Malaria entomology and vector control

GUIDE FOR TUTORS
The revision of this document was made possible through the Russian Federation grant for malaria capacity development in Africa.
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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.

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## Abbreviations

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

This module was produced by the WHO Global Malaria Programme (GMP), with participation of current and former staff from WHO Headquarters and Regional Offices. WHO also gratefully acknowledges the following experts who contributed to the development of this document:

- T. A. Abeku and P. Herath originally prepared the module with technical inputs from M. Aregawi, E. Renganathan and MC. Thuriaux. Y. Ye-ebiyo contributed to the development of the Learning Unit on malaria stratification. M. Zaim provided inputs and background (unpublished) WHO documents on judicious use of insecticides and R. H. Zimmerman contributed to the finalization of this original version of the module.

- H. Vatandoost who spearheaded the updating of the current version of the module and S. Lindsay who reviewed it as an independent expert. Also Y. Rassi, M.A. Oshaghi, M.R. Abai from Tehran University of Medical Sciences for their input in the module.

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The revision process was coordinated by M. Warsame; technical editing of the module was by L.J. Martinez.

The revision of the module was made possible through the Russian Federation grant for malaria capacity development in Africa.
Development of the module

The content of the module is based on the 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 development 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 *Guide for Tutors* is designed primarily to help those responsible for the training on entomology and vector control of health personnel involved in the planning, implementing and evaluation of malaria vector control activities. The *Guide for Tutors* is arranged in a series of Learning Units organized by topics and learning objectives. The sections correspond to the Learning Units in the *Guide for Participants* and are set out in the sequence in which they should be taught. For the tutor, it is important to clarify and elaborate on the technical content of the *Guide for Participants* by using examples, giving demonstrations and responding to questions; the tutor should be adequately prepared to do this. Short presentations are needed and the guide indicates when these should be presented. After completion of the training course the participants should each receive a copy of the *Guide for Tutors* for future reference.

Responsibility for running the course

The tutor is responsible for organizing and running the course. The job will be simplified, and teaching will be more effective, if the tutor is assisted by colleagues who act as facilitators throughout the course. The facilitators should have knowledge and experience in the subject. The class can be divided into small groups of 4–8 participants, with one facilitator allocated to each group. This allows greater interaction between the participants and the facilitators resulting in better learning and understanding. The facilitator’s role is to explain the questions asked in the small group exercises, as required, and facilitate the discussions to keep them focused.

As the overall manager of the this training module, the tutor will be responsible for designing the timetable, explaining the learning tasks to the participants and facilitators, and providing what help they may need. The facilitators are not necessarily trained as teachers; their task is to explain or demonstrate a particular activity and to watch the performance of the participants. The facilitators should also be ready to admit to participants when they are unable to answer a question and refer it to the tutor. They should be reassured that no one person can be expected to know everything about every subject.

Many problems can be avoided by giving the facilitators plenty of time to read the *Guide for Participants* and *Guide for Tutors* and to discuss any part that may need clarification. It would be useful for the tutor and the facilitators to go through the module together; the tutor could then test their knowledge by asking appropriate questions.

Why a *Guide for Participants* is provided

Providing participants with a full set of notes ensures that:

- All participants have exactly the same basic materials and guidelines on how to proceed with exercises;
- Tutor and facilitators can refer to any part of the *Guide for Participants* knowing that all participants can find the right page quickly;
Participants can spend more time reading the notes, and therefore have a greater opportunity for thinking, discussion and formulation of ideas; there is no possibility of participants making errors in note-taking; after the course, each participant can keep a copy of the Guide for Participants and the Guide for Tutors as a helpful reference in daily work and perhaps also to use in teaching others.

Running the training course

The tutor and the facilitators

The tutor should have extensive experience in entomology and vector control and be able to help the participants to solve a wide range of problems. Facilitators who work with the tutor will collaborate with participants to achieve the objectives outlined above. Facilitators will lead discussions and provide general help to individuals and to small groups.

Presentations

Formal presentations (e.g. lectures) will usually be kept to a minimum and each session will be as short as possible. Most of the information provided in such sessions is already contained in this guide and the participants will not need to take extensive notes. A lecture will usually be combined with a demonstration and practical exercises.

Demonstrations

Demonstrations will be used to illustrate equipment and techniques relevant for malaria entomology and vector control: procedures for identification of malaria vectors, sampling of malaria vectors, monitoring insecticide resistance, and demonstration of vector control interventions.

Practical sessions

The course will include as many practical sessions as feasible in order to provide as much practical experience as possible in all aspects of malaria entomology. These will include both laboratory practical sessions on identification of malaria vectors, sampling of malaria vectors, monitoring insecticide resistance as well as a field trip to practice mosquito collection techniques and other relevant practical activities.

Small group work

The small group discussions are considered to form a particularly valuable component of the course. The participants are encouraged to take full advantage of these sessions and to contribute actively in discussions. At each group work session there should be a change of moderator and reporter to ensure that every participant gains experience in these roles and that the tasks are shared equitably. These sessions provide opportunities for the participants to give their individual opinions, to develop ideas and to learn from one another.

Training facilities

The training facility should provide: (i) a classroom large enough to accommodate the entire class including the tutor and facilitators, (ii) an entomological laboratory equipped with
microscopes, and (iii) an insectary where local vector species are reared. Some vector control equipment, including compression sprayers, will be needed for demonstration.

For lecture sessions and demonstrations, some of the following equipment will ideally be available: a chalkboard or whiteboard, a screen (or plain white wall), one or more flipcharts, a TV set (preferably large screen), a video or DVD player, a computer with a projector attached.

A reference collection of mosquitoes should be available, consisting of preserved specimens of adult anopheline and culicine mosquitoes, larvae and pupae, and including all vector species that occur in the country or area.

Transport for up to 35 people will be required for a fieldwork. Details of other equipment needed for laboratory and fieldwork are given in later sections of this guide.

Due to the short time allocated to this course, it will be necessary to plan ahead and organize activities as efficiently as possible. Therefore, it is important that every evening (including the evening before the start of the training course) the tutor should:

- explain the plan for the next day’s activities to the facilitators and discuss how to carry out the activities efficiently;
- prepare equipment and materials required for the next day of the training;
- assign responsibilities to the facilitators regarding each task to be accomplished the next day.

