Basic malaria microscopy

PART I
Learner’s Guide
Preface

This training module on basic malaria microscopy is in two separately bound parts. Part I, the Learner’s Guide, contains all the technical information that will be needed by trainees in this field. Part II is the Tutor’s Guide, which gives extensive advice for those responsible for organizing, running and evaluating training programmes.

The module is one of two published by the World Health Organization concerned with different aspects of the control of malaria. It can stand alone as a medium for teaching malaria microscopy to public health and laboratory personnel, or can be used as an integral part of a longer and more comprehensive programme of training in malaria.

In 1988 WHO published Bench aids for the diagnosis of malaria, which comprise all the colour plates that appear in the Learner’s Guide and much of the core information. These bench aids are published in the form of separate laminated sheets, which are very robust and easy to use at the laboratory bench. While it is not essential to provide these for use during training courses, they are recommended for use by all health workers engaged in routine malaria microscopy.

The need for this module was identified by Member States in the Eastern Mediterranean, South-East Asia and Western Pacific Regions of the World Health Organization, and the project was conceived by Dr McWilson Warren, former Team Leader of WHO’s Interregional Secretariat for the Coordination of Malaria Training in Kuala Lumpur, Malaysia. Work on the module was one of the major activities of the Secretariat. The original text was written by Mr John Storey and the colour plates in Part I are taken from watercolour paintings meticulously prepared by Mr Yap Loy Fong.

The text, particularly that of Part II, has been reviewed by numerous individuals and revised by Dr P. F. Beales, Dr C. W. Hays, Dr D. Payne and Mr W. Rooney. Editing of the entire module was undertaken by Professor Michael Colbourne.

WHO wishes to acknowledge the collaboration and financial support provided by the United States Agency for International Development for this and other activities of the Interregional Secretariat for the Coordination of Malaria Training.

1 Also available: Enzyme-linked immunosorbent assay (ELISA).
2 A second edition of these bench aids will be published in 2000 (see inside back cover for details).
Introduction

This Learner's Guide, Part I of the publication Basic malaria microscopy, is made up of teaching material on each of the activities involved in diagnosing malaria by microscopy. Together with Part II, the Tutor's Guide, it forms one component — or module — of a series of teaching materials on malaria. The Guide is designed to be used throughout a formal period of training and provides information and instructions in a simple, easily understandable form. It is also intended to be used as a reference after training. Reference materials of this type are sometimes called "job aids". The information contained in this Learner's Guide has been made as complete as possible, which reduces the need for note-taking during lectures, demonstrations and other exercises.

For whom is the Learner's Guide designed?

The Guide is designed for general health service and laboratory personnel who will carry out the activities described.

Objectives

At the end of the training programme based on this Learner's Guide you should have acquired the skill and competence that will enable you to:

- appreciate the importance of malaria as a disease
- recognize the common signs and symptoms of malaria
- record details about patients on the appropriate forms
- make thick and thin films of blood taken from people with suspected malaria
- stain blood films for examination with Giemsa stain
- maintain the microscope in good working order
- use the oil immersion objective and the correct ocular to examine thick and thin blood films, and:
  - recognize the various components of normal blood
  - recognize and measure the density of malaria parasites, and correctly identify their stage and species
- record accurately the results of your examination on the appropriate form
- inform those people responsible for the treatment of malaria patients of your findings
- use the information in this Guide to teach other public health workers to make thick and thin blood films
- submit reports and requests for supplies when necessary
- recognize the need to take special precautions when handling blood to prevent transmission of blood-borne viral infections.

How this subject will be taught

Facilitators

Facilitators are people who work with the tutor to help you to achieve the objectives outlined above. The tutor has wide experience in malaria
microscopy and is able to help you to solve a wide range of problems. Facilitators will lead discussions and provide general help to individuals and to small groups of learners.

Presentations

Formal presentations of information, in the form of lectures for example, will usually be kept to a minimum and each session will be as short as possible. The information that will be given in such sessions is already contained in this Guide, so there will be very little need for you to take notes. A lecture presentation will usually be combined with a demonstration.

Demonstrations

Demonstrations will either be used to illustrate activities that you will later carry out yourself or consist of looking at specimens and equipment that you need to know about and be able to use.

Practical sessions

There will be as many practical sessions as possible. They are intended to help you to gain as much practical experience as you can in all aspects of malaria microscopy. In some, each facilitator will work with a small group of four or five learners. Because there are only a few learners in each group, the facilitator will be able to give a great deal of attention to each individual: this increases your opportunities to practise and to learn.

Role-play

In a role-play exercise you will be asked to pretend to be a person in a situation that may arise in your job. For example, you may be asked to play the part of a laboratory worker preparing a blood film from a patient suspected of having malaria. Another learner will play the part of the patient. Afterwards, members of the group will discuss what was said and done. Much can be learned from this enjoyable type of exercise.

Small group discussions

In these exercises, a facilitator will lead discussions on particular subjects. These sessions provide good opportunities for you and the other learners to give your opinions, develop your ideas and learn from one another.

Field work and visits to work places

A number of these types of visit may be arranged. They are designed to give practical experience of real-life situations and allow you to learn about the problems you may meet in the course of your daily work.

Evaluation

Evaluation of the learner

The evaluation of individual progress and achievement will be carried out by the tutor, the facilitators and you yourself. It will include:
• **Spot tests**

At regular intervals, a series of "spots" will be set out for you to comment on. The spots may be microscopic specimens or other items linked to what you have learned. They are designed to help you and the tutor assess how well you have mastered the skills and developed the competence to carry out your work.

Correct answers will be supplied after the spot tests and a discussion will take place. This is intended to improve the process of learning and help you to identify those activities in which you need further practice.

• **Multiple-choice quizzes**

In multiple-choice quizzes, each question is provided with a list of possible answers from which you must select the one you think is correct. At the end of these sessions you will not necessarily be given the correct answer to each question, but the tutor will analyse the results to identify topics that were not clearly understood. The tutor may also tell you where you made mistakes and point out areas where you need to improve.

This part of the evaluation is designed to help you and the tutor to assess how well you understand the non-practical aspects of the course. Multiple-choice tests will take place regularly, often during the same session as spot tests.

**Evaluation of the training by the learner**

By means of a questionnaire, the tutor will ask you, the learner, how you think the training has helped you and how it might be improved. This evaluation will take place at the end of the training period in order to provide as much feedback from the learners as possible. You may sign the questionnaire or not, as you wish, but you should feel completely free to make suggestions for improvements on the part of the tutor and facilitators as well as in the content of the course and the training facilities.

**Use of the Learner's Guide**

This Learner's Guide consists of instructional materials designed to enable you to achieve the objectives stated earlier. The Guide is divided into chapters called Learning Units. You must acquire the skills and knowledge contained in one Unit before progressing to the next, otherwise you may have difficulty in achieving the objectives of subsequent Learning Units.
Notes
LEARNING UNIT 1

Malaria, the disease

Learning objectives

By the end of this Unit you should:

- recognize the importance of malaria as a disease
- be able to recognize the common clinical signs and symptoms of malaria
- know that some people can have malaria without clinical symptoms
- know that malaria is caused by the presence of parasites in a patient’s blood
- know that a female anopheles mosquito can transmit malaria to people
- realize that, to diagnose malaria accurately, you must be able to find and identify parasites in a stained blood film examined under the microscope.

The importance of malaria

Malaria is a serious public health problem in many parts of the world. Attacks of the disease can be very severe and can even lead to death if they remain untreated.

Malaria can be responsible for people spending many days away from school or work and so may affect:

- the amount that they learn at school
- the quantity of food they are able to grow
- the money they can earn.

Malaria is caused by a very small living organism called a parasite, which infects a person’s blood. The disease is transmitted from one infected person to another by the bite of a female anopheles mosquito. This can occur only after the parasite has been inside the mosquito for at least a week.

You will learn more about the malaria parasite and how the disease is transmitted in a later Learning Unit.

Clinical signs and symptoms of malaria

In people who have had very few attacks of malaria, the disease is fairly easy to recognize by the presence of one or more of the following clinical signs and symptoms:

- high fever
- headache
• severe chills
• general body pains.

In some cases the following symptoms are also present:
• vomiting
• diarrhoea.

As a learner, you may be confused because these signs and symptoms are also found in other diseases. Further observations are needed for accurate diagnosis.

It is more difficult to diagnose malaria in people who have had several attacks of the disease. This is because their bodies are more used to the disease and so the clinical signs and symptoms are not always present. Similarly, if patients have treated themselves with some medication before you see them, the signs and symptoms may be modified. A patient may have only a mild headache and nothing else, or a very slight fever that causes little discomfort.

**How to diagnose malaria**

Many people do not know that malaria is caused by a parasite in the blood. The parasite is very small and can be seen only with the aid of a microscope.

Before the parasite can be seen in a patient’s blood, a blood film must be made. The dry blood film is then stained with Giemsa stain and examined under the microscope, using the oil immersion objective lens. If stained parasites are seen by the examiner, the patient is confirmed as having malaria.

You should therefore understand that the only correct way to diagnose a case of malaria is by examining the patient’s stained blood film with a microscope. This is a highly skilled job. The following Units of this Learner’s Guide will take you through the steps necessary to acquire the skills you need.
LEARNING UNIT 2

Cleaning and storing microscope slides

Learning objectives

By the end of this Unit you should be able to:

- describe how microscope slides for blood films are correctly cleaned, wrapped and stored
- distinguish slides that are suitable for making blood films from those that are not
- demonstrate how to wash, dry, wrap and store slides for blood films.

