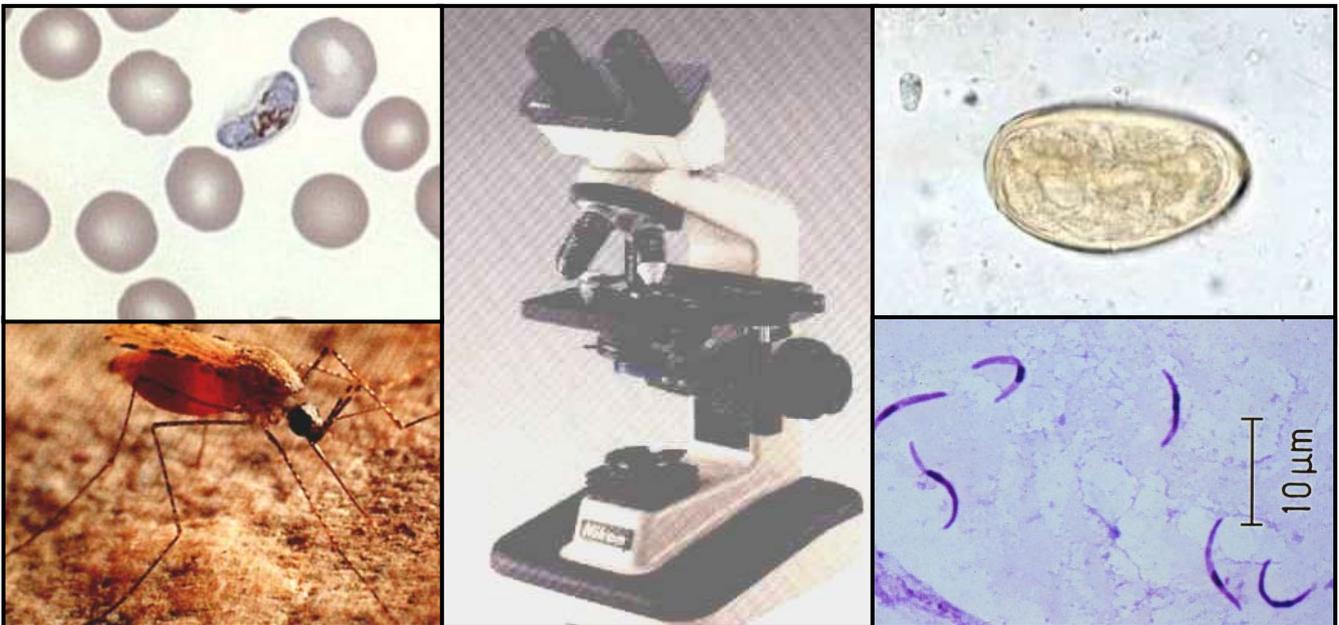




# *LABORATORY MANUAL FOR LABORATORY TECHNICIAN TRAINING*



2002  
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Thailand

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### **FRONT COVER PICTURES:**

Upper left: *Plasmodium falciparum* gametocyte on a Giemsa stained thin blood film.

Upper right: *Paragonimus westermani* egg in sputum. Wet preparation, X40 objective.

Down left: *Anophele* female mosquito feeding.

Down right: *Plasmodium falciparum* sporozoites in a smear of mosquito's salivary gland, Giemsa stain.

## **Preface**

This laboratory manual is a selected review of the main laboratory techniques used for the training of laboratory technicians working in the camp's laboratory along the Thai – Myanmar border. All the tests presented in the manual correspond to the disease most commonly found along the border. The techniques presented in the manual are those most appropriate to use in the camp laboratories and are feasible to standardize in all camps along the border. Most of all equipment and supplies can be purchased and maintained in Thailand. This manual is intended to prepare displaced persons from Myanmar to organize and maintain laboratories as well as train additional laboratory technicians on their own.

## 1. HEALTH AND DISEASE

### WHAT IS A HEALTHY PERSON?

Somebody, who feels well, has no pain, eats well, and sleeps well. A healthy child grows well. A healthy adult has normal activity.

### WHAT IS A DISEASE?

Anything that disturbs the normal function of the body. A person who has a disease will feel various **symptoms**; for example: headache, abdominal pain, chills.