**Principles of the training programme**

The training programme in this Guide for Tutors is based on the following principles:

- The purpose of the training is the acquisition of essential knowledge and the development of basic skills in malaria entomology.
- Effective learning is encouraged if participants understand the purposes of training and feel that the goals are important in their own malaria control programmes.
- Effective learning is encouraged if participants are actively involved in learning by themselves and from others, as well as from information provided by the tutor. The number of lectures should therefore be kept to a minimum, and participants encouraged to find information for themselves.
- Effective learning is encouraged if participants can feel that they are making progress. It is therefore important for the tutor to recognize any difficulties that participants may encounter and help to overcome them.

The learning and teaching methods to be used include:

- Reading by participants: they should be asked to read the relevant units for the next day before coming to class.
- Presentation by the tutor of key points in each unit using PowerPoint projection or overheads.
- Demonstration by tutor and facilitators of equipment and techniques.
- Field trip to a rural locality to practise mosquito collection techniques and other relevant practical activities.
Laboratory practical sessions.

Group discussions followed by plenary discussions.

Use of the Guide for Tutors

Participants will follow the group training activities using the Guide for Participants plus other materials provided by the tutor. A copy of the Guide for Tutors will be given to each participant at the end of the training. The way in which the tutor and facilitators should make the best use of the guides will become apparent in working through the training module.

The two guides may be used together for small group training when qualified facilitators are not available. In this case the tutor must, to the extent possible, replace the facilitators. The guides may also be used in combination by individuals for study and reference.

Evaluation

Judging whether or not the course was successful involves answering the following questions:

- How well did the participants learn?
- How did the participants view the training?

Evaluation of the participant

Whether this module is used for group training or individual learning, assessment of progress made by the trainee in gaining skills and competence in the subject matter is essential. This can be accomplished by means of a pre-test and a post-test using a multiple-choice questionnaire (MCQ). The pre-test will be given before the trainee reads the Guide for Participants and to be carried out alone. The post-test will be administered after all the Learning Units have been completed. Since the answers to the questions and to the exercises are included in this Guide for Tutors, it is essential that participants do not have access to it until after the training activity has been completed. During the pre-and post-test evaluations, participants must be seated apart from one another and work alone.

The results of the pre-test can be used in two ways. The tutor may use the test to ascertain the general level of knowledge on the subject in the group, and have an indication of common weak areas that need emphasis or re-emphasis. It could also be used to identify individuals who might be used as facilitators for specific subject areas. The other major use for the pre-test is as an individual base-line comparator for measuring the gain in knowledge, skills and competence at the end of the training as revealed by the post-test.

If the test results are to be valid, the questions in the pre-tests and post-tests should be of the same degree of difficulty and both tests should be given under the same conditions and the same length of time. The only way to ensure that the questions in the post-test are of equal difficulty to those in the pre-test is to give the same questions in a different order, and in the case of MCQ questions, with the answers also in a different order. It is therefore essential that the pre-test papers be collected and retained (not handed back to the participants). The participants do not need to know the results of the pre-test until the end of the training when it is used to assess progress.
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, and the quality of the teaching, competence of the tutors and facilitators will be assessed by the participants through a questionnaire, and at a plenary feedback and discussion session after the completed questionnaires have been analysed. This evaluation will take place at the end of the training period in order to provide as much feedback from the participants as possible. 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.

**Certificate**

The attendance and performance of each participant should be noted during the course and the record retained for future reference. Participants should receive a certificate of successful completion of the training course.

*Note: it is important to stress to the participants that they must take time to read each Learning Unit carefully before attending the class in which it will be considered. The time allotted for the course is based on the assumption that the corresponding unit in the Guide for Participants has been studied in advance.*
### Suggested timetable

#### Short version for programme managers

<table>
<thead>
<tr>
<th>TIME</th>
<th>DAY 1</th>
<th>DAY 2</th>
<th>DAY 3</th>
<th>DAY 4</th>
<th>DAY 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>08:00– 10:00</td>
<td>Pre-test</td>
<td>LEARNING UNIT 4</td>
<td>LEARNING UNIT 5 (cont'ed)</td>
<td>LEARNING UNIT 6</td>
<td>LEARNING UNIT 8 (cont'ed)</td>
</tr>
<tr>
<td>10:00– 10:30</td>
<td>BREAK</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10:30– 12:30</td>
<td>LEARNING UNIT 1 (cont'ed)</td>
<td>LEARNING UNIT 4 (cont'ed)</td>
<td>LEARNING UNIT 5 (cont'ed)</td>
<td>LEARNING UNIT 6 (cont'ed)</td>
<td>LEARNING UNIT 8 (cont'ed)</td>
</tr>
<tr>
<td>12:30– 14:00</td>
<td>BREAK</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14:00– 15:00</td>
<td>LEARNING UNIT 2</td>
<td>LEARNING UNIT 5</td>
<td>LEARNING UNIT 5 (cont'ed)</td>
<td>LEARNING UNIT 7</td>
<td></td>
</tr>
<tr>
<td>15:00– 15:30</td>
<td>BREAK</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15:30– 16:30</td>
<td>LEARNING UNIT 3</td>
<td>LEARNING UNIT 5 (cont'ed)</td>
<td>LEARNING UNIT 5 (cont'ed)</td>
<td>LEARNING UNIT 7 (cont'ed)</td>
<td></td>
</tr>
<tr>
<td>16:30– 17:30</td>
<td></td>
<td></td>
<td></td>
<td>LEARNING UNIT 8</td>
<td></td>
</tr>
</tbody>
</table>
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
The aim of this unit is to introduce participants to the field of malaria entomology, emphasizing the importance and purpose of entomological monitoring in malaria control programmes.

1.1 Equipment and support

An insectary with live *Anopheles* eggs, larvae, pupae and adults should be available for this learning unit (and other units).

1.2 Teaching and learning methods

Presentation

The tutor should start the session by asking participants what they know about malaria, its transmission and control, and whether they had any experience in malaria entomology or any entomological activities before coming to the training course.

To facilitate a smooth introduction to the field of entomology, the tutor will give a presentation on an overview of malaria and its transmission, control measures, and the life-cycle of the *Anopheles* vector, followed by a plenary discussion. It is useful to show a film on malaria entomology during this plenary session.