Cleaning slides

Microscope slides are usually supplied in boxes of 50 or 72. They may be described on the box as "washed" or "pre-cleaned", but they will still need to be properly washed, dried and wrapped. It is not possible to make good quality blood films on dirty microscope slides. Blood films made on dirty or greasy slides will wash off easily during staining. It is therefore best to discard slides that:

- have an iridescent bloom or appear white or opaque
- are not properly cleaned
- are old, with surface scratches or chipped edges.

In order to clean slides you will need:

- a large plastic basin
- gauze or cotton wool
- a good quality detergent (powder or liquid)
- 2-4 clean, dry, lint-free cotton cloths
- clean water.

New slides

All new slides should be washed with detergent and clean water. After being soaked for between 30 minutes and 1 hour, the slides should be rinsed under running tap water or in several changes of clean water. Each slide should be individually wiped dry and polished with the clean, dry, lint-free cloths.

Cleaned slides should be handled only by the edges to avoid finger marks or grease being deposited on the surfaces.

Used slides

Used, dirty slides should be soaked for a day or two in water containing detergent. (Warm water should be used whenever possible.) After soaking, the
slides should be cleaned one by one with a small piece of gauze or cotton wool. All traces of the blood film and oil (used during microscopy) should be removed from the slides.

Do not leave the slides in the detergent for too long; soaking should be for a few days only, not weeks. If slides are left in the detergent solution for long periods, the water will evaporate, leaving a deposit on exposed slides that is impossible to remove.

After cleaning, the slides should be transferred to a fresh solution of detergent and later rinsed under running water or in several changes of clean water. They should be individually dried with the clean cotton cloths as described previously.

Slides that are slightly scratched and considered unsuitable for blood films may be handed over to other sections of the laboratory service for routine use.

**Wrapping cleaned slides**

To wrap cleaned slides correctly you will need:
- sheets of thin, clean paper, about 11 cm × 15 cm in size
- empty cardboard slide boxes (of the type new slides are packed in)
- rubber bands or adhesive tape.

Cleaned slides should be wrapped with thin paper in packs of 10. Each pack can be secured with adhesive tape or a rubber band. Packs can then be placed in the cardboard slide boxes for later use or dispatch to the field.

Slides should be stored in a dry place such as a warm-air cupboard. If stored at room temperature with high humidity, the slides will stick together after a few weeks. It will then not be possible to use them unless they are rewashed and dried.
LEARNING UNIT 3

Keeping accurate records

Learning objectives

By the end of this Unit you should be able to:

- identify which record form(s) or register(s) should be used to record information about patients
- record accurately all the required information on the appropriate record form
- select the correct copy of the record form for dispatch to your supervisor.

To make sure that you can trace patients easily, it is important to record all the required information when they attend the clinic or when you see them in their villages. Information is usually recorded on specially designed forms. Although these forms may differ from country to country or health district to health district, the information required is usually the same and may include the following:

- region, province, district and zone where the work is being done
- town or village where the patient lives
- street and house number where the patient lives or through which he or she can be easily contacted
- patient’s name, age and sex
- blood film number
- results of the examination:
  - negative for malaria parasites
  - positive for malaria parasites
  - species of malaria
  - parasite stage(s) seen.

Your tutor or facilitator will show you the forms that you will routinely use and will advise you on how to fill them in. You will also have plenty of practice in completing them during field collections that you will undertake later.

Remember that mistakes in completing forms can be just as serious as mistakes in examining blood films.
LEARNING UNIT 4
Blood films

Learning objectives

By the end of this Unit you should be able to:

- name the diseases that can be transmitted by contaminated blood
- list the precautions that must be taken to prevent contamination
- list all the materials required for making blood films
- demonstrate under field conditions the preparation on the same slide of a thick and a thin blood film, each of good enough quality for malaria microscopy
- explain the reasons why a blood film should be correctly labelled
- demonstrate the correct labelling of blood films
- recognize and select thick and thin blood films of good quality
- identify the causes of common faults in both thick and thin blood films.

Diseases carried in the blood

Some people may carry a disease in their blood even if they do not appear to be ill. You cannot easily see the diseases in the blood, and sometimes the tests to demonstrate the diseases are very complicated. The principal diseases are:

- hepatitis
- acquired immunodeficiency syndrome (AIDS)
- malaria.

The collection and handling of blood samples presents a potential risk of blood from a patient infected with one of these diseases accidentally contaminating another patient or a health worker. However, this risk can be reduced to a negligible minimum by taking the following precautions:

- Wear protective gloves when handling blood or taking blood samples.
- Avoid getting blood, including that from unstained slides, on your fingers or hands.
- Cover any cuts or abrasions on your hands with adhesive dressings.
- Take care not to prick yourself or others with any sharp instrument that has been in contact with blood.
- Never use disposable lancets more than once.
- Always wash your hands with soap and water after completing any task that involves the handling of blood.
- If blood does get on to your skin, wipe it off quickly with cotton wool dampened with alcohol and wash the affected area with soap and water as soon as possible.
- Any materials contaminated with blood, such as lancets, cotton swabs and discarded slides, should be boiled for 20 minutes, or placed in a solution of bleach or sodium hypochlorite (available chlorine level 10 000 parts per million), then disposed of safely by burial or incineration.

**Kinds of blood film**

Two kinds of blood film — thick and thin — are used in malaria microscopy.

**Thin film**

The thin film consists of a single layer of red blood cells and is used to assist in the identification of the malaria species, after the parasites have been seen in the thick film. It is also used as a label to identify the patient.

**Thick film**

The thick film is made up of large numbers of dehaemoglobinized red blood cells. Any parasites present are concentrated in a smaller area than in the thin film and so are more quickly seen under the microscope.

**Preparation of thick and thin blood films on the same slide**

The following items are needed for preparation of blood films:

- cleaned and wrapped slides
- sterile lancets
- methylated spirit and water
- absorbent cotton wool
- slide box (or a cover to keep flies and dust off the slides)
- clean, lint-free cotton cloth
- record form or register.

The techniques for taking blood samples and preparing thick and thin blood films are detailed in Plate 1. Note that, whereas a thick and a thin film from the same patient may be made on the same slide, it is not recommended to make blood films from more than one individual on the same slide.
Plate 1. **Preparation of thick and thin blood films on the same slide**

After details about the patient have been recorded in the appropriate form or register, the blood films are made as follows:

1. **Holding the patient's left hand, palm upwards, select the third finger from the thumb. (The big toe can be used with infants. The thumb should never be used for adults or children).**

   Clean the finger with a piece of cotton wool lightly soaked in alcohol, using firm strokes to remove dirt and grease from the ball of the finger.

   Dry the finger with a clean cotton cloth, using firm strokes to stimulate blood circulation.

2. **Puncture the ball of the finger with a sterile lancet, using a quick rolling action.**  

   Apply gentle pressure to the finger to express the first drop of blood and wipe it away with a dry piece of cotton wool. Make sure that no strands of cotton remain on the finger to be later mixed with the blood.

3. **Working quickly and handling clean slides only by the edges, collect the blood as follows.**

   Apply gentle pressure to the finger and collect a single small drop of blood, about this size ●, on the middle of the slide. This is for the thin film.

   Apply further pressure to express more blood and collect two or three larger drops, about this size ●●, on the slide, about 1 cm from the drop intended for the thin film (see illustration).

   Wipe the remaining blood away from the finger with a piece of cotton wool.
4. Thin film. Using a second clean slide as a “spreader” and, with the slide with the blood drops resting on a flat, firm surface, touch the small drop with the spreader and allow the blood to run along its edge. Firmly push the spreader along the slide, keeping the spreader at an angle of 45°. Make sure that the spreader is in even contact with the surface of the slide all the time the blood is being spread.

5. Thick film. Always handle slides by the edges or by a corner to make the thick film as follows.

Using the corner of the spreader, quickly join the drops of blood and spread them to make an even, thick film. The blood should not be excessively stirred but can be spread in circular or rectangular form with 3 to 6 movements. The circular thick film should be about 1 cm (1/3 inch) in diameter.

6. Label the dry thin film with a soft lead pencil by writing across the thicker portion of the film the patient’s name or number and the date. Do not use a ball-point pen for labelling the slide. Allow the thick film to dry with the slide in a flat, level position, protected from flies, dust and extreme heat.

7. Wrap the dry slide in the patient’s record form and dispatch it to the laboratory as soon as possible.

8. The second slide used for spreading the blood films may now be used for the next patient and another clean slide from the pack will be used as a spreader.

Example of well made and correctly labelled thick and thin films
Common faults in making blood films

A number of faults are common in making blood films. These can affect the labelling, the staining or the examination, and sometimes more than one of these.

Badly positioned blood films

Care should be taken that the blood films are correctly sited on the slide. If they are not, it may be difficult to examine the thick film. Also, portions of the films may even be rubbed off during the staining or drying process.

Too much blood

After staining films made with too much blood, the background to the thick film will be too blue. There will be too many white blood cells per thick film field, and these could obscure or cover up any malaria parasites that are present. If the thin film is too thick, red blood cells will be on top of one another and it will be impossible to examine them properly after fixation.

Too little blood

If too little blood is used to make the films, there will not be enough white cells in the thick film field and you will not examine enough blood in the standard examination. The thin film may be too small for use as a label.

Blood films spread on a greasy slide

The blood films will spread unevenly on a greasy slide, which makes examination very difficult. Some of the thick film will probably come off the slide during the staining process.