These **symptoms** are only caused by the disease. **They are not the diseases.**

### CAUSE OF DISEASE:

- 1) A Disease can be caused by a lack or an excess of something, for example of food.
  - Lack of food for child: no growth, œdema, weakness...
  - Lack of essential vitamins: eye problems (vitamin A), skin disease (vitamin B)....
  - Drinking too much alcohol: liver problems
- 2) A disease can also be caused by infectious agents. Infectious agents are **parasites, bacteria, viruses** or **other small living things** that you cannot see with your eyes that enter our body from the outside and cause diseases.
  - Some examples of parasites are worms and the parasite that causes malaria
  - Some examples of bacteria that causes diseases are the bacteria that cause tuberculosis and the bacteria that cause wounds to get infected (cause pus in wound).
  - Some viruses cause diseases like the common cold and measles.
- 3) A disease can also be caused by abnormality inside the body such as diabetes, cancer...

### WHAT IS THE ROLE OF A DOCTOR OR A MEDIC?

The doctor or the medic will examine the patient to find **clinical signs** of the disease. For example; big liver, hard belly, noise in the lungs, fast pulse....

For some disease, examining only the clinical signs and symptoms is enough for a medic to treat the patient. Sometimes it is not enough, so they need the help of a laboratory for other examinations before giving treatment.

### WHAT IS THE ROLE OF THE LABORATORY?

The laboratory will find the infectious agents that cannot be seen only by eyes, because they are very small in size. To find them, the laboratory uses a microscope.

The laboratories can also check for some bodily substances, especially in blood and urine. For example: some kind of sugar in the blood or protein in urine.

While doctors and medics examine the whole body, laboratory technicians examine only **samples** from the body such as blood, urine, sputum and stool. These samples are also called **specimens**.

| PATIENT  | DOCTOR   | LABORATORY  |
|--|--|---|
| <p><i>Symptom</i><br/>Example: (fever)<br/>→</p> | <p><i>Clinical Signs</i><br/>(Big liver) →</p> | <p><i>Infectious Agents</i><br/>(Malaria)<br/>↓</p> |
| <p>← <b>Healthy</b></p>                          | <p>← <b>Treatment</b></p>                      | <p>← <b>Result</b></p>                              |

## 2. JOB DESCRIPTION

### A. LABORATORY TECHNICIANS

1. **Collect** the specimen: - Sputum / Blood
2. **Prepare** the specimen: - Thin and thick blood smears  
- Dry and wet sputum smear
3. **Make** the staining
4. **Examine:** for
  - Malaria parasites
  - TB Bacteria
  - Lung parasite (Paragonimus)
  - Microfilaria parasite
  - Haemoglobin or haematocrit measurement
  - Blood grouping
  - HIV
  - Hepatitis B
5. **Record** each specimen with the result in the register book.
6. **Return the result** to the medic or nurse. The laboratory technician is responsible for the result that he / she gives. In emergency cases, he / she must give the result directly to the medic as quickly as possible.
7. **Keep** the laboratory clean
  - Clean the room. Tables, chairs, shelves, floor and boxes
  - Clean the laboratory Equipment
  - Clean the old slides
  - Properly dispose of all waste
8. **Maintain** the microscope properly; dry the desiccant (silica gel) for reusing.
9. **Regularly check** the stock of reagents, equipment, and items and fill out the inventory (stock) and the order form that you will give to the in – charge.
10. **Do the statistics** at the end of the month and fill in the laboratory statistic form.
11. **Collect the slides** for quality control every month and send to the lab supervisor.

### QUALITIES OF THE LAB TECHNICIANS

Conscientious, Honest, Neat and Reliable

*Lab technicians should be aware that the patient's life depends on the results  
he / she gives*

## **B. LABORATORY SUPERVISORS**

The supervisors should be able to:

1. Perform all the laboratory activities described in the laboratory workers job description.
2. Communicate:
  - 2.1 Between the laboratory and the other departments (OPD, IPD...):
    - Discuss the result of all IPD patients with the medics.
    - With the medic, organise a meeting if there is a problem to solve.
    - Teach the nurses and medics for the proper collection of specimen especially thin and thick blood smear in case the lab technicians cannot do it by their own. Bad smears can lead to wrong results, so lab technician supervisor should show the medics and nurses how to make a good smear and explain that the quality of the result depends on it. However, blood collection should be done by the lab technician as much as possible.
  - 2.2 Among the laboratory staff:
    - Organise meeting with the staff if there is a problem.
    - Organise the staff duties.
  - 2.3 With the NGO laboratory technician in – charge:
    - Discuss the problems met during the month.
    - Give new ideas to improve the work.
    - Complete and send the statistic forms, quality control form, quality control slides, and laboratory order form on time.
    - Discuss about the monitoring record.
3. Monitor the laboratory activities:
  - 3.1 Check the collection of the specimens:
    - Blood.
    - Sputum.
  - 3.2 Evaluate all the techniques done in the laboratory:
    - Thin and thick smears.
    - Giemsa staining.
    - TB smears.
    - TB staining.
    - Paragonimus smears.
    - Haemoglobin or haematocrit measurement.
    - Blood grouping.
    - HIV tests.
    - Hepatitis B tests.