Demonstration

Demonstrations of the life-cycle of *Anopheles* mosquitoes should take place in the laboratory or insectary. The participants will visit the insectary in groups of 10, where possible. Together with the insectary supervisor:

- show live specimens of each of the stages of the *Anopheles* life-cycle
- explain how an insectary functions (for more details see Annex 1 in the *Guide for Participants*)

Answer

Exercise 1.1

Entomological monitoring generates information on the characteristics of malaria transmission in an area as well as the behaviour and habitats of the specific vector species. In the context of the roles of malaria control, entomological monitoring serves to:

- identify the vectors responsible for transmission of the malaria parasite;
- provide basic information on the behaviour and habitats of vector species for purposes of planning effective control measures;
- monitor the impact of vector control measures by determining changes in vector population density, rates of infection, susceptibility of vectors to insecticides, and residual effects of insecticides on treated surfaces.
- Investigate the factors which, in some areas, have contributed to the failure of the vector control measures.

1.3 Guidelines for assessment

During the plenary discussions, the tutor should ask the participants how and why they need to carry out entomological monitoring in malaria control programmes in their respective districts or countries, giving examples of their own entomological activities.
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 Equipment and support

Live and preserved Anopheles and Culex eggs, larvae, pupae and adults (both male and females), dissecting and compound microscopes, Anopheles wing mounted on a slide, Petri dishes, beakers, mosquito cages, a copy (for each participant) of Anopheles species identification keys relevant for the local area/region (adult females and larvae), forceps, slides, cover slips, droppers.

2.2 Teaching and learning methods

Presentation

Using PowerPoint or overhead transparencies, the tutor will present the characteristics that help distinguish Anopheles mosquitoes from other insects and other mosquito genera at different stages of their life-cycle. Using clear diagrams, describe the external structures of both the adult and larval stages of Anopheles mosquitoes that are useful for species identification. It is not useful to spend time in explaining parts that are not normally used for identifying species. Use mounted specimens for distinguishing different parts of mosquitoes at all stages. If possible tutors will demonstrate the equipment used for new methods of species identification, such as PCR. Two DVDs entitled “The mosquitoes of Europe” and “The mosquitoes of Afro-Tropical Region” prepared by IRD France are useful for training.

Demonstration

In the laboratory, the tutor should show live and preserved specimens of anopheline and culicine mosquitoes at the various stages of their life-cycle. To demonstrate the differences in the positions of live larvae, use beakers or enamel trays. Similarly, use cages to demonstrate the resting positions of adult anophelines and culicines. Use preserved or pinned specimens to show differences at all stages of the life-cycle.

Answers

Exercise 2.1

<table>
<thead>
<tr>
<th></th>
<th>Anophelines</th>
<th>Culicines</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eggs</td>
<td>Float separately and each of them has floats</td>
<td>Clump together in a raft</td>
</tr>
<tr>
<td>Larvae</td>
<td>With siphon</td>
<td>Without siphon</td>
</tr>
<tr>
<td></td>
<td>Rest parallel to and immediately below the surface</td>
<td>Hang down from the water surface using its siphon</td>
</tr>
<tr>
<td>Pupae</td>
<td>Short and wide breathing trumpet</td>
<td>Long and slender breathing trumpet</td>
</tr>
<tr>
<td>Adult (live mosquitoes)</td>
<td>Rest at an angle between 50° and 90° to the surface</td>
<td>Rest parallel to the surface</td>
</tr>
<tr>
<td>Head of the adult mosquito</td>
<td>In female, palps are as long as the proboscis</td>
<td>In female, palps are shorter than the proboscis</td>
</tr>
<tr>
<td></td>
<td>In male, palps are as long as the proboscis with club-shaped at the tip</td>
<td>In male, palps are longer than the proboscis with tapered tips</td>
</tr>
</tbody>
</table>
Exercise 2.2
Organize the participants to work in pairs. Provide each pair with a compound and a dissecting microscope, forceps, dissecting needles and two freshly pinned adult female anophelines and two larval specimens on slides. Ask each participant to identify one adult and one larva to the species (or species complex) level. The third day of the training programme is devoted to fieldwork. Participants will also have another opportunity to identify anophelines after the fieldwork.

2.3 Guidelines for assessment
At the end of the practical session, ask the participants how they identified the species of the specimens they were given.
Ask the participants to draw and label an outline diagram of anopheline adult female and anopheline larva, with emphasis on the parts that are used for species identification.
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 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 Equipment and support
Sucking tube, torch, paper cups with covering net, cotton wool, rubber bands, mosquito cages, chloroform, towels, white cotton sheets, pyrethrin solution, kerosene, small petri dishes, hand lens, forceps, a card box container (preferably a picnic box) for transporting mosquitoes, live adult females in cages, live larvae and pupae in enamel trays, dippers, pipette, vials, 70% alcohol solution, cotton wool, safety match or lighter.

3.2 Teaching and learning methods

Presentation
Explain the different mosquito collection techniques, including:
- hand collection of indoor-resting mosquitoes,
- spray-sheet collection of indoor-resting mosquitoes,
- hand-collection of outdoor-resting mosquitoes,
- direct collection from human and animal baits,
- human and animal trap-net collections,
- light trap collection,
- collection of larvae and pupae.
Also explain how to transport live adults, larvae, and pupae and the techniques used to kill and preserve specimens.

Demonstration
During the presentation, demonstrate each item of equipment used in mosquito collection.
In the laboratory, demonstrate the following:
- how to use the sucking tube to pick up mosquitoes from a cage and how to put them in paper cups,
- how to pick live larvae and pupae using a dropper and put them in a vial,
- how to kill and pack (preserve) larvae.

Answers

Exercise 3.1
The participants should work in small groups to indicate the points for the main methods of mosquito collection using the schematic representation of malaria vector’s life-cycle (Fig. 3.17 in the Guide for Participants). After this has been completed, provide the Figure 3.1 (in the Guide for Tutors) to the participants. Time should be allowed for comparison of the version produced by the groups with Figure 3.1 and for discussion any major differences. The tutor should then invite participants to ask questions and respond accordingly.
Exercise 3.2

**Practical session:** Each participant should practice in the laboratory the following:

- Collecting adult mosquitoes from a cage using a sucking tube, and putting them in paper cups with netting lids.
- Collecting live larvae and pupae using droppers and putting them in vials.
- Killing and preserving larvae and pupae.

The fieldwork will take place on an appropriate day of the course.