Edge of spreader slide chipped

When the edge of the spreader slide is chipped, the thin film spreads unevenly, is streaky and has many "tails". The spreading of the thick film may also be affected.
Thin film too big, thick film in the wrong place

If the thin film is too large, the thick film will be out of place and may be so near the edge of the slide that it cannot be seen through the microscope. During staining or drying, portions of the thick film will probably be scraped off by the edges of the staining trough or drying rack. It may be very difficult, or impossible, to position the thick film on the microscope stage so that it can be examined.

Other common faults

Other faults that occur commonly in the preparation of blood films include the following:

- Flies, cockroaches or ants eat the dry blood and damage the films.
- Blood films are made on badly scratched slides.
- The thick film is allowed to dry unevenly.
- Autofixation of the thick film occurs with the passage of time or through exposure to heat, and staining then becomes difficult or unsatisfactory.
- Slides are wrapped together before all the thick films are properly dried, and the slides stick to one another.

Drying the blood films

Blood films must be stored correctly in order to allow the thick film to dry evenly. They should also be protected from flies and dust.

The best kind of box, both in the field and the laboratory, is that shown below:
Slides are stored horizontally, which allows the thick film to dry level and with even thickness. There is a door to keep out flies and dust and a handle for carrying the box. Only after 100 slides have been collected is it necessary to empty one side of the box before more slides can be collected. At the speed slides are usually taken in field collections, the first 50 will be dry before they need to be packed.

When the thick film is completely dry, the slides are stored front to back in the empty cardboard slide box previously used for the cleaned, wrapped slides. In warm humid climates, however, autolysis of unstained slides occurs quite rapidly and all slides should be stained as soon as possible, at the latest within 3 days of collection. When long storage is unavoidable, the slides can be kept in a desiccator to delay autolysis. Finally, it is important to ensure that the slides are packed correctly and that they are not put into strong sunlight or near to a source of heat (e.g. the exhaust pipe of a vehicle in the field).
LEARNING UNIT 5

Staining blood films with Giemsa stain

Learning objectives

By the end of this Unit you should:

• be able to operate the simple chemical balance used in your laboratory
• be able to make up buffered water suitable for mixing with Giemsa stain, by correctly weighing the buffer salts and by the proper use of the Lovibond Comparator
• be able to make up 2% correcting fluids to adjust the pH of water for Giemsa stain
• know why it is essential for buffered water to be at pH 7.2 for good quality staining of blood films with Giemsa stain
• know when the regular method of staining or the rapid method should be used
• be able to demonstrate the use of Giemsa stain and the correctly buffered water to stain blood films suspected of containing malaria parasites, using either the regular or the rapid method
• be able to demonstrate the preparation of thick and thin blood films for staining
• know the correct and incorrect ways of handling and using Giemsa stain
• be able to demonstrate the correct drying and storage of stained slides.

Buffered water

Before you can stain blood films correctly, you first need to prepare the buffered water that is to be mixed with the stain. This buffered water needs to be at the correct pH to ensure that the quality of staining is good. Any malaria parasites that are present in blood films will then be properly stained and can be clearly seen under a microscope.

The term "pH" is used to express the acidity or alkalinity of a liquid. It is based on a scale of near 0 (very acid) to 14 (very alkaline). Liquids that are neither acid nor alkaline are called neutral and are indicated by a pH of 7.0.

The pH can be measured with a pH meter or with a colour indicator such as the Lovibond Comparator, which you will later learn how to use.

Water can be made more acid or more alkaline by the addition of certain buffer salts. These salts may be bought separately and then combined in the correct proportions, in a fixed volume of water, to give the required pH, or they may be bought as specially formulated tablets that produce a particular pH when added to a stipulated volume of water, e.g. 100 ml or 1 litre. If you plan to
prepare your own buffer salts, you will need to weigh them using a balance. You must also ensure that the buffer salts have been properly stored and have not been affected by absorption of moisture from the air.

Making up buffered water

Equipment

One chemical balance, readable to 0.01 g or better (a two-pan trip balance is ideal)
Two filter papers, 11 cm in diameter
One conical flask, capacity 1000 ml
One beaker, capacity 250 ml
Two wooden spatulas (wooden tongue depressors may be used)
Distilled or deionized water, 1000 ml
Potassium dihydrogen phosphate (anhydrous) (KH₂PO₄)
Disodium hydrogen phosphate (anhydrous) (Na₂HPO₄)

Method

Step 1 Make sure that the pointer of the balance is set to zero. If it is not, adjust the balancing screw on the right-hand arm until the needle points to zero; your tutor or facilitator will help you to do this.

Step 2 Place one filter paper in each pan of the balance. Set the balance to zero again, by moving the gram weight along the gram scale arm.

Step 3 Now move the gram weight a further 0.7 g along the scale arm, ready for weighing the potassium dihydrogen phosphate.

Step 4 Using a wooden spatula, take some of the potassium dihydrogen phosphate from the container and place it on the filter paper in the left-hand pan. Watch the balance pointer. Continue to add or subtract the salt until the balance pointer is again at zero.

Step 5 Transfer the 0.7 g of potassium dihydrogen phosphate to the beaker and add about 150 ml of water. Stir with the second spatula until the salt is dissolved.

Step 6 Replace the filter paper in the left-hand pan with a new one.

Step 7 After resetting the balance to zero to allow for the weight of the filter paper, adjust the gram weight to 1 g for the disodium hydrogen phosphate.

Step 8 Using the dry wooden spatula, add the disodium hydrogen phosphate to the filter paper, balancing the weight as described in step 4.

Step 9 Dissolve the disodium hydrogen phosphate in the water already in the beaker from step 5.

Step 10 When the salt is dissolved, add the fluid from the beaker to the conical flask; add water up to the neck of the flask (this makes approximately 1 litre).

The buffered water is now ready for adjusting to pH 7.2. However, before checking and adjusting the pH, you must have the correcting fluids ready for use; they will need to be made up next.
Making up the 2% correcting fluids

**Equipment**

One chemical balance, readable to 0.01 g or better (a two-pan trip balance is ideal)
Two filter papers, 11 cm in diameter
Two glass-stoppered bottles, capacity 100 or 150 ml
Potassium dihydrogen phosphate (anhydrous) (KH₂PO₄)
Disodium hydrogen phosphate (anhydrous) (Na₂HPO₄)
Distilled or deionized water, 200 ml
Two wooden spatulas
Two beakers, capacity 250 ml
One measuring cylinder, capacity 100 ml
Labels

**Method**

**Step 1** Follow steps 1 and 2 of the method for making buffered water, then move the gram weight along the scale arm a further 2 g.

**Step 2** Weigh 2 g of disodium hydrogen phosphate and add it to 100 ml of water in the beaker; stir with the wooden spatula until the salt has dissolved.

**Step 3** Pour the solution into one of the glass bottles and label the bottle “2% disodium hydrogen phosphate”.

**Step 4** Repeat steps 1 and 2 above, this time weighing out 2 g of potassium dihydrogen phosphate. Pour the solution into the second glass bottle and label it correctly.

*Note:* When not being used, the bottles should be stored in a cool place, away from sunlight.

Checking and adjusting the pH of the buffered water

It is very important that you check the pH of the buffered water before you use it. To alter the pH you will need to add small quantities of one of the correcting fluids: 2% Na₂HPO₄ if the pH is below 7.2 (too acid) or 2% KH₂PO₄ if the pH is above 7.2 (too alkaline). If the pH is not 7.2, it may be adjusted following the method outlined below.

**Equipment**

One conical flask containing the buffered water
Correcting fluids (2% Na₂HPO₄ and 2% KH₂PO₄)
One Lovibond Comparator fitted with 2/1H bromothymol blue disc
Two Lovibond glass cells
One bottle of bromothymol blue indicator
One pipette, capacity 1 ml

**Method**

**Step 1** Pour the buffered water from the conical flask into each of the Lovibond glass cells until the 10-ml mark is reached. Place one cell in the left-hand compartment; this is the control cell.
Step 2 Pipette 0.5 ml of bromothymol blue indicator into the other cell. Mix the colour indicator and place the cell in the right-hand compartment.

Step 3 Holding the Lovibond Comparator up towards a clearly lit background, turn the colour disc until its colour matches that of the fluid in the right-hand cell.

Step 4 Adjust the pH of the water remaining in the conical flask by adding small quantities of the 2% correcting fluids. To make the water more alkaline you need to add the solution of disodium hydrogen phosphate; to make it more acid add the solution of potassium dihydrogen phosphate. Recheck pH using the Lovibond Comparator.

Staining the blood films

Regular method, for 20 or more slides

Equipment
Stock of Giemsa stain
Methanol
Absorbent cotton wool
Staining troughs (to hold 20 slides, placed back to back)
Distilled/deionized water, buffered to pH 7.2
Measuring cylinder, capacity 100–500 ml (depending on the number of slides to be stained)
Measuring cylinder, capacity 10–25 ml (depending on the amount of stock stain to be measured)
Flask or beaker (capacity will depend on the amount of stain to be made up)
Timing clock
Slide-drying rack

Method

Note: For this method, it is better if slides have dried overnight.

Step 1 Fix each thin blood film by dabbing it gently with a pledget (small piece) of cotton wool dampened with methanol or by dipping it in a container of methanol for a few seconds. Avoid methanol, or its fumes, coming into contact with the thick film, otherwise fixation may take place and will prevent proper staining.

Step 2 Place the slides, back to back, in a staining trough, making sure that all thick films are at one end of the trough.

Step 3 Prepare a 3% solution of Giemsa stain by adding 3 ml of Giemsa stock solution to 97 ml of buffered water.

Step 4 Pour the stain gently into the trough until the slides are totally covered. Avoid pouring the stain directly on to the thick films.