3.3 Control the registration of the results in each book and on forms:

- Malaria book.
- TB book.
- Haemoglobin or haematocrit book.
- Blood transfusion book.
- Lab request forms.
- Statistics forms.
- Quality control forms.

3.4 **Confirm the results** from the lab technicians if they are not sure.

3.5 **Control the laboratory inventory** and ordering system

- Recheck the inventory form done by the lab technician
- Realisation of the laboratory order

3.6 Do **quality control** of malaria and TB slides

3.7 Fill **out** general monitoring record.

3.8 Supervise the cleaning and maintenance of the lab items and the laboratory room

### 3. SAFETY IN THE LABORATORY

The main accidents associated with medical laboratory work are:

- ⊗ INFECTIONS
- ⊗ CUT
- ⊗ BURNS
- ⊗ HARMFUL EFFECTS OF TOXIC CHEMICALS
- ⊗ WASTES

#### INFECTIONS

**Laboratory specimens are often infectious:**

- Sputum of TB patients contains bacteria that may infect the lab technicians.
- Urine of patients with urinary tract infection (UTI) contains bacteria. If the lab technician touches this urine, then touches his / her mouth or his / her eyes, he / she may get an infection.
- Stool may contain parasites such as roundworm eggs. If swallowed, they will give roundworm infection.

**To avoid this, you must:**

- Put a mask on before smearing a sputum specimen.
- Put gloves on before you work with any specimens or when cleaning equipment.
- Always wash your hands after handling specimens.
- Be very careful with needles, blood lancets and dispose of them properly.

*Using these safety methods is called **UNIVERSAL PRECAUTIONS**. If you follow this you will protect yourself from the diseases that can infect and kill you. Anytime that you handle any specimens for laboratory tests, you must treat them as if they were infectious. Do not treat only the specimens that you think are infectious. You must assume that every patient has an infection. This way you will protect yourself from the possibility of getting an infection.*

#### CUTS

- Lab work requires some glassware: slides, beakers, cylinders, pipettes, bottles.
- You must be careful with cracked glassware, especially when you wash slides.

## **BURNS**

- Different kinds of alcohol are used in the laboratory. All of them are flammable.
- Acid alcohol, used in TB test, contains acid that causes burns on skin and clothes.

## **HARMFUL EFFECTS OF TOXIC CHEMICALS**

- All the chemicals used in the laboratory are toxic if swallowed.
- Mouth pipetting should be avoided. Never mouth pipetting for acid.
- Avoid skin contact with xylene, and do not breath it.

## **WASTES**

Before washing used slides (especially unstained slides) or specimen containers, throw the wasted specimen into the latrine. Then soak these slides and containers with disinfectant (solution used for destroying the infectious agents), such as chlorine and hypochlorite.

Old slides and used blood lancets must be collected in closed bottles or boxes (or old infusion bottles) and thrown into the latrines or wasted wells.