Exercise 3.3

**Fieldwork:** The tutor should organize a one-day field trip to allow participants to practice the mosquito collection techniques that were demonstrated in class. It is important to ensure that home-owners have provided their full and informed consent for the collections in advance (well before the students arrive at the collection site). In the field, participants will work individually and in groups to carry out the following activities:

- Using sucking tubes, torches and paper cups, each participant will search for indoor-resting mosquitoes in three houses.
- Using sucking tubes, torches and paper cups, 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.

---

**Figure 3.1** Schematic representation of malaria vector’s life-cycle and main vector collection methods

---

(1) In natural or artificial shelters
(2) By hand collection (alive) or pyrethrum spray collection (dead)
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.

- Using dippers, vials and pipettes, 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).
- Participants will transport live specimens to the laboratory.

**Exercise 3.4**

*Practical session:* Working in pairs, participants will demonstrate how to kill anophelines, determine their abdominal conditions and identify them to species level. They will also practice dissecting ovaries and salivary glands of the field collected mosquitoes. The tutor should ensure that the participants acquire these skills.

### 3.3 Guidelines for assessment

This learning unit is based on demonstrations and a practical. During the practical, evaluate the performance of the participants and correct any errors in mosquito collection, preservation, and identification.
LEARNING UNIT 4

Vector incrimination and malaria control

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

1. Describe the methods used to incriminate malaria vectors
2. Describe the methods and applications of mosquito age grading and salivary gland dissection
3. Identify the entomological indicators of malaria transmission
4. Calculate the entomological indicators associated with resting and feeding habits, human-vector contact, and entomological inoculation rates for malaria
5. Measure the components of the vectorial capacity model and understand its value for malaria transmission and control
6. Interpret the entomological measurements and their implications for malaria vector control
This Unit is based on practical laboratory activities. Participants should be able to dissect ovaries and salivary glands of female anophelines and identify the abdominal conditions of females correctly.

This Unit puts together the applications and implications of the entomological techniques learned so far in this course. Some of the materials are advanced and the tutor may prefer to demonstrate or describe the examples rather than having them carried out by the participants.

A major objective of the Unit is to illustrate how entomological information can be used to plan an effective vector control programme. It is based on a real example of an entomological study carried out in Ethiopia during the early stages of the country’s malaria eradication programme in the 1960s.

4.1 Equipment and support

Female anophelines at different abdominal stages (with a majority of unfed mosquitoes), dissecting microscope, compound microscope, dissecting needles, fine forceps, slides, dropper, distilled water and 0.65% saline solution. Flip charts for group discussions are also needed.

4.2 Teaching and learning methods

4.2.1 Vector incrimination techniques

Presentation

Give a brief presentation with slides on the abdominal conditions of adult females and how to determine whether they are infected. Mention oocyste examination, although this will not be covered in the module. Figure 4.1 shows the midgut of a mosquito infected with oocysts.

Demonstration and practical

At the start of the practical session, the tutor should introduce the participants to the dissecting microscope and make sure that they can use it correctly. The tutor should explain and demonstrate the correct methods for dissection, and demonstrate ovaries which have been dissected.

Answers

Exercise 4.1

Recognizing abdominal conditions: The participants, working in pairs, should kill anophelines and determine the abdominal conditions of several mosquitoes. They should gain practice in dissecting ovaries and salivary gland of the mosquitoes collected in the field.
Exercise 4.2

Dissection of ovaries and determination of parity: In pairs, participants should dissect ovaries of unfed and freshly-fed females, demonstrating to each other. Observe their techniques and point out errors individually. Give the participants plenty of opportunity for individual practice. Make sure that each participant carries out dissection.

The tutor should explain how ovaries are dried and how to protect them from ants and flies, and demonstrate parous and nulliparous ovaries under the microscope. All participants should be given time to practice dissections correctly and plenty of individual opportunities to classify dried ovaries as parous or nulliparous until they can make these distinctions accurately.

Exercise 4.3

Dissecting salivary glands: This requires more practice than dissecting ovaries. First demonstrate how to dissect the salivary glands and show the size and shapes of the lobes under the dissecting microscope. In pairs, the participants should dissect out salivary glands. Demonstrate the glands under the compound microscope; all participants should be able to do so with their own specimens.

Demonstrate how to flip the cover slip with adhesive in order to look for infection under microscopic examination of salivary glands, and how to stain.

Exercise 4.4

There will be a field trip to allow participants to practice the various mosquito collection techniques covered in Learning Unit 3, and the dissection techniques demonstrated in the present unit. In the field, the participants should carry out the following activities:

- Using sucking tubes, flashlights and paper cups, search for indoor-resting mosquitoes in three houses, for at least 30 minutes in total.
- Using sucking tubes, torches and paper cups, spend at least 30 minutes searching for outdoor-resting mosquitoes.
- In groups of four, carry out spray-sheet collections in one house per group.
- Using dippers, vials and pipettes, collect larvae and pupae from natural breeding sites for at least 30 minutes.
- Practise sitting with bare legs indoors and outdoors as would be done during night-landing collections. (If carried out at night, it is necessary to ensure that participants are taking appropriate malaria prophylaxis.)
- Transport live specimens to the laboratory.

Exercise 4.5

When the participants return from the field to the laboratory they should work in pairs and kill the mosquitoes they collected during the field trip. They should also identify the abdominal conditions and species and then practice dissecting ovaries and salivary glands of the field-collected mosquitoes. The tutor must ensure that each participant has gained skills in dissecting ovaries and glands of mosquitoes.
Exercise 4.6

Through practical sessions, each pair of participants should prepare filter paper for ELISA. Using each filter paper for only one species obtained from the same type of resting site (indoor or outdoor, prepare the filter paper for ELISA as follows:

- 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.
- Place a killed or anaesthetized freshly-fed female mosquito on the filter-paper. Squash the abdomen using a blunt needle or the corner of a slide or glass rod. Ensure that the squashed abdomen remains inside the area labeled 1.
- Place a second female mosquito in the area labeled with the number 2 and squash it. Continue in this way until all 16 areas of the filter paper have been used.
- Ensure that the blood-meal squashes from one specimen is not transferred to another (contamination)
- Allow the blood-meal squashes to dry, making sure that the filter papers are protected from ants and humidity.

The tutor should ensure that all participants acquire the necessary skills to prepare mosquito blood meal squashes on filter for ELISA.

4.3 Entomological indicators of transmission

The tutor should present a short introduction to this part of the learning unit and, if necessary, go through an example of the exercises. The calculations of the entomological indicators should be carefully reviewed with the participants. For example, do a “worked-out solution” for the calculation of \( f \) (proportion of blood meals taken on human and followed by indoor resting), longevity and infectivity of mosquitoes and the entomological inoculation rate (EIR).

