procedure

▸ Into each of the holding tubes, insert a piece of clean white paper (12x15 cm). The papers should rolled into a cylinder to line the wall of each tube and fastened in position with a spring-wire clip (silver). Attach the slides to the tubes.

▸ Aspirate gently adult females of known susceptibility and age from the mosquito cage (see Fig. An7.1). Non-blood fed adult females, 24–48 hours post emerged should be used. In each aspiration not more than 5 mosquitoes should be collected. Gently release mosquitoes into each holding tube through the filling-hole (see Fig. An7.2). Insert a cotton pad into the filling hole. Fill one holding tube with 15–25 mosquitoes. After filling, remove the cotton pad and then close the slide unit. At least 4–5 replicates of 20–25

Figure An7.1   Aspirating mosquitoes from the cage

mosquitoes per test tube representing at least 100 female mosquitoes should be prepared. If it is not possible to collect this number of mosquitoes on a single occasion, multiple tests over a few days should be done to reach this total.

▶ Position the holding tubes upright for one hour. At the end of this time remove all the damaged insects.

▶ Into each of the exposure tubes introduce a sheet of paper, and then a piece of impregnated net rolled into a cylinder to line the wall and fastened into position with an appropriate copper spring-wire clip.

▶ 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 down into the exposure tube. Close the slide. Detach the holding tube and set it aside.
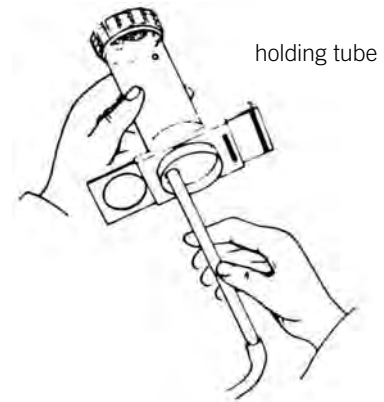
holding tube

Figure An7.2 **Releasing of mosquito to the tubes**

▶ During the exposure period (3 minutes) for all insecticides, all the exposure tubes should be held in a vertical position.

▶ At the end of the exposure period, transfer the mosquitoes to the holding tube by reversing the above procedure. 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.

▶ Keep all the holding tubes for 24 hours in a shaded place, preferably in an insectary. Store all the tubes under condition of moderate, diffuse illumination and adequate humidity. All the tubes can be stored in a container and covered with a damp towel. Temperature and humidity should be recorded during the recovery period.

▶ Mortality is measured 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 that have lost legs or wings should be considered as a dead, since such mosquitoes can easily be caught by predators and ants. The results should be recorded on the form provided.

▶ For the controls, and treated nets should be used and the test replicated four times.

A7.2.2 Test using WHO cones
Composition of test kit

▶ Conical chambers of transparent plastic, 8.5 cm in diameter at the base and 5.5 cm high

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

▶ 1 role of self-adhesive plastic tape or one sheet of label tag

▶ Instruction sheet

▶ 3 sheets of log-probit paper for plotting regression line for calculating LT_{50}, using variable times with constant concentration

▶ Counter, to count the mosquito while releasing in the cones or calculating the knock-down.

Treatment of netting

Pieces of netting material measuring 25 x 25 cm should be folded and each put into a disposable plastic Petri dish. Separate Petri dishes are used for the treatment of each net, and later disposed of correctly. The required diluted formulation should be carefully and evenly dropped by pipette on to the net. The net should then be soaked for a few seconds using the fingers protected by plastic gloves, to ensure that all of the insecticide solution is absorbed. Each net sample should be left in the same dish to dry. Net samples should be treated the day before the bioassay, and not more than 3 days before the test (see Fig. An7.3). Ideally the treated nets should be used within 1 to 3 days, and not later than 1 week, after treatment.

Figure An7.3   Treatment of netting

Bioassay

Standard WHO bioassays use standard susceptible 1–3 day old, non-blood fed *Anopheles* females exposed to netting under WHO cones for 3 minutes. The bioassay is used, (i) 1–3 days after treatment of the netting materials; (ii) 24 hours after each net washing; and (iii) at different intervals of time after dipping the insecticide treated nets. Four cones should be gently fitted on the net. Five female mosquitoes are introduced in each cone with 8 replicates per net sample (i.e. 40 mosquitoes tested). The time interval between each "4 cone" set should be as brief as possible (see Fig. An7.4. Mosquitoes from the first 4 cones tested are grouped in one plastic cup (total of 20 mosquitoes). The same procedure is followed for the second series.

The knock-down (KD) is recorded at regular intervals during the 20–30 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. Sucrose will be provided to each cup, added on a cotton plug. In addition to KD rate at 60 minutes post-exposure, mortality is recorded after 24 hours (see Fig. An7.5).

Figure An7.4   Bioassay test in conical

Figure An7.5   Introducing the mosquito into a cup for knock-down time and recovery period

A7.2.3   Test using netting apparatus with wire frame sphere

Composition of test kit

▶ Wire framed apparatus with 15 cm diameter (see Fig. An7.6)

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

▶ 1 role of self-adhesive plastic tape or one sheet of labels

▶ Instruction sheet

▶ 3 sheets of log-probit paper for plotting the regression line for calculating LT_{50}, using variable times with constant concentration

▶ A counter, to count the mosquitoes while releasing in the wire frame or calculating the knock-down (see Fig. An7.7).

Figure An7.6   Netting apparatus with wire frame

Figure An7.7   Composition of test kit using netting apparatus with wire frame

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Test procedure

All the procedures will be carried out as described above for the conical test.

In summary: for mortality 5 mosquitoes are introduced into each chamber for a 3-minute exposure period (see Fig. An7.8). Mortality is counted after a 24-hour recovery period. For knock-down 11 mosquitoes are introduced into each chamber and the time of knock-down for the 6th mosquito will be recorded. This time is considered to be the median knock-down time. As each mosquito is knocked down, it is sucked into an aspirator to avoid confusion with mosquitoes which recover and are knocked down subsequently.

This method has been found to give a sensitive indication of the effect of washing and re-treating nets, whereas a standard 3 minute exposure of a susceptible strain/population to an alpha-cyano pyrethroid tends to give 100% mortality in all tests. In this method the mortality and knock down assessment can be undertaken in parallel using the netting apparatus. For any test, adequate replicates are needed to examine the variation in bio-efficacy, on different

Figure An7.8   Introducing mosquito into netting apparatus

parts of the same net and between nets. Ideally about 50 female mosquitoes should be tested by adequate replications.

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.

Interpretation of the test results

Percentage mortality should be recorded on the report form after the 24-hour recovery period. If the control mortality is in the range 5–20%, the percentage mortality should be corrected by applying Abbott's formula as follows:

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

If control mortalities exceed 20%, the results should be recorded and test repeated. For calculating KD50, the probit analysis should be used.