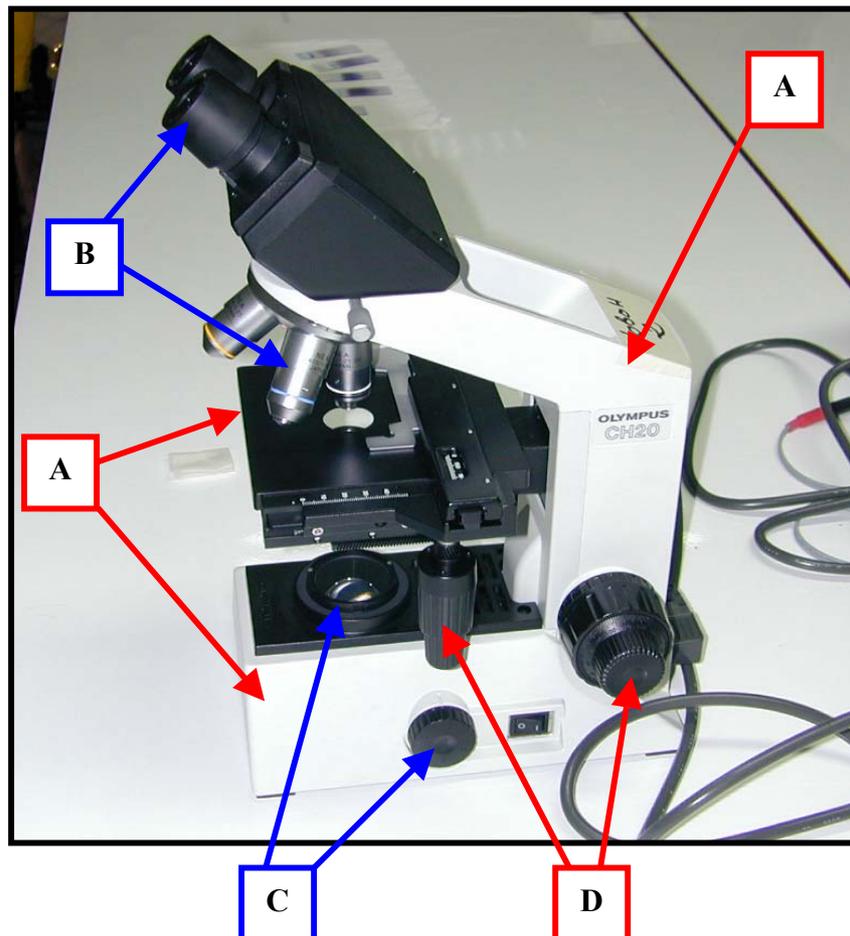
***NEVER LEAVE USED SLIDES or BLOOD LANCETS LYING ON THE FLOOR***

## 4. THE MICROSCOPE

Microscope is used to see something so small that you cannot see it with your eyes. A microscope can magnify the object up to 1000 times its real size.

The parts of the microscope can be classified into 4 systems:

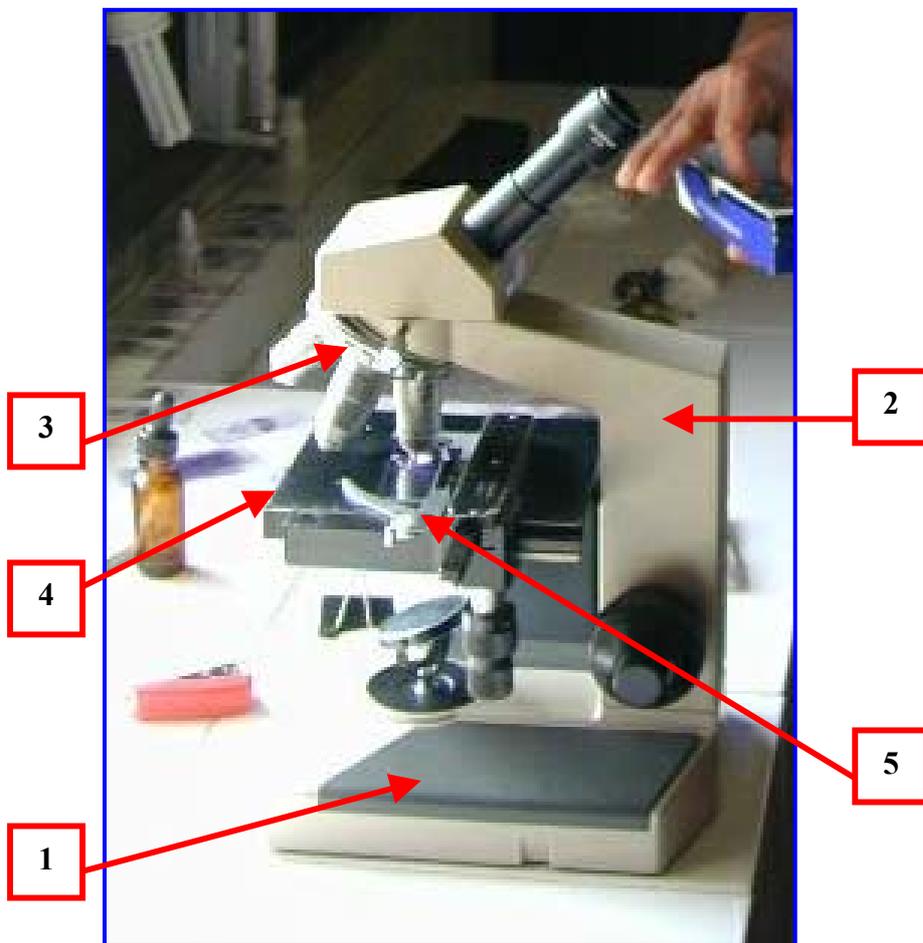
- A) THE SUPPORT SYSTEM
- B) THE MAGNIFICATION SYSTEM
- C) THE ILLUMINATION SYSTEM or THE LIGHT SYSTEM
- D) THE ADJUSTMENT SYSTEM