Step 5 Leave the slides in the stain for 30–45 minutes. Experience will indicate the correct time for each batch of slides.

Methanol (methyl alcohol) is highly toxic and can cause blindness or death if swallowed. It should be stored in a lockable cupboard.
Step 6 Pour clean water gently into the trough to float off the iridescent "scum" on the surface of the stain. The water should be poured into the end of the trough where the thin films are, to avoid undue disturbance of the thick films.

Alternatively, gently immerse the whole trough in a bowl or basin filled with clean water.

Step 7 Gently pour off the remaining stain and rinse again in clear water for a few seconds. Then pour off the water.

Step 8 Remove the slides one by one and place them, film side downwards, in a drying rack to drain and dry, making sure that the thick film does not touch the edge of the rack.

Rapid method

The rapid staining method is generally used for between 1 and 5 slides at a time. You would use this method when you need to check urgently whether or not a patient has malaria. Much larger quantities of stain are required than for the regular method.

Equipment

Giemsa stain in a 25-ml bottle
Methanol\(^1\)
Absorbent cotton wool
Test tubes, capacity 5 ml
Distilled/deionized water, buffered to pH 7.2
Pasteur pipette, with rubber teat
Curved plastic staining tray or plate
Slide-drying rack
Timing clock
Small electric hair-drier or spirit lamp

Method

Thick blood films must be thoroughly dry before they are stained. They can be dried more quickly with warm air blown from a small hair-drier or by exposing them to the heat from a spirit lamp. However, great care must be taken to avoid making slides hot to the touch, otherwise films will be heat-fixed and will not stain properly.

Step 1 Fix the thin film by dabbing it with a pledget of cotton wool dampened with methanol or by dipping it in a container of methanol for a few seconds. Avoid methanol, or its fumes, coming into contact with the thick film, otherwise fixation may take place and will prevent proper staining.

Step 2 Use a test-tube or small container to hold the prepared stain. Make up a 10% Giemsa solution with distilled/deionized water buffered to pH 7.2. If only one slide is to be stained, you will require about 3 ml of prepared stain. Allow 3 drops of stock Giemsa solution (from the

\(^1\) Methanol (methyl alcohol) is highly toxic and can cause blindness or death if swallowed. It should be stored in a lockable cupboard
Pasteur pipette) to each millilitre of buffered water to give a 10% solution.

**Step 3** Gently pour the stain on to the slides (or use a pipette to drop the stain on to the slide).

**Step 4** Stain the film for 5 to 8 minutes. Experience will indicate the correct time for each slide (or batch of slides).

**Step 5** Gently flush the stain off the slide by adding drops of clean water. Never pour the stain off the slides, otherwise the surface scum will stick to the film and spoil it for microscopic examination.

**Step 6** Place the slide in the drying rack, film side downwards, to drain and dry. Make sure that the thick film does not touch the edge of the rack.

**Use of Giemsa stain**

Giemsa stain, which is a mixture of eosin (pink-staining) and methylene blue, will be provided to you as a made-up stock solution in bottles of 100 ml, 250 ml or larger capacity.

There are a number of things that you should do, and other things you should not do, with the stock solution of Giemsa stain.

What you should do

- When the bottle of stock Giemsa is not being used, keep the stopper screwed tightly to prevent evaporation of the solvent and oxidation of the stain; the stock solution will then last longer.
- Keep the stain in a dark glass bottle and store it away from direct sunlight.
- Measure a small quantity of stain into a smaller bottle for one or two days' use: again, the stock solution of stain will last longer.
- Store the stock solution in a cool dry place at all times.

What you should not do

- Never add water to the stock solution of stain: the smallest amount of water will cause deterioration of the solution so that it will no longer stain properly.
- Do not shake the bottle of stain before use: you will resuspend very small, undissolved crystals of stain, which can settle on the blood films during staining and obscure parts of the microscope field during examination.
- Never return unused stain to the stock bottle: it is better to measure out a small quantity for one or two days' use.

**Care of glassware**

Glassware such as measuring cylinders, pipettes and staining troughs must always be clean and dry before use.

Any glassware that has been used for Giemsa stain should be rinsed in clean water immediately after use to remove as much of the stain as possible. It should then be soaked for some time, preferably overnight, in a detergent solution.
Washing glassware in detergent gives satisfactory results provided that you rinse it thoroughly in clean water. Deposits of detergent left on glassware can upset the pH of buffered water and spoil the staining, so always make sure that glassware is properly rinsed before being dried for future use.

Any stain deposits that are allowed to dry on glassware will become difficult to remove and may spoil the staining of subsequent blood films. They can be removed by soaking the glassware in methanol and then washing it with detergent in the normal way.
Notes
LEARNING UNIT 6

The microscope

Learning objectives

By the end of this Unit you should:

- be able to demonstrate the use of the microscope with artificial or natural light
- be able to demonstrate use of the oil immersion objective
- be able to operate the mechanical stage
- know the names of the main components of the microscope
- know how to maintain the microscope and its components in good working order
- know what should not be done to the microscope and its components
- know how to store the microscope
- know how to pack the microscope for transportation from one place to another.

You cannot do your job without a microscope. It is important that you learn how to use it properly, that you understand its limitations and that you know what needs to be done to keep it in good condition.

The microscope that you will use is called a compound microscope. You will need to know the names of some of its component parts:

- so that you can easily carry out instructions during the practical exercises that are part of your training
- so that you can accurately describe parts that may need attention or replacement during the course of your work.

Parts of the compound microscope

All the main parts of a typical compound microscope are illustrated in Fig. 1.

Main tube and body tube

The main tube and body tube are often collectively called the head of the microscope. The head generally slopes towards the user for greater comfort and is then called an inclined head. Polished glass prisms are fitted inside the body tube of an inclined head; these allow the light to bend so that the image reaches the user’s eye.

The eyepiece, or ocular, is located at the top of the main tube. Most compound microscopes are fitted with binocular heads, which means they have two oculars — one for each eye. Some, however, have only one ocular and are referred to as monocular microscopes.
Fig. 1 Parts of a typical compound microscope

1. Main tube
2. Body tube (prism) \{ inclined head
3. Revolving nosepiece
4. Objective
5. Stage (mechanical stage)
6. Substage condenser with iris diaphragm
7. Mirror
8. Base (foot)
9. Ocular (eyepiece)
10. Arm (limb)
11. Coarse adjustment
12. Fine adjustment

Revolving nosepiece

A number of objective lenses of different magnifications are screwed into the nosepiece of the microscope, which can then be revolved to increase or decrease the magnification of the specimen being examined.

Objectives

All parts of the microscope are important, but the objective lenses — the lower, magnifying lenses — must be treated with particular care. The lenses are of the best quality and need to be handled very carefully. Sometimes two lenses are glued together, and you must be careful not to use solvents such as strong alcohol solutions or acetone, which could dissolve the glue or cement.

Objectives are referred to by their magnifying power, which is marked on the side. The microscope you will use has the following objectives:
- \times 10
- \times 40
- \times 100 (this objective is often called the oil immersion objective; sometimes it has a black or red ring around it for easy identification).

You will notice in the diagram above that the size of the front lens of the objective decreases as magnifying power increases.

As the magnification differs between objectives, so does the working distance. The working distance is the distance between the front lens of the objective and the specimen on the stage (when the specimen is in focus). The higher the magnifying power of the objective, the shorter is the working distance. Working distances for the standard objectives are likely to be as indicated below (depending on the make of the microscope):

- \times 10 \quad 15.98 \text{ mm}
- \times 40 \quad 4.31 \text{ mm}
- \times 100 \quad 1.81 \text{ mm} (this is the oil immersion objective).

The mechanical stage

The mechanical stage holds the slide secure and allows the specimen to be moved smoothly backwards, forwards or sideways. Sometimes a scale is fitted to two sides of the stage to show the extent of the movement. This is called the Vernier scale, and it is useful to know how to use it; it can be used to trace a part of the blood film that you need to re-examine or show to your supervisor.
Substage condenser (with iris diaphragm)

The substage condenser is made up of a number of lenses. These centre the light from the mirror, or electric light source, to a central spot on the field. The substage condenser can be raised or lowered to give maximum or minimum illumination.

Inside the condenser is the iris diaphragm. This is used to control the amount of light passing through the condenser. The iris diaphragm consists of a number of interlocking leaves made of a thin metal. It is adjusted by means of a lever.

Filter holder and blue filter

Beneath the iris diaphragm is the filter holder. This is where a blue filter is placed when you use an electric light source for illumination. It has the effect of making the microscope field white rather than yellow.

Mirror

The mirror is used to direct light from the light source to the microscopic field. The mirror has two sides, one of which is a plane or flat surface and is used with the substage condenser. The other surface is concave and is used without the condenser (the curved surface itself acts as a condenser).

Note: Some microscopes with a built-in illuminator do not use a mirror but have a built-in prism instead, which directs light from the illuminator to the objective/ocular lens system. Others have a removable illuminator, which can be replaced by a mirror when necessary.

Ocular (eyepiece)

The ocular or eyepiece fits into the upper end of the main tube and is what the microscopist looks through when using the microscope. The ocular has its magnifying power marked on it. Magnifying power is the number of times by which it will magnify the image produced by the objective. For instance, with an ocular of ×7 and an oil immersion objective of ×100, the total magnification of the specimen would be 7×100 = 700.

Oculars are available in a range of powers. In malaria microscopy, an ocular of ×7 magnifying power is preferred. An ocular of ×6 could also be used, but one of ×10 magnifying power is not recommended.