Exercise 4.7

a. Indoor resting density

The participants should be divided into groups of 5 and asked to calculate the indoor resting density per house per day for each species for the month of October 1964 (see Table 4.2 in Unit 4 of the Guide for Participants). They can be asked to calculate for other months and compare their results by month.

Indoor resting density can be calculated by dividing the total number of females of a particular species by the total number of houses inspected.

Solution: density/house/day:  
\[ \text{gambiae s.l.} = \frac{1765}{18} = 98.06; \]
\[ \text{pharoensis} = \frac{91}{18} = 5.06 \]

b. Feeding habits

Solution: the results indicate that both species will bite freely outdoors throughout the night if a suitable host is found there, which shows a more exophagic behaviour. The ratio of outdoor to indoor biting is 30:2 = 15 for \( A. \text{pharoensis} \) and 136:50 = 2.7 for \( A. \text{gambiae} \) s.l. \( A. \text{pharoensis} \) is thus more exophagic than \( A. \text{gambiae} \) s.l.
c. Human-biting rates

\[ \text{A. gambiae} \]

Aug.: \[ M = M_x + M_y = 360.25 + 4.75 = 365.0 \]

Sept.: \[ M = M_x + M_y = 4.13 + 1.5 = 5.63 \]

\[ \text{A. pharoensis} \]

Aug.: \[ M = M_x + M_y = 23.10 + 4.63 = 27.7 \]

Sept.: \[ M = M_x + M_y = 18.4 + 17.9 = 36.3 \]

4.3.1 Resting habit

Exercise 4.8

Solution: 97% of the \textit{A. gambiae} s.l. feeding on humans rested indoors (species highly endophilic), whereas only 4.0% of the resting \textit{A. pharoensis} fed indoors (species highly exophilic).

4.3.2 Longevity and infectivity

Exercise 4.9

The working groups will answer the two questions in the Guide for Participants. They should present their results in plenary.

Solutions:

a) \textit{A. gambiae} s.l. is more important as a vector in the area compared to \textit{A. pharoensis}, because: (i) it lives longer; (ii) it has been found infected, whereas no infection was confirmed in the latter species; and (iii) it has more human-vector contact (average of 13.3/person/night versus 10.8/person/night).

b) From the following Table 4.1, it can be seen that most human-vector contact with \textit{A. gambiae} s.l. takes place indoors, in spite of the fact that this species tends to be exophilic if given equal opportunity indoors and outdoors. However, from the indoor resting densities and human-biting rates, it can be seen that the month of May is the best time to apply the insecticide, and that the insecticide can be applied once a year, even though the residual activity is only 6 months, due to low densities and human-biting rates after the sixth month until the month of June of the following year.

This result also shows how the night-time habits of the local people affect transmission, despite the basic feeding habit of the vector. The use of insecticide-treated nets may be feasible in this particular case.
Table 4.1  Night landing collections of A. gambiae s.l. in Awasa Sector (Abello Wondo station), 1964–1965

<table>
<thead>
<tr>
<th>Month &amp; Year</th>
<th>No. of nights of catch</th>
<th>No. of baits</th>
<th>Total catches indoors</th>
<th>Total catch outdoors</th>
<th>Human-biting rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Indoor component (3+8 h)</td>
<td>Outdoors component (1 h)</td>
<td>Total (12 h)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Indoors</td>
<td>Outdoors</td>
<td>18.00–22.00</td>
<td>22.00–06.00</td>
<td>18.00–22.00</td>
</tr>
<tr>
<td>Jun. ’64</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Jul. ’64</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>12</td>
<td>84</td>
</tr>
<tr>
<td>Aug. ’64</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>81</td>
<td>340</td>
</tr>
<tr>
<td>Sept. ’64</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>Oct. ’64</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>4</td>
<td>21</td>
</tr>
<tr>
<td>Nov. ’64</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Dec. ’64</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Jan. ’65</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Feb. ’65</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mar. ’65</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Apr. ’65</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>May. ’65</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

**Closure discussion**

This learning unit is based on demonstrations and a practical. During the practical, evaluate the performance of the participants and correct any errors in mosquito collection, preservation, and identification.

4.4  **Guidelines for assessment**

The participants should be able to:

- differentiate unfed, freshly fed, half gravid and gravid mosquitoes correctly,
- dissect both ovaries completely and without damage,
- accurately discriminate between parous and nulliparous ovaries,
- dissect salivary glands without damaging the lobes,

The groups should also be able to work out all of the exercises. During the plenary, a representative of each group can be asked to give solutions to one exercise question.
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 classes of 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 fogging machine)
- 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)
This unit serves as an introduction to malaria vector control. A considerable amount of time has been allocated to the unit, as extensive discussions, demonstrations and practical exercises are required to provide sound technical knowledge and skills for the various vector control options. The tutor will give presentations based on the materials in the Guide for Participants.

5.1 Equipment and support

Presentation of equipment and materials are needed in this section, including the relevant videos on spraying techniques if available.

5.1.1 Indoor residual spraying

Compression sprayers, buckets, measuring cylinders (1 litre capacity), and a training wall for spraying will be needed. A well-trained vector control technician should demonstrate the parts and operations of a spray pump, preparation of insecticide solutions, and spraying techniques.

The field station should prepare in advance a sprayable surface of 19 m² for spraying practice. It is particularly important to stress the need to ensure safe handling and disposal of insecticide.

5.1.2 Insecticide-treated mosquito nets

Bednets, insecticides for bednet treatment, treating basins, measuring cylinders, balance (1 litre capacity and 10 ml capacity) are required for demonstration and practical work on treatment of bednet with insecticide.

5.1.3 Space spraying

A thermal fog is required for demonstration and explanation of different parts and exercise on fogging.

5.1.4 Larvicides

At least one type of biological control agent, and one chemical larvicide should be available. Demonstrate application methods and dosage calculations.

5.1.5 Biological agents

The local larvivorous fish could be kept in a glass jar for demonstration and feeding them with mosquito larvae.

Different formulations of Bacillus thuringiensis israelensis should be applied by the students to jars containing larvae and inspected for live mosquito larvae the following day.