## A) THE SUPPORT SYSTEM

Consists of:

1. The **foot**
2. The **body**
3. The **objective holder**
4. The **stage** to place the slide or the object
5. The **mechanical stage**, which holds and controls the movement of the object slide



## B) THE MAGNIFICATION SYSTEM

Consists of a system of lenses:

1. **The eyepieces:**  
They multiply the size of the object by 10.



2. **The objectives:**  
X 10 objective multiplies the size of the object 10 times.  
  
X 40 objective multiplies the size of the object 40 times.  
  
X 100 objective multiplies the size of the object 100 times  
***With x 100 objective, we use immersion oil on the slide.***

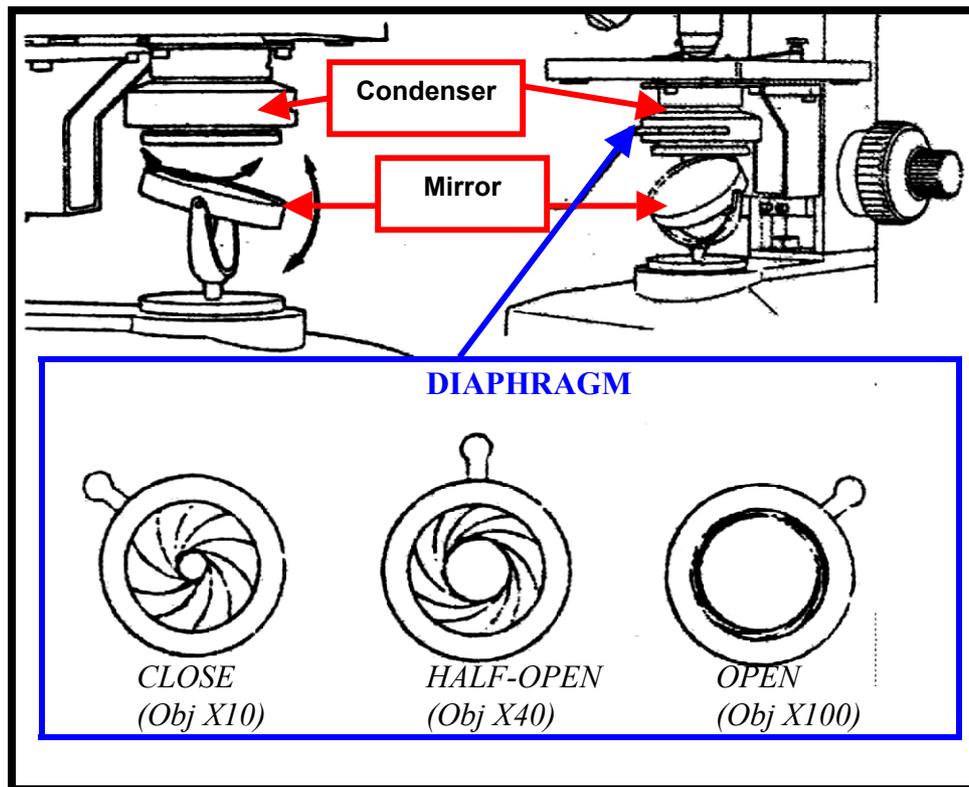
Example: If the x 40 objective and the x 10 eyepiece are used, the total magnification will be 40 times (objective) multiplied by 10 times (eyepieces):  $40 \times 10 = 400$ . It means that the image will be magnified 400 times from real eyesight image.