Oculars fitted to binocular microscopes are called paired oculars and are specially made to suit the microscope in question. On the rim of the oculars you may see the marking "×7P", which denotes a paired set of eyepieces of ×7 magnifying power.

Arm

The arm forms a rigid support for the main tube and stage of the microscope. It is strongly made and can be used to carry the microscope around the laboratory. It is recommended, however, that you also support the microscope at the base, with your other hand.

Coarse and fine adjustment

The two adjustment systems — coarse and fine — are used to focus the specimen being examined. The coarse adjustment is for rapid and relatively
large movements of the stage (and therefore the specimen); the fine adjustment is for the finer focusing required when the higher powered objectives are used.

It is normal to focus the specimen first with the coarse adjustment and then to use the fine adjustment while the specimen is being examined.

With the oil immersion objective, the coarse adjustment is used in a different way. This will be explained later.

Base (foot)

Whatever the shape of the base of the microscope (usually U-shaped or rectangular), it must rest on a firm, flat bench or table. It is essential that the microscope does not wobble while it is being used.

A threaded hole can be seen on the underside of the base. This is to take a screw that secures the microscope inside its storage box during transportation.

Use of the microscope

In the practical sessions you will learn how to use the microscope. You will see how the image (of the specimen) appears larger as you increase the magnification by changing objective lenses.

Initially you will examine specimens given to you by your tutor or facilitator. Some of these will be living organisms in water, others will be everyday objects with which you are familiar (although they will look very different under the microscope).

During these exercises you will learn how to adjust the illumination, and see how to make the best use of the substage condenser and iris diaphragm. You will also be able to use the mechanical stage and the Vernier scale.

You will have time to practise on a monocular microscope and will notice that its illumination is very good. This is the microscope that should be used with the ×100 oil immersion objective when only natural light is available. The binocular microscope, though more restful for long hours of work, needs a reliable electricity supply for illumination; with only natural light available, it is less efficient than the monocular when used with the oil immersion objective.

The light source

A good source of light is needed to examine specimens properly. This may be either daylight or electric light. The electric current may be provided by mains supply or by a battery or generator. Light that is either too bright or too dim will interfere with examination of specimens.

Light from the light source travels the following path, via the substage mirror or the substage lamp:

light source → mirror (if there is one) → substage condenser and diaphragm (if the source is artificial) → specimen → objective → ocular
When artificial light is used, a blue filter must be placed between the source and the substage condenser. If a mirror is employed with artificial light, the flat side of the mirror should be used; when daylight is the light source, the concave mirror should be used, without the substage condenser.

Obtaining even illumination

To obtain good, even illumination, the procedure is as follows:

**Step 1** Place the specimen slide on the mechanical stage. Using the coarse adjustment, focus on the specimen with the ×10 objective.

**Step 2** Making sure that the iris diaphragm is completely open, raise the substage condenser to the point where the field is brightest.

**Step 3** Remove the eyepiece and, while looking down the tube, adjust the mirror until the objective lens is fully illuminated.

**Step 4** Replace the eyepiece and focus sharply on the specimen using the fine adjustment.

**Step 5** Remove the eyepiece again and close the iris diaphragm until the aperture of the objective is two-thirds visible; this will make the specimen appear clearer — it gives maximum resolution.

**Step 6** Replace the eyepiece and revolve the nosepiece to select the objective of the required power; you may need to focus slightly at each change of objective. Specific directions concerning the use of the oil immersion objective are given below.

Illumination can be easily adjusted by increasing or decreasing the aperture of the iris diaphragm.

Using the oil immersion objective

When setting up the microscope for use with the oil immersion objective, the following procedure is the best:

**Step 1** After arranging the illumination as described in the previous section, observe the rest of the process from the side of the microscope.

**Step 2** Using the coarse adjustment, rack up the main tube.

**Step 3** Place the slide on the stage of the microscope with the blood film uppermost.

**Step 4** When you can see that there will be sufficient space between the stage and the ×100 objective, turn the revolving nosepiece so that the ×100 objective is over the specimen.

**Step 5** Place 1–2 drops of immersion oil on the blood film in the area which is to be examined.

**Step 6** Using the coarse adjustment, carefully lower the objective until the lens is in contact with the immersion oil. Then raise the lens slightly, but allow the lens and oil to remain in contact.

**Step 7** Focus the specimen using the fine adjustment, making sure that the lens does not come into contact with the slide. You may alter the illumination if necessary by adjusting the iris diaphragm.

Immersion oil is used between the microscope slide and the objective lens to reduce scattering of the light transmitted by the mirror or illuminator. The oil
has to reproduce the optical properties of the glass used for the lenses, and
must therefore have a refractive index of 1.515, i.e. approximately 1.5 times the
refractive index of water.

When immersion oil is used, the objective lens and the slides must be cleaned at
the end of the day’s work. You can use a soft cotton cloth or lens tissue for the
objective lens but remember not to use it to clean other lenses on the
microscope. From time to time, dried oil should be removed from the oil
immersion objective using xylene (but no other solvent). Oil can be washed off
the slides with a small amount of xylene; if no xylene is available, the oil smear
can be carefully dabbed with absorbent paper.

Immersion oil can be obtained commercially. In some countries, however,
anisole is used for work with the oil immersion objective; this product has the
same refractive index as immersion oil. Anisole evaporates from the blood film
after some time, so that the film does not need to be cleaned and there is less
chance of its being damaged or wiped off. Use of anisole also means that the
objective lens does not need to be cleaned.

**Care of the microscope**

Provided that normal care and common sense are exercised, the laboratory
microscope will be useful for many years.

**Removing dust and grease**

When not in use during the day, the microscope should be kept covered with a
clean cloth or plastic cover to protect the lenses from dust that settles out of the
air. Overnight, or if it is to remain unused for long periods, the microscope
should be placed inside its box with the door tightly closed. To protect the
objective lenses, the ×10 objective should be rotated to line up with the ocular.

Oil and grease from eyelashes and fingers are easily deposited on lenses and
oculars as the microscope is used; these parts should be cleaned with lens tissue
or with very soft cotton cloth.

The oil immersion objective should be cleaned after use. If it is not cleaned, the
oil will harden and make the objective useless. A lens tissue or soft cotton cloth
is usually sufficient for the purpose. However, the tissue or cloth should never
be used to clean other objectives, the oculars or the mirror, otherwise oil will be
transferred to these components.

**Preventing the growth of fungus**

In warm, humid climates it is very easy for fungal growths to become
established on lenses and prisms. These growths can cause problems and may
even become so bad that the microscope can no longer be used. The lenses
may need to be repolished by the manufacturer, which is very expensive and
may take several months.

Fungus cannot grow on glass when the atmosphere is dry, and every effort
should therefore be made to store the microscope in a dry atmosphere when it
is not being used. One of the following methods should be used:
- Keep the microscope in a continuously air-conditioned room.
  
  *Note:* It is pointless to store the microscope overnight in a room where the air-conditioner operates only during the day.

- Place the microscope in a "warm cupboard", i.e. an airtight cupboard in which one or two 25-watt bulbs are constantly alight.

- Keep all lenses and prism heads in an airtight box or desiccator where the air is kept dry by means of active silica gel.

  *Note:* Silica gel is a desiccant — a compound with the ability to absorb water vapour from the air. Self-indicating silica gel is blue when active but becomes pink when it has absorbed all the water it can. It can then be reactivated by heating; it turns blue again as it becomes reactivated. When the silica gel cools it can be returned to the airtight container. Only self-indicating silica gel should be used.

- In locations without electricity but where a kerosene refrigerator is used, place the microscope box on a small shelf sited 20–30 cm above the refrigerator chimney. Heat from the chimney will keep the box sufficiently warm and dry to prevent fungus growing on the microscope lenses.

**Transporting the microscope**

When the microscope is to be transported from one location to another, it is important to ensure that it is properly secure inside its box. The best way to do this is by means of the securing device, which screws into the base of the microscope.
EXAMINING UNIT 7
Examining blood films

Learning objectives

By the end of this Unit you should be able to:

- list the components of normal blood
- examine thick and thin blood films with the oil immersion objective and the x7 ocular
- recognize and classify the normal components of blood
- recognize artefacts (contaminants, etc.) that may be mistaken for malaria parasites and so cause confusion in the diagnosis of malaria.

Components of normal blood

When taken directly from a vein, and collected in a test-tube, blood is a red liquid.

If the test-tube is allowed to stand for 5–20 minutes, you will see that the blood separates into two different layers, as shown in the diagram below. The serum layer is a pale yellow fluid. The blood clot is a semisolid substance that is now dark red or almost black in colour. It contains red blood cells, white blood cells and platelets. These components are much too small to see without the aid of your microscope and can be seen clearly only after making blood films which are then stained and dried.

![Diagram of blood components](image)

Appearance of normal blood components

You need to be able to recognize the various components found in blood films. Their appearance differs slightly in thin and thick films.
Blood in thin films

When you examine thin blood films with the ×100 objective and ×7 ocular, you will see the following:

- red blood cells (or erythrocytes)
- white blood cells (or leukocytes)
- platelets (or thrombocytes).

You will see examples of all these blood components in thin films. You may use Plate 2 to help you to identify the various types of white blood cell. Your tutor will help you throughout this exercise.

Red blood cells

The shape of the red blood cell, or erythrocyte, is described as a biconcave disc; this is illustrated in the diagram on the right. The erythrocyte is the commonest cell that you will see in the thin blood film. There are about 5 000 000 red blood cells in each microlitre (μl) of blood.1 With good Giemsa staining, the red cell should be a pale greyish-pink. It measures about 7.5 micrometres (μm) in diameter.1

![Red blood cell or erythrocyte](image)

The red cell does not have a nucleus. However, some cells may contain material that has stained differently; such cells may appear larger than the normal red blood cells, or normocytes (see Plate 2, p. 47).