5.2 Teaching and learning methods

Presentation

Using PowerPoint or overhead transparencies, the tutor should briefly present vector control options and their advantages and limitations, currently used insecticides, importance of geographical reconnaissance in malaria control. The tutor should also explain integrated vector management (IVM) and its role in vector control.
Demonstration
In the field station, the tutor should demonstrate competence in (i) applying insecticides for indoor spraying and (ii) operation, storage and maintenance of the vector control equipment.

Exercise 5.1
Solution: A possible solution is given below.

Exercise 5.2
Solution: Aspects of the vector (and components of vectorial capacity) expected to be affected by different types of vector control methods are shown in Table 5.1 below.
Table 5.1  Aspects of the vector (and components of vector capacity) that are likely to be affected by various vector control methods

<table>
<thead>
<tr>
<th>Method</th>
<th>Larval density (m)</th>
<th>Adult density (m)</th>
<th>Adult survival (p)</th>
<th>Human biting habit (a)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Larval control</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Source reduction</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Larvivorous fish</td>
<td></td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Larviciding</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Reducing human-vector contact</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insecticide-treated mosquito nets and other materials</td>
<td>-</td>
<td>+/–</td>
<td>+/-</td>
<td>+</td>
</tr>
<tr>
<td>Improved housing</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Repellents and mosquito coils</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Adult mosquito control</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insecticide-treated mosquito nets and other materials</td>
<td>-</td>
<td>+/–</td>
<td>+/-</td>
<td>+/-</td>
</tr>
<tr>
<td>Indoor residual spraying</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Space spraying</td>
<td>-</td>
<td>+</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

**Exercise 5.3**

The advantages and limitations of malaria vector control can be presented in different ways. Examples are given in the tables below.

Table 5.2  Reduce human-vector contact

<table>
<thead>
<tr>
<th>Approach</th>
<th>Advantages</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Environmental management</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Improve housing (screening windows, closing eaves)</td>
<td>Long-lasting, effective and efficient Low maintenance</td>
<td>Large initial cost Periodic maintenance Vector biting behaviour outside houses decreases effectiveness Success or failure depends on the participation of the people at risk</td>
</tr>
<tr>
<td><strong>Chemical control</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insecticide-treated nets Repellents</td>
<td>Can be community-based Effective</td>
<td>Vector biting behaviour outside houses decreases effectiveness Success or failure depends on the participation of the people at risk Short duration of repellents Requires a large well-run control programme</td>
</tr>
<tr>
<td><strong>Biological control</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Not applicable</td>
<td></td>
</tr>
</tbody>
</table>
### Table 5.3 Reduce vector density

<table>
<thead>
<tr>
<th>Approach</th>
<th>Advantages</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Environmental management</strong></td>
<td><em>Eliminate or modify breeding sites</em></td>
<td><em>Large initial cost (although it may be paid for outside the health department)</em></td>
</tr>
<tr>
<td></td>
<td>Long-lasting effective and efficient</td>
<td>Potential negative effect on the environment in some cases</td>
</tr>
<tr>
<td></td>
<td>Low maintenance</td>
<td>Periodic maintenance required</td>
</tr>
<tr>
<td></td>
<td>Can be community based</td>
<td></td>
</tr>
<tr>
<td><strong>Chemical control</strong></td>
<td>Space spraying</td>
<td>Short duration</td>
</tr>
<tr>
<td></td>
<td>Larviciding</td>
<td>Need specialized personnel for space spraying or larvicide application</td>
</tr>
<tr>
<td></td>
<td>Effective in some circumstances</td>
<td>Risk of contamination of environment (but not for microbial larvicides)</td>
</tr>
<tr>
<td></td>
<td>Larvicides are easy to apply</td>
<td>Potential effects against non-target species (but not for microbial larvicides)</td>
</tr>
<tr>
<td></td>
<td>Larviciding can be community-based</td>
<td>Larviciding requires a large-well-run control programme</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Breeding sites may be extensive</td>
</tr>
<tr>
<td></td>
<td></td>
<td>In urban areas both anophelines and culicines should be controlled.</td>
</tr>
<tr>
<td><strong>Biological control using fish</strong></td>
<td>Larvivorous fish</td>
<td>Breeding sites may be extensive</td>
</tr>
<tr>
<td></td>
<td>Effective in some circumstances</td>
<td>Requires an understanding of fish cultivation</td>
</tr>
<tr>
<td></td>
<td>Can be community-based</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Little environmental impact</td>
<td></td>
</tr>
</tbody>
</table>

### Table 5.4 Increase adult vector mortality

<table>
<thead>
<tr>
<th>Approach</th>
<th>Advantages</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Environmental management</strong></td>
<td>Not applicable</td>
<td></td>
</tr>
<tr>
<td><strong>Chemical control</strong></td>
<td>Indoor residual spray</td>
<td>High cost</td>
</tr>
<tr>
<td></td>
<td>Very effective if used correctly</td>
<td>Requires periodic application</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Inadequate sprayable surfaces</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Need well-trained personnel for spraying</td>
</tr>
<tr>
<td></td>
<td>Insecticide-treated nets</td>
<td>Success or failure depends on the participation of the people at risk</td>
</tr>
<tr>
<td></td>
<td>Very effective if used correctly</td>
<td>Success depends on the relationship between the activity of the vector and humans</td>
</tr>
<tr>
<td></td>
<td>Low environmental impact</td>
<td></td>
</tr>
<tr>
<td><strong>Biological control</strong></td>
<td>Not applicable</td>
<td></td>
</tr>
</tbody>
</table>
In a plenary session, the tutor will lead a discussion on the criteria that are used to select vector control methods. Participants will be asked how control methods are selected in the place where they work, and whether they have any experience of situations in which the criteria were not used. If so, these experiences should be discussed.

**Exercise 5.4**

The working groups should use the reading at the end of the Learning Unit 5 of the *Guide for Participants* as a guide to this exercise. They will need to include their plan, outcomes anticipated, and advantages and limitations of their implementation strategy. Ask the following question to close this exercise and lead into the next section on integrated control.

a) *What are the consequences if the strategy does not work?*

b) *What would you do?*

**Exercise 5.5**

The participants should work in small groups to answer the questions in the *Guide for Participants*. The tutor should check their responses against the possible answers given below.

a) *If the insecticide used has a repellent action, spraying of cattle sheds without fully spraying human residents may divert mosquitoes towards the human dwellings, which could increase transmission.*

b) *In addition to the refusal of spraying by inhabitants, vector behaviours of outdoor biting and outdoor resting may diminish the effectiveness of IRS.*

c) *True: IRS reduces the average life span of mosquito vectors to the extent that the complete development of the parasite cycle in the mosquito (sporogonic cycle) is not achieved.*

d) *False: An “all or nothing” principle is the principle behind IRS. In the example of 50% of coverage, the degree of protection will be close to zero, since the level of coverage is much less than the desired threshold of 100%. On the other hand, if coverage is sufficiently high, people in a few unsprayed houses are also protected.*

e) *True: Coverage of IRS depends on the number of house structures with sprayable surface.*

**Exercise 5.6**

*Possible solution*

1. Three important stakeholders/collaborators for IVM are:
   - Agriculture – use of pesticides, coordination in registration and monitoring/managing resistance;
   - Environment – health impact assessment of development projects;
   - Municipality/local government – control of urban pests and use of insecticides to control them.

The key word here is intersectoral collaboration and coordination.
2. No: There are other important examples i.e. intersectoral collaboration etc. and the use of different interventions is only recommended where evidence for cost-effectiveness has been demonstrated.

3. Yes: Because it is the only way available resources can be used rationally – no duplication of efforts where complementarity and synergy of resources can be ensured.

4. See the advantages from Q 1 and 3 above. As for disadvantages: IVM is a concept that has not been understood by many and requires advocacy and political commitment from decision makers.

**Exercise 5.7**

The tutor should provide spray pump and required amount of insecticide for 19 m² wall. Each participant should spray successfully the wall as shown in Fig. 5.8 in the Learning Unit 5 of the *Guide for Participants*. A total 60 minutes should be allocated for this exercise.

**5.3 Guidelines for assessment**

The groups should be able to demonstrate understanding of the basic skills required for vector control.

Towards the end of the session, the tutor should ask participants at random some of the important roles of vector control in malaria control programmes and issues to be considered during implementation of vector control, in order to assess their level of understanding. Discuss the safety, maintenance and operational issues related to the demonstration of vector control methods.
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 importance
- Determine the residual efficacy of an insecticide-treated net
This unit includes demonstrations and some laboratory activities. Because of time constraints, it will be necessary for each participant to be assigned part of the experiment, and the data from the different participants will be combined for analysis and interpretation.

6.1 Equipment and support

Unfed and live female anophelines, susceptibility test kit, thermometer, wooden box with large holes, towels, cotton wool, paper cups with cover nets, rubber bands, markers or wax pencils, mosquito cage, bioassay kit, cardboard paper, adhesive tapes, insecticide-treated net, insecticide-free net.

6.2 Teaching and learning methods

Presentation

Explain briefly the principles and procedures of susceptibility tests and bioassays in the class while demonstrating the required equipment.

Demonstration and practical

During the presentation, demonstrate each item of equipment used in susceptibility testing for adult mosquito. In the laboratory, demonstrate how to introduce adult mosquitoes into the tubes and how to transfer mosquitoes from one tube to another as well as other aspects of susceptibility testing for adult mosquitoes.

Answers

Exercise 6.1

Susceptibility tests on adults mosquito:

- The participants will be provided with live unfed female mosquitoes. It is recommended to use insectary bred unfed 2–3 day-old females.
- Working in pairs, participants should prepare holding tubes and each pair should introduce 15 unfed females to the holding tubes.
- When all groups have introduced the mosquitoes, ask half of the groups to prepare exposure tubes and the other half to prepare control tubes.
- After all tubes have been prepared, ask all groups to transfer the mosquitoes to the exposure and control tubes and ask them to label each tube with the group number indicating whether it is an exposure or a control tube, and the time of the day.
- After one hour of exposure, each group should transfer the mosquitoes back to the holding tubes for 24 hours observation. After 24 hours, all results should be combined to calculate mortality rates.
- For calculation of LT50, set a series of exposure times, e.g. 2, 4, 8, 16, 32 minutes, and ask participants to individually perform each exposure time with 2 replicates. After a recovery period of 24 hours, calculate LT50 according to probit mortality analysis.
Exercise 6.2

Bioassay on ITNs:

Working in pairs, the participants will carry out the residual efficacy of ITNs. Each group of participants will be provided with a piece of netting material measuring 25 x 25 cm and required diluted insecticide formulation and four cones. They will carry out the following steps:

- Half of the group will impregnate the piece of the net carefully and evenly using the required diluted insecticide formulation. Soak the net for a few seconds and ensure that all of the insecticide solution is absorbed and let it dry.
- Half of the group will attach WHO cones on the treated nets and half on untreated nets.
- Each pair should transfer 5 standard susceptible 1–3 day old, non-blood fed Anopheles females to each cone.
- After 3 minutes the mosquitoes should be taken out and placed in paper cups for 24 hours observation.
- Record the knock-down rate at 60 minutes post-exposure.
- Record mortality after 24 hours.
- Results should be combined to calculate mortality rates.

Exercise 6.3

Working in pairs, the participants will carry out the following:

- Use only 3rd or early 4th instar larvae.
- Prepare insecticide test solutions by adding 1 ml of stock solution of insecticide (1000 ppm) to a large beaker containing 224 ml of water (total volume 225 ml).
- Wait for 15–30 minutes after preparing the test solution. Temperature of the solution should be between 20 °C and 30 °C.
- Add 25 ml of water containing 25 larvae into a large beaker containing the test solution.
- Leave the larvae in the test solution for 24 hours.
- Discard the larvae that have pupated during the test.
- Record the number of dead and moribund after 24 hours.

Guidelines for assessment

This unit is based on active involvement of the participants in the practicals and performance should be assessed while they are doing their assigned tasks. Any shortcomings should be corrected accordingly.
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 eco-epidemiological strata
The main objective of this unit is to acquaint participants with malaria stratification and the appropriate vector control options for each stratum. Participants are expected to understand the important characteristics of the various eco-epidemiological types of malaria and how to select appropriate vector control methods that maximize the benefits of protection.

### 7.1 Equipment and support
Flip charts should be provided for group discussions.

### 7.2 Teaching and learning methods

**Presentation**
The tutor’s presentation should cover the classifications of malaria situations into different strata. The following points should be covered in the presentation:

- Concept of stratification;
- Stratification of malaria situation by risk of malaria;
- Stratification of malaria situation by endemicity using spleen and parasite rates;
- Stratification of malaria by eco-epidemiological types;
- Selection of appropriate vector control options for different malaria strata.

The participants should have a good understanding of these strata. During the presentation the participants can be asked questions concerning the types of malaria situations in their area of work. For example:

1. Using Table 7.1 in Learning Unit 7 of the Guide for Participants, ask the participants whether their malaria programme classifies endemicity based on spleen and parasite rates.
2. What type of malaria is most prevalent where they work or in their country?
3. Is there an area where urban malaria is present? If so what vectors are involved?

The tutor should lead discussions on the main entomological and environmental features of the different strata of malaria situations such as unstable and stable malaria; hypoendemic, mesoendemic, hyperendemic and holoendemic malaria; and the different eco-epidemiological types of malaria. The tutor should ask how these factors determine the distribution of infection and disease in the population.

The tutor should ask the participants how malaria is stratified in their place of work/country.

**Exercise 7.1**

**Possible solutions**
1. (i) In areas with unstable/epidemic malaria, parasite prevalence is normally very low and only increases during the transmission season. Due to the short transmission and/or low level of transmission, immunity to the disease is generally very low or absent. All age groups are affected by malarial disease and mortality.
(ii) In areas with stable malaria, parasite prevalence is very high, with little seasonal fluctuation. Due to the intense transmission, immunity to the disease is generally very high in the adult population. Children < 5 years and pregnant women are most affected by malarial disease and mortality.

2. In areas of stable malaria, vectorial capacity is usually very high. This is a result of vectors’ long survival time, allowing them to bite people more than once in their life span. It is not easy to reduce the transmission of malaria in such areas.

3. Factors that may affect vector density in urban settings include limited breeding sites as the result of construction of buildings and streets. In rural settings, on the other hand, the number of houses are much fewer than the potential breeding sites.

Exercise 7.2
The tutor should divide the participants into 5 working groups and assign one stratum for each working group. The participants are to select the vector control options that they consider feasible for each stratum. Each group should list the results on a flip chart and the reason for their selection. They will then present the results in plenary.

Possible solutions
Vector control options in different malaria situations (strata):

1. Vector control options in areas with unstable malaria
Almost any vector control measure appropriately applied to specific situations of unstable malaria areas will have an impact in reducing the risk of malaria. The main objective of vector intervention in such low endemicity areas is to reduce the degree of transmission. For example reducing the EIR from one per year, which is common in such areas, to 0.5 per year will have a considerable impact on prevalence of infection, disease and death due to malaria. Individual protection methods could also have a considerable impact when applied correctly and accepted by the public. The choice of appropriate vector control measure in such areas is therefore dependent on vector behaviour, cost, and other socioeconomic factors. In areas where malaria is unstable vector control is an indispensable tool to protect the population from the risk of malaria epidemics.

Indoor residual spraying is a highly effective technique to prevent or reduce the negative impact of epidemics. In such areas, houses or shelters are sprayed with insecticides with residual life of 3–12 months. For maximum killing effect insecticides with low irritant effect are preferred. However, chemicals with repellent property should also provide a reasonable degree of protection by driving the mosquitoes to the hostile outdoor environment, thereby lowering mosquito survival and parasite development.

Individual protection with insecticide-treated materials and other repellents can also be highly effective. ITNs have been proved to reduce illness and death due to malaria significantly in low, medium and high transmission areas. Long-lasting insecticidal nets are effective for about 3 years. However, it is important to make sure that there is sufficient awareness and acceptability in the community before choosing ITNs over other vector intervention methods. One important drawback of ITNs in low transmission areas is the fact that the low nuisance
effect of mosquitoes for a longer part of the year may affect the appropriate use and acceptability of ITNs – people may see no need for nets if mosquitoes are not a significant nuisance.

Larviciding and source reduction, as supplementary control measures to ITNs and IRS, are effective if breeding sites are fixed, few and findable. It is also an excellent alternative where highly exophilic vectors are involved and where there are significant cultural or other objections to indoor house spraying.

2. Vector control options in areas with stable malaria

Stable malaria is characterized by intensive transmission, often far greater than is needed to saturate the prevalence of infection in a community.

ITNs and IRS (where appropriate) are currently the most effective and practical vector control options in areas where malaria is highly endemic. Recourse to both interventions are effective in significantly reducing child mortality in sub-Saharan African countries where transmission occurs all the year round or is seasonally intensified.

3. Vector control options in urban settings

Because fewer open spaces are available, vector breeding sites in the urban environment are usually few, fixed and findable. Therefore:

- vector control in urban area must focus on the reduction of human-vector contact and on environmental measures directed at eliminating the vector and its breeding sites. The main recommended interventions in urban settings are ITNs/LLINs, focal IRS, larval source management.

4. Vector control in development projects

- Selection of suitable housing sites for the labour force and adequate protective measures such as screening of houses must be considered in the planning of projects.
- Environmental disturbances that favour vector breeding must be avoided and precautionary measures must be included in the design of the projects.
- Use of ITNs/ LLINs should be encouraged.
- IRS might also be used depending on the housing conditions, cost, and other epidemiological factors. Policies and legislation must incorporate some of the above precaution measures in the planning of development projects.

5. Vector control options in humanitarian emergencies

The choice of vector control interventions will depend on local factors such as the type of shelter available, human habits, vector behaviour and malaria endemicity.

In humanitarian emergencies, priorities are prompt diagnostic testing 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.

**Epidemic threshold chart and timing of insecticide spraying**

The participants should be asked to bring their past 5 years of malaria data for preparing the graph as shown below. Such a chart should show the epidemic threshold. The following chart indicates the current malaria situation and timing of vector control. The planned IRS or distribution of ITNs should be carried out prior to the epidemic-prone period.

**Discussion**

To close this learning unit the tutor should summarize the relationships between epidemiological strata and vector control. The tutor should also present the graph below and explain why the timing of vector control in relationship to epidemiological indicators is so important.

![Graph showing Adult mosquito density and Morbidity](image)

Spraying at this time point is effective

Spraying at this time point is not effective

Finally, the following proposition can be introduced and the participants asked whether they consider it to be true or false.

*Even a large effort (e.g. by applying residual spraying to reduce transmission) may have very little impact on prevalence of infection due to the high vectorial capacity or basic reproduction rate of malaria vectors in areas of stable malaria transmission.*

The tutor should lead a discussion on this aspect of vector control.
Malaria entomology and vector control

GUIDE FOR TUTORS

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