### C) THE ILLUMINATION SYSTEM or THE LIGHT SYSTEM

#### Consists of:

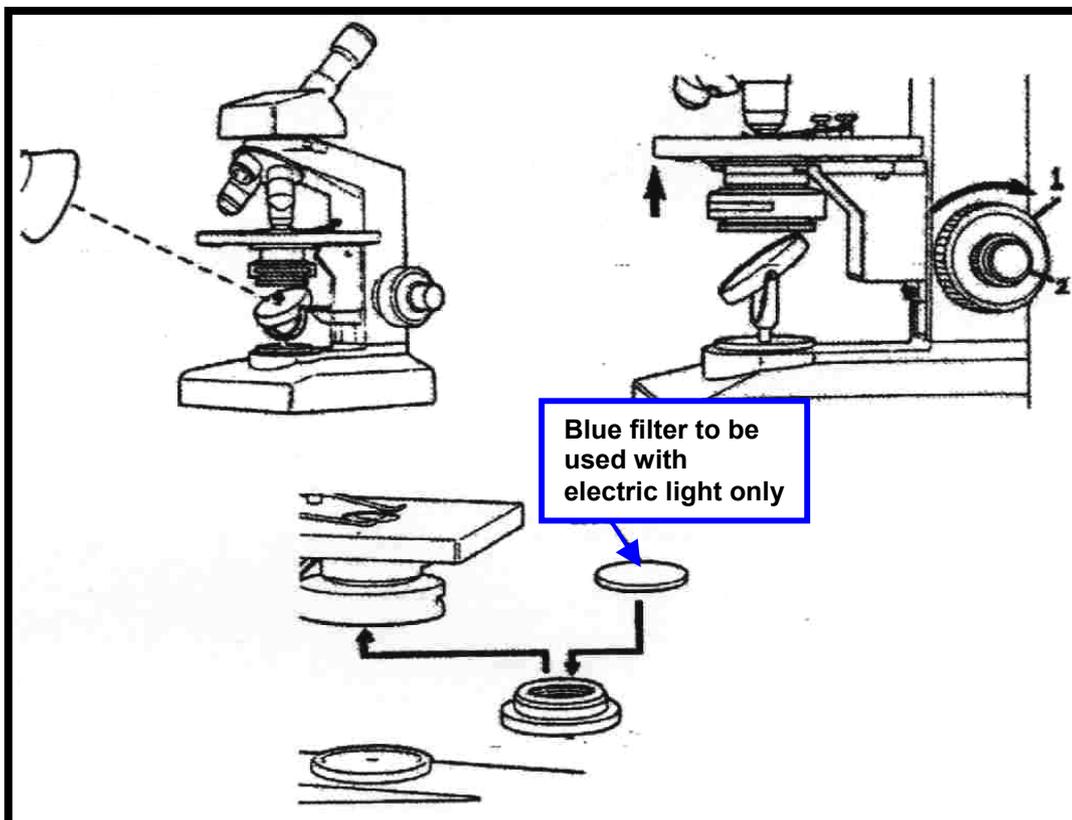
1. The **mirror** that sends the light to the object.
2. The **condenser** that focuses the light.
3. The **diaphragm**, which is fixed within the condenser.  
It reduces or increases the light before passing through the condenser.



## D) THE ADJUSTMENT SYSTEM

### Consists of:

4. The **coarse adjustment screw**  
This is the largest screw. It is used to adjust approximately
5. The **fine adjustment screw**  
This moves the objective more slowly. It is used to adjust perfectly.
6. The **condenser screw** (some models do not have this)  
This is used to raise the condenser for greater light or to lower it to reduce the light:  
Using x 10 objective: condenser down  
Using x 40 objective: condenser half way  
Using x 100 objective: condenser up
7. The **iris diaphragm lever**  
This is a small lever inside the condenser. It is moved to close or open the diaphragm to reduce or increase the intensity of light
8. The **screw of the mechanical stage**  
One screw moves the slide to the right or left.  
One screw moves the slide forward or backward.



### **ROUTINE MAINTENANCE OF THE MICROSCOPE**

The microscope needs daily attention to keep it in good working order to ensure reliable laboratory result

- 1- Remove the oil from x100 objective with lens paper or soft tissue paper.
- 2- Clean the eyepieces with lens paper or soft tissue paper.
- 3- Clean the condenser, the mirror and support stage with a soft cloth.
- 4- Cover the microscope with a clean plastic or cloth cover in order to protect it from dust.
- 5- Put it in a sealed microscope box with an opened box of blue desiccant to protect from fungus developing on the microscope especially on the lens surface

***Note:***

***Desiccant must be renewed regularly by heating it. Its color will turn from pink to blue and ready to be used again.***

## 5. THE CELL

The cell is the basic unit of all living things. A cell is an important part of all animals or plants. It usually works together with other cells to form various organisms (it performs like a leaf, which is one component of an entire roof). Some organisms are formed of only one cell but others, like the human body or plants, have billions of cells.