You will often see the abbreviation RBC used for red blood cells.

White blood cells

The total number of white blood cells or leukocytes in a microlitre of blood is about 6000–8000, which is much lower than the number of red blood cells. There are several different types of leukocytes, which stain differently from each other. With practice, it is quite easy to distinguish between them.

Before you go any further in this exercise, you need to know the main parts of a white blood cell. These are illustrated in the diagram below.

![Typical white blood cell](image)

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1 A microlitre is one-millionth (1/1,000,000) of a litre, it used to be referred to as a cubic millimetre (mm³). A micrometre is one-millionth of a metre, and is sometimes also known as a micron (μ).
You will see that every leukocyte has a nucleus surrounded by cytoplasm. Sometimes the cytoplasm is granular in appearance. Some leukocytes have a multilobed nucleus. The leukocytes can be divided into two groups, as follows.

**Group 1. Multilobed (polymorphonuclear) leukocytes**

- **Neutrophils**
  Neutrophils make up 65% of the total white cell count in the blood of a healthy person. They have well-defined granules in the cytoplasm and nuclei that stain deep purple. In cases of malaria it is common to see neutrophils containing malaria pigment, which is all that remains of the malaria parasite “eaten” or phagocytosed by the neutrophils.

- **Eosinophils**
  Eosinophils make up about 1–4% of the total white cell count in the blood of a healthy person. The granular nature of the cytoplasm is very distinctive, with the granules taking on the pinkish colour of eosin. (If you are not familiar with this colour, you will see examples in the practical exercises.)

- **Basophils**
  Basophils are rare leukocytes, usually making up less than 1% of the total. Large blue or mauve granules can be seen in the cytoplasm after staining.

**Group 2. Non-multilobed leukocytes**

- **Monocytes**
  Monocytes are the largest of the white blood cells — about 12–18 μm in diameter. The large nucleus is kidney- or bean-shaped and the cytoplasm may contain a few granules that stain pinkish or red. Monocytes make up 2–10% of the total white cell count in the blood of a healthy person; like neutrophils, they can phagocytose malaria parasites.

- **Lymphocytes**
  The two types of lymphocytes — large and small — make up 20–45% of the total white cell count.

  The nucleus of the large lymphocyte is round and appears a deep mauve colour in well-stained blood films. The large amount of cytoplasm stains a clear water-blue and may contain a few mauve-staining granules.

  The small lymphocyte is slightly larger than a normal red blood cell. It has very little cytoplasm and its nucleus stains a dark blue-black colour.

**Platelets**

Platelets are small, red-staining bodies of irregular shape and without nuclei; they number about 100,000 per microlitre of blood. They often appear in groups of 5–10 but may clump together in larger numbers if a blood film has been poorly made. It is important to be able to identify them; they may be confused with malaria parasites by inexperienced microscopists.
Blood in thick films

When you examine thick blood films with the ×100 objective and the ×7 ocular, you will see the following:

- the remains of red blood cells
- white blood cells (or leukocytes)
- platelets (or thrombocytes).

You will remember that a thick blood film consists of many dehaemoglobinized red blood cells packed together in a thick mass. When the thick film is stained with Giemsa stain, the water in the stain acts on the unpreserved red blood cell: the contents of the cell dissolve in the water. The main component of the red blood cell is called haemoglobin and so this process is called dehaemoglobinization. You can see this take place if you put a thick blood film into a dish or staining tray containing water. Within 1–2 minutes the red colour of the haemoglobin starts to flow out of the thick film, which becomes pale and opalescent.

This process happens during staining, so that all that remains on completion of staining are the remnants of the red blood cells, plus white cells and platelets.

**Fig. 2. The appearance of blood elements in thin and thick blood films**
The white blood cells and platelets have an appearance very similar to that in thin films. Because they have not been spread in a single layer on the slide, the white blood cells appear to be smaller, with the cytoplasm more compact around the nuclei. This can be seen in Fig. 2.

In this exercise you will learn to recognize the different kinds of leukocytes, the platelets, and the red cell remnants as they appear in Plate 2. If at any time you see something that you think is abnormal, bring it to the attention of your tutor or facilitator. A number of features that may appear in blood films and confuse you are illustrated in Plate 3; you will learn more about these “artefacts” in a later Learning Unit.
Notes
Examining blood films

LEUKOCYTES

N = Neutrophil, E = Eosinophil, M = Monocyte, L = Lymphocyte, P = Platelets

ERYTHROCYTES

NC = Normocyte, MC = Micocyte, PM = Polychromatic macrocyte, PC = Pkilocyte, PB = Punctate basophilia,
CR = Cabot's ring, HJ = Howell-Jolly bodies, RC = Reticular 'clouds' and chromatoid bodies in severe anaemia

pH

6.4 6.8 7.2 7.6

MALARIA STAINING AND pH

Plate 2. **Components of the blood**
Plate 3. *Artefacts that may cause confusion in diagnosis*
Examining blood films for malaria parasites

**Learning objectives**

By the end of this Unit you should be able to:

- name the various parts of the malaria parasite
- distinguish malaria parasites in thin blood films, and recognize and name the three stages of trophozoite, schizont and gametocyte
- distinguish between the malaria species and recognize and name the stage and species of malaria parasite seen in thin blood films
- distinguish between the malaria species, and recognize and name the stage and species of parasite seen in thick blood films.

**Recognition of a malaria parasite**

Malaria parasites take up Giemsa stain in a special way in both thick and thin blood films. You must be able to distinguish the various parts of the parasite, as shown in the diagram that follows.

![Diagram of malaria parasite](image)

Parts of a malaria parasite inside a red blood cell

Malaria parasites pass through a number of developmental stages. In all stages, however, the same parts of the parasite will stain the same colour:

- Chromatin (part of the parasite nucleus) is usually round in shape and stains a deep red.
- Cytoplasm occurs in a number of forms, from a ring shape to a totally irregular shape. It always stains blue, although the shade of blue may vary between the malaria species.

More will be said about the malaria species later in this Learning Unit.
Stages of the malaria parasite

Stages of the malaria parasite that you will see in blood films are described below. For this exercise, they are shown inside the red blood cell.

During the practical exercises you will learn to recognize the various stages of the parasites in thin blood films. You should ask your tutor or facilitator to confirm the stage of parasite that you identify. You can also use the key on pages 51 and 52 to help you identify whether what you see is a parasite or not and, if it is, what stage it is. (Remember, the key is for thin films only, and it does not show the effect of the parasite on the red blood cell.)

The trophozoite stage

This stage is the most commonly seen; it is often called the ring stage, although it sometimes takes the form of an incomplete ring.

The ring stages of malaria parasites

Because the trophozoite stage is a growing stage, the parasite within the red blood cell may vary in size from small to quite large. Pigment appears as the parasite grows. Malaria pigment is a by-product of the growth or metabolism of the parasite. It does not stain, but has a colour of its own, which may range from pale yellow to dark brown or black.

The schizont stage

At the schizont stage the malaria parasite starts to reproduce. This reproduction is referred to as asexual because the parasite is neither male nor female but reproduces itself by simple division. There are several obvious phases in this stage, ranging from parasites with two chromatin pieces to parasites with a number of chromatin dots and definite cytoplasm. These are clearly seen in the diagram that follows.

Stages of schizont growth
Note: The process of forming schizonts, which takes place in the liver and in blood, is referred to as schizogony.

The gametocyte stage

The gametocyte stage is sexual in that the parasites become either male or female in preparation for the next stage, which takes place in the stomach of the female anopheline mosquito. Gametocytes may be either round or banana-shaped, depending on the species. The way in which the parasite takes up the stain will also help you to identify whether what you see is male (microgametocyte) or female (macrogametocyte).

![Male and female gametocytes](image)

Key to identifying malaria parasite stages in thin blood films

This key applies to those features inside red blood cells which appear to be parasites.

1. Are there one or more red-stained chromatin dots and blue cytoplasm?  
   Yes: go to 2  
   No: what you see is not a parasite

2. Are the size and shape right for a malaria parasite?  
   Yes: what you see is probably a malaria parasite; go to 3  
   No: what you see is not a parasite

3. Is there malaria pigment in the cell?  
   Yes: go to 7  
   No: go to 4

4. Does the parasite have one chromatin dot attached to blue cytoplasm in the form of a regular ring with a vacuole?  
   Yes: this is a trophozoite stage  
   No: go to 5

5. Does the parasite have one chromatin dot attached to blue cytoplasm in the form of a small solid or regular ring or with a vacuole?  
   Yes: this is a trophozoite stage  
   No: go to 6
6. Is the parasite with one chromatin dot irregular or fragmented?
   Yes: this is a trophozoite stage
   No: go to 8

7. Does the parasite with malaria pigment have one chromatin dot?
   Yes: go to 8
   No: go to 9

8. Does the parasite have a vacuole or is it still fragmented in some way?
   Yes: this is probably a late trophozoite stage
   No: go to 11

9. Does the parasite have two chromatin dots attached to a ring and also have a vacuole?
   Yes: this is a trophozoite stage
   No: go to 10

10. Does the parasite have between 2 and 32 chromatin dots and pigment?
    Yes: this is a schizont stage

11. Is the parasite rounded or banana-shaped?
    Rounded: go to 12
    Banana-shaped: go to 14

12. Does the rounded parasite have clearly stained chromatin and deep blue cytoplasm?
    Yes: this is a female gametocyte
    No: go to 13

13. Does the rounded parasite have a reddish overall colour to the staining, so that the chromatin cannot be clearly seen?
    Yes: this is a male gametocyte

14. Does the banana-shaped parasite have densely stained blue cytoplasm and bright red chromatin?
    Yes: this is a female gametocyte
    No: go to 15

15. Does the banana-shaped parasite have a reddish overall colour to the staining, so that the chromatin is indistinct?
    Yes: This is a male gametocyte
Species of malaria parasite

You have been learning how to recognize malaria parasites and their stages in thin films and have so far concentrated only on their appearance. However, the effect the parasite has on red blood cells is also important because it will help you to identify the malaria species.

The four species of malaria

There are four species of malaria that affect humans:

- *Plasmodium falciparum*: the commonest species in the hotter parts of the world and responsible for much sickness and even death.
- *P. vivax*: the commonest species in the cooler parts of the tropics, the largest of the malaria parasites found in humans, and the cause of much illness.
- *P. malariae*: a less common species but one that occurs throughout much of the world.
- *P. ovale*: a relatively rare species but reported from time to time in many countries, especially in Africa: sometimes confused with *P. vivax*.

Appearance of parasite species in thin blood films

The simplest guide to distinguishing between the four species of malaria is the effect the parasite has on infected red blood cells. Features to concentrate on include the size of the red blood cell (whether or not it is enlarged) and whether or not staining reveals Schüffner’s dots or Maurer’s dots (also known as Maurer’s clefts) within the cell.

The diagnostic features outlined in Fig. 3 will help you to decide which species of malaria parasite you have found. If you have any problem using this key, your tutor or facilitator will help you. You should also consult the colour plates (Plates 4–7) in this Guide. The left-hand side of each plate shows the various stages of the different species of malaria parasite as they appear in thin blood films, and the right-hand side shows their appearance in thick blood films.

You will be given plenty of time to practise identification of species in thin films. Once your tutor considers that you are able to identify accurately the stage and species of malaria parasite, you will move on to the examination of parasites in thick blood films.

Appearance of parasite species in thick blood films

Just as the appearance of both red and white blood cells differs in thin and thick blood films, so too there are differences in the appearance of malaria parasites.

The first difference is obvious as soon as you look at a thick film with the ×100 oil immersion objective and the ×7 ocular: there are no red blood cells. The malaria parasites, however, can be seen, although, like the white blood cells, they appear to be smaller than in thin blood films. You may need to look quite carefully before you see them. You will need to refocus, using the fine adjustment, each time you move the microscope field: this will allow you to examine the thick film at different depths.

The fine rings of cytoplasm of the trophozoites may appear incomplete or broken. This is the normal appearance of trophozoites in thick blood films.
Fig. 3. **Species differentiation of malaria parasites by host-cell change in Giemsa-stained thin blood films**
Plate 4. Appearance of *Plasmodium falciparum* stages in Giemsa-stained thin and thick blood films
Plate 5. Appearance of *Plasmodium vivax* stages in Giemsa-stained thin and thick blood films
Plate 6. Appearance of *Plasmodium ovale* stages in Giemsa-stained thin and thick blood films
Plate 7. Appearance of *Plasmodium malariae* stages in Giemsa-stained thin and thick blood films
Fig. 4. **Species differentiation of malaria parasites by cytoplasmic pattern of trophozoites in Giemsa-stained thick blood films**

- **Trophozoites**
  - **Regular cytoplasm**
    - **Uniform**
    - **Compact**
      - *P. falciparum*
      - Associated stages: banana-shaped or rounded gametocytes with rice-grain-like dark pigment, sometimes with pinkish extrusion body or "tongue" (Schizonts usually not seen except in heavy infection with many ring forms.)
    - *P. malariae*
      - (abundant pigment with yellow tinge in older forms)
  - **Irregular cytoplasm**
    - **Markedly fragmented**
      - Associated stages: schizonts and gametocytes usually seen
      - "Ghost" of host cells, with faint Schüffner's dots seen at film edge
    - *P. vivax*
    - (irregular wisps or fragmented cluster)
    - Associated stages: schizonts and gametocytes usually seen
      - "Ghost" of host cells, with prominent Schüffner's dots at film edge
    - *P. ovale*
<table>
<thead>
<tr>
<th>Species</th>
<th>Stage of parasite in peripheral blood</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Plasmodium falciparum</em></td>
<td>Trophozoite: Young growing trophozoites and/or young schizonts usually seen. Size small to medium; number: often numerous; shape: ring and comma forms common; chromatin: often two dots; cytoplasm: regular; fine to fleshy; mature forms: sometimes present in severe malaria, compact with pigment as few coarse grains or a mass.</td>
<td>Schizont: Usually associated with many young ring forms. Size: small, compact; number: few. Common: usually in severe malaria; mature forms: 12–30 or more merozoites in compact cluster; pigment: single; dark mass.</td>
</tr>
<tr>
<td><em>Plasmodium malariae</em></td>
<td>All stages seen.</td>
<td>Size: small; number: few to moderate; shape: broken ring to irregular forms common; chromatin: single, occasionally two; cytoplasm: irregular or fragmented; mature forms: compact, dense; pigment: scattered, fine.</td>
</tr>
<tr>
<td><em>Plasmodium ovale</em></td>
<td>All stages seen.</td>
<td>Size: may be smaller than <em>P. vivax</em>; number: usually few; shape: ring to rounded, compact forms; chromatin: single, prominent; cytoplasm: fairly regular, fleshy; pigment: scattered, coarse.</td>
</tr>
<tr>
<td><em>Plasmodium vivax</em></td>
<td>All stages seen.</td>
<td>Immature and certain mature forms difficult to distinguish from mature trophozoites. Size: small; number: usually few; shape: ring to rounded, compact forms; chromatin: single, large, cytoplasm: regular, dense; pigment: scattered, abundant, with yellow line in older forms.</td>
</tr>
</tbody>
</table>

Plate 8. *Species identification of malaria parasites in Giemsa-stained thick blood films*
Similarly, the absence of red blood cells may make the Schüffner's dots difficult to see; in fact, in the thicker parts of the film, it may not be possible to see the stippling at all. However, the "ghosts" of red cells can usually be seen surrounding parasites in the thinner parts of the films, often towards the edge, and this will help you make your diagnosis.

*Note:* The Maurer's dots of *P. falciparum* cannot be seen in thick films.

Fig. 4 provides a key for species identification in thick films; you can also get help from Plates 4–7 (right-hand side) and from Plate 8. With practice, you will soon become competent at identification of species.
Artefacts in blood films

Learning objectives

By the end of this Unit you should:

- know that blood contaminants may be confused with malaria parasites
- recognize and be able to distinguish between malaria parasites and common artefacts
- know the various ways in which artefacts can contaminate blood films
- know how to prevent artefacts from contaminating blood films.

By this stage in your training you are likely to have seen a number of objects in blood films that have caused you some confusion; if you could not identify them as parasites you have probably wondered what they are.

Blood films may contain many features that can cause confusion and problems in diagnosis. Such features are known as artefacts. Some are more common than others; some are easier to prevent than others.

Fungus will show up as artefacts on blood films. The best way to prevent fungal growths on slides is to stain blood films as soon as possible after making and drying them — within 48 hours at most. Unfortunately, this is not always practicable.

Other contaminants will be picked up from the environment. Dust particles floating in the air will settle on blood films while they are drying; either before or after staining. Specks of dirt may be transferred from a patient's finger when a blood sample is taken, or the original slide may not be perfectly clean.

Plate 3 (page 47) shows a variety of artefacts that may be seen in both thick and thin blood films. The examples are not exhaustive but give a good idea of the size, shape and colour of different kinds of artefact.

You may occasionally see microfilariae (e.g. Wuchereria bancrofti) in blood films you are examining. These are not, strictly speaking, artefacts and you should report what you see, both on your record form and directly to your supervisor.
Notes
LEARNING UNIT 10

Routine examination of blood films for malaria parasites

Learning objectives

By the end of this Unit you should:

- know that thick and thin blood films must be examined in a specific way for consistency
- know that the thin film is examined only exceptionally for malaria parasites and know what the exceptions are
- be able to demonstrate the skills needed for systematic examination of both thick and thin films by the standard methods
- know why parasite counts must be recorded
- be able to use accurately the two methods described in this Unit for establishing parasite density.

Examining the thin film

Since it takes almost 10 times as long to examine a thin film as to examine a thick film, routine examination of thin films is not recommended. Only a very few could be properly examined in a day’s work.

However, examination of thin films is recommended in the following circumstances:

- when it is not possible to examine a thick film because it is too small, has become autofixed, or is unexaminable for some other reason;
- when it is necessary to confirm the identification of a species.

When a thin film does have to be examined, this should be done in a systematic, standard way as follows:

Step 1 Place the slide on the mechanical stage.

Step 2 Position the ×100 oil immersion objective over the edge of the middle of the film (as shown by the × mark in the diagram on page 66).

Step 3 Place a drop of immersion oil on the edge of the middle of the film.

Step 4 Lower the oil immersion objective until it touches the immersion oil (as described on page 38).

Step 5 Examine the blood film, following the pattern of movement shown in the diagram, that is by moving along the edge of the thin film, then moving the slide inwards by one field, returning in a lateral movement and so on.
Step 6  Continue the examination for approximately 100 fields to determine whether the blood film is positive or negative for malaria. If doubtful diagnosis makes it necessary, more fields (up to 400) may be examined.