Most of the organisms that cause diseases are one-cell living things, which are too small to be seen with the eye.

Examples of these living things are *yeast, malaria protozoa and bacteria*.

Aside from these living things, there are free-living things, which are so small that we cannot see them even with an ordinary microscope. They are called Virus and they are not complete cells.

**ONLY SOME OF EACH KIND OF THESE ORGANISMS CAN CAUSE DISEASE IN HUMANS**

Although cells vary in shape, size, and function, they are similar in their main structure.

Commonly a cell consists of 2 major parts:

1. **Nucleus**
2. **Cytoplasm**

1. **NUCLEUS** is the inner part of the cell. Inside the nucleus, there is a very small material called *Chromatin* that acts like the brain of the cell
2. **CYTOPLASM** is the fluid that surrounds the nucleus and is enclosed by a *Cell membrane* or/and *Cell wall*. Inside the cytoplasm, there are various components, which help the cell to perform its activities.

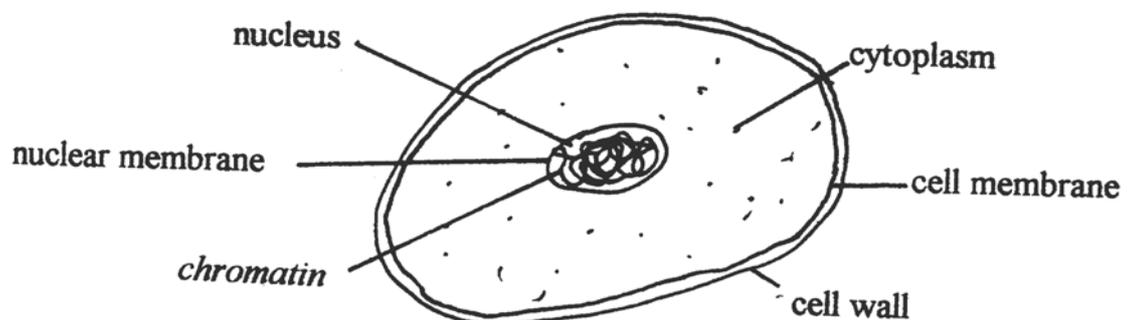


Fig 5. 1: Structure of a cell.

## 6. BLOOD CELLS

Blood is a red liquid solution, which contains several different kinds of cells. These cells are:

- 1) Red Blood Cells
- 2) White Blood Cells
- 3) Platelets

### 1) THE RED BLOOD CELLS (RBC)

The RBC carries oxygen (good air) from lungs to all body tissue cells.

Size: 7  $\mu\text{m}$  (micrometer or micron. 1000  $\mu\text{m}$  = 1 mm)  
It is seen about 7 mm by the x100 objective.

Shape: Round as a biconcave disc, occasionally slightly irregular.  
Mature RBC is the only kind of cell in our body that has no nucleus.

Coloring by Giemsa stain: Periphery is pink-grey color  
Center is pale pink-grey color or almost colorless.

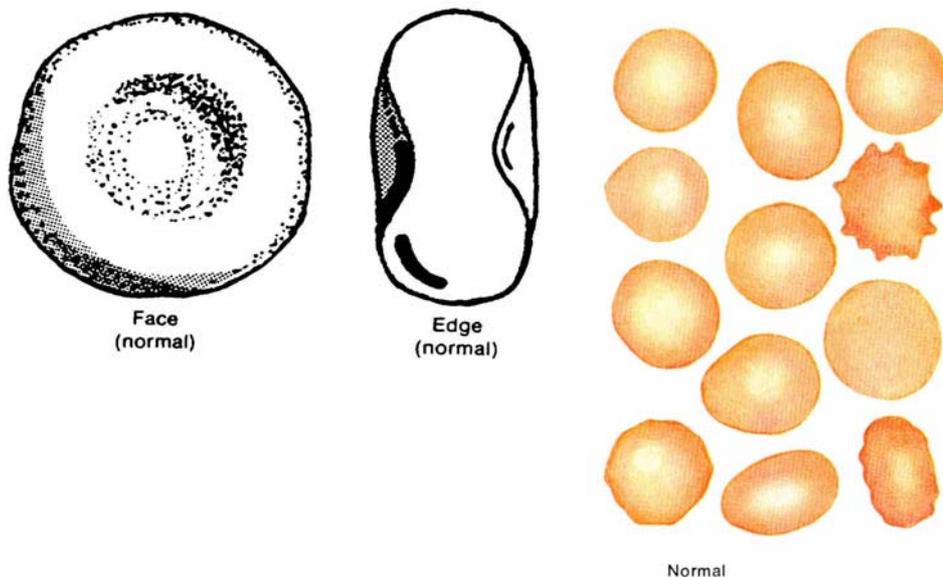


Fig 6. 1: Normal RBC as seen on a thin smear.

## 2) THE WHITE BLOOD CELLS (WBC)

The WBC defends the body against infection. They act as the soldiers of our body.

There are 5 main types of WBC, which differ in size, shape of the nucleus, color of cytoplasm and **granules** in the cytoplasm (granules look like spots).

**We will study only 4 kinds of WBC:**

### 2.1) Neutrophil

Size: 12 – 15  $\mu\text{m}$

It is seen about 12 – 15 mm by x100 objective.

Shape: Round

#### Coloring by Giemsa stain:

Cytoplasm: Pinkish

Nucleus: Several (2 to 5) lobes, linked by strands of chromatin.  
The chromatin forms a deep purple mass.

Granules: Mauve and very small, blue, numerous but separate.

For patients suffering from severe malaria the cytoplasm may contain brownish – black masses. This is malaria pigment (see figure 7. 20).

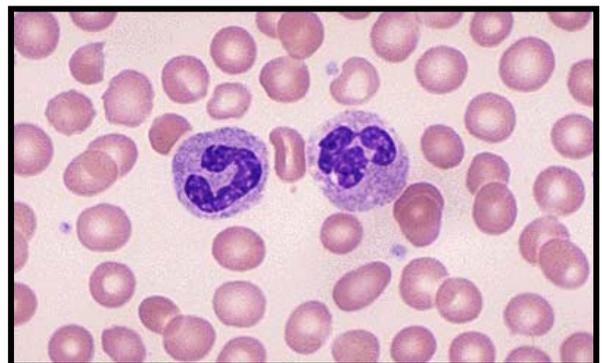
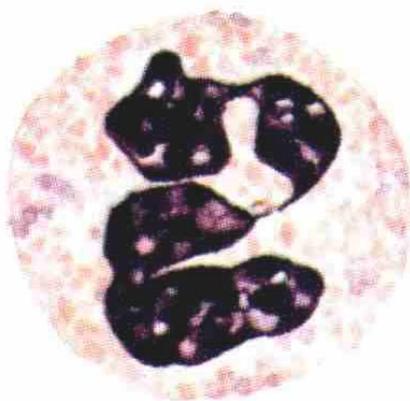


Fig 6. 2: Neutrophil

## 2.2) Eosinophil

Size: 12 – 15 $\mu$ m

Shape: Round

### Coloring by Giemsa stain

Nucleus: Usually 2 lobes in a deep purple mass.

Granules: Large, round, orange, numerous and closely packed.  
Sometimes the cell appears damaged, with scattered granules.

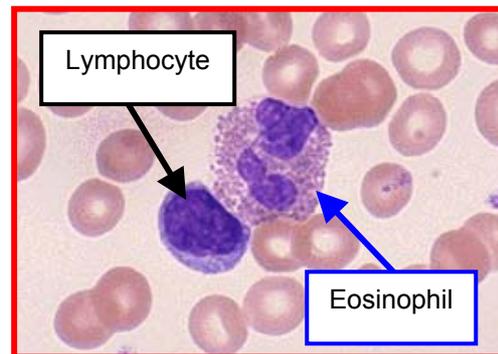
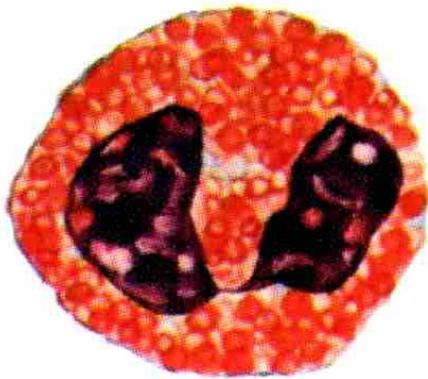


Fig 6. 3: Eosinophil

## 2.