Examination of a thin blood film

Examining the thick film

Routinely, it is thick blood films that are examined. Provided that they have been well made and stained before autofixation could take place, there should be no problems in identifying the species of malaria parasites. Occasionally, however, you may find that it is difficult to tell the difference between the later, mature trophozoites and the gametocytes of P. vivax and between P. malariae trophozoites and rounded P. falciparum gametocytes. Also, it is not possible to distinguish between late trophozoites and gametocytes of P. malariae in thick films, but the need to know whether gametocytes are present in blood is usually confined to P. falciparum, and this is a relatively easy diagnosis to make.

Routine examination of a thick film is based on examination of 100 good fields. That is, a slide can be pronounced negative only after no parasites have been found in 100 fields of the blood film. If parasites are found, a further 100 fields should be examined before a final identification of species is made. This ensures that there is little possibility of a mixed infection (more than one species present in the blood film) being overlooked.

The technique for thick film examination is as follows:

Step 1  Using the ×40 objective, scan the film for any microfilariae that may be present. At the same time, select a part of the film that is well stained, free of staining debris, and well populated with white blood cells. If the film is well made and of even thickness, this should present no problems; poorer quality films may need to be quite extensively searched.

Step 2  Place immersion oil on the thick film.

Step 3  Swivel the ×100 oil immersion objective over the selected portion of the blood film.

Step 4  Lower the objective so that it touches the immersion oil.

Step 5  Confirm that the portion of the film selected is acceptable and continue to examine the slide for 100 oil immersion fields. Move the blood film by one oil immersion field each time, following the pattern shown in the diagram on page 67. Remember to use the fine adjustment for focusing.

Step 6  To assist with your examination, you should use a hand tally counter to count the fields as they are examined. (You will also use this to help you carry out a parasite density count later, in another exercise.)
Examination of a thick blood film

At the end of the examination, record your findings on the appropriate record form. Your result will probably include a parasite count.

Establishing a parasite count

It is necessary to establish a parasite count for the blood film for the following reasons:

- The physician may want to know how severe the malaria is.
- The physician may need to know whether the malaria parasites are responding to the antimalarial treatment being given. This can be monitored over time by plotting the parasite count on the day of treatment and comparing it with the count in a blood film made at some specified later time.
- Parasite counts are especially important in P. falciparum infections which are potentially fatal.
- The district health officer needs to know the severity of malaria infections being seen in the local health facilities.
- The data may be needed for special purposes, such as testing the sensitivity of parasites to antimalarial drugs.

Two methods are used to establish the parasite count. You would not start this procedure until you had completed your 100-field examination and identified the parasite species and stages present.

Method 1: parasites per microlitre of blood

This is a practical method of reasonable and acceptable accuracy. The number of parasites per microlitre of blood in a thick film is counted in relation to a standard number of leukocytes (8000). Although there are variations in the number of leukocytes between healthy individuals and even greater variations between individuals in ill health, this standard allows for reasonable comparisons.

You will need two tally counters, one to count parasites and the other to count leukocytes.

**Step 1 (a)** If, after 200 leukocytes have been counted, 10 or more parasites have been identified and counted, record the results on the record form in terms of the number of parasites per 200 leukocytes.

(b) If, after 200 leukocytes have been counted, 9 or fewer parasites have been counted, continue counting until you reach 500 leukocytes on your tally counter; then record the number of parasites per 500 leukocytes.
**Step 2** In each case, the number of parasites relative to the leukocyte count can be converted to parasites per microlitre of blood by the simple mathematical formula:

\[
\text{number of parasites} \times 8000 \div \text{number of leukocytes} = \text{parasites per microlitre}
\]

In effect, this means that if 200 leukocytes are counted, the number of parasites is multiplied by 40 and if 500 leukocytes are counted the number of parasites is multiplied by 16.

*Note:* It is normal practice to count all the species present and to count and record separately the gametocytes of *P. falciparum* and the asexual parasites. This is particularly important when monitoring the response to schizontocidal drugs, which would not be expected to have any effects on the gametocytes.

**Method 2: the plus system**

A simpler method of counting parasites in thick blood films is to use the plus system. This system is less satisfactory, however, and should be used only when it is not possible to carry out the more acceptable count of parasites per microlitre of blood.

The system entails using a code of between one and four plus signs, as follows:

- + = 1–10 parasites per 100 thick film fields
- ++ = 11–100 parasites per 100 thick film fields
- +++ = 1–10 parasites per single thick film field
- ++++ = more than 10 parasites per single thick film field
LEARNING UNIT 11

Life cycle of the malaria parasite

Learning objectives

By the end of this Unit you should be able to:

- describe the life cycle of the malaria parasite in humans
- describe that part of the life cycle which is spent in the female Anopheles mosquito (the vector of malaria)
- recall the various stages of the malaria parasite found in human blood.

In humans

The liver phase

When an infected female Anopheles mosquito bites a human being, malaria parasites are introduced into that person.

The parasites move quickly to the liver, where each invades a liver cell. Over a period of 7–21 days the parasite grows and reproduces. Finally the liver cell bursts, releasing parasites into the bloodstream, where each attaches to and invades a red blood cell.

This description is invariably true of Plasmodium falciparum and P. malariae infections. In P. vivax and P. ovale infections, however, some of the parasites remain in the liver and do not reproduce immediately. It is these dormant parasites that are responsible for the relapses that occur in patients with P. vivax and P. ovale infections.

The blood phase

From the work you have done in earlier Learning Units you will already be familiar with the different stages of the blood phase, which are shown diagrammatically in Fig. 5.

In the mosquito

The sexual phase of the malaria life cycle takes place in the stomach of the mosquito. Soon after the female Anopheles mosquito has ingested blood from an infected person, the male gametocytes (microgametocytes) each produce 4–8 flagella. These flagella enter, and fertilize, the female gametocytes (macrogametocytes). The mobile products of this fertilization burrow through the stomach wall and develop into cysts in the lining of the abdomen. When
the cysts rupture, they release sporozoites which eventually enter the salivary glands. After a period of time which varies according to the species of mosquito and the ambient temperature but which is usually between 7 and 14 days, the anopheline mosquito is able to transmit malaria.
Supervisory aspects of malaria microscopy

**Learning objectives**

By the end of this Unit you should:

- understand why supervision of your work is necessary
- be aware that supervision can be carried out in a number of ways
- understand what you must do in order to help your supervisor in his or her job.

**The need for supervision**

Supervision is necessary for a number of reasons:

- It confirms that you are doing your job as you have been trained to do it.
- It enables you to make minor but necessary corrections to your work.
- It should indicate whether you need retraining or are suitable for further and more advanced training.
- It provides a good opportunity for you to discuss with your supervisor any difficulties you may be having in your work.

**Types of supervision**

There are two basic types of supervision — direct and indirect.

**Direct supervision**

In direct supervision your supervisor is able to be in constant touch with you over a period of time. That period may be a single day, if your supervisor is visiting your place of work, or longer. The supervisor is able to see what you do in your job and how you do it. You have the opportunity to discuss important aspects of your work, and this is helpful to both of you.

**Indirect supervision**

In indirect supervision the supervisor can judge how well you are working only from the records that you submit regularly. However, he or she also needs to see how you are dealing with your slides, what the quality of your staining is, and how accurately slides are being examined, and may also want to know whether or not your microscope and other equipment are in good working order.

To assess the quality of staining and blood film examination, the supervisor will need to re-examine, or cross-check, a number of your slides. Usually, you
will send all the positive slides to your supervisor so that the species and density of parasites can be confirmed and to ensure that you are not mistaking artefacts for parasites. You will also send 10% of the slides that you have found to be negative, and these will be re-examined to make sure that you are not overlooking the low-density positives.

Selecting 10% of the negative slides is not difficult. The most common system is for your supervisor to indicate selection by the last digit of the slide number. For example: you might be instructed to select all slides whose number ends with 5. Provided that you have consecutively numbered your slides for the month, this is an easy task. (If any of the slides whose number ends with 5 happens to be positive, simply select the slide next to it, say the one ending in 6.) The selected slides will then be sent to your supervisor, with the forms and the short report that you may have written.

It is your supervisor’s task to examine your slides as soon as possible after receiving them. If there is any disagreement about your diagnoses or parasite counts, this should be discussed with you at the earliest opportunity. Preferably, you should see the slide or slides in question, so that you can be made aware of where you went wrong.

Referring to the Learning Units, diagrams and colour plates in this Guide will continue to help you in your daily work. Remember that you have a very important job to do and that you must try to do it as well and as accurately as you can. Even though your supervisor may be far away, he or she should always be informed of any problems you may encounter: it is the supervisor’s job to help you when difficulties arise.
Effective management of malaria relies heavily on accurate and timely diagnosis, which in turn depends on sufficient numbers of health workers being trained in appropriate microscopy techniques. Recognition of the increasing need for such personnel prompted the publication of this comprehensive, two-part training module on basic malaria microscopy. The module is one of three, each concerned with a different aspect of the fight against malaria, and is intended for use in training courses devoted exclusively to diagnostic microscopy or as part of broader teaching programmes.

Part I – the Learner’s Guide – consists of a series of Learning Units, with clearly stated learning objectives, which take trainees through each step of the diagnostic routine in the correct sequence. Topics covered include preparation of microscope slides, blood sampling, use of the microscope, and identification of malaria parasites. The information contained in the Learning Units is designed to accompany a programme of lectures, demonstrations, group discussions, field work and – most importantly – practical laboratory work. Its completeness is intended to obviate the need for trainees to make more than the briefest notes during the course and to provide them with a valuable reference work when they return to their jobs. Detailed colour plates are included in the Guide, which will be of particular assistance in the correct identification of the species and stage of malaria parasite observed in blood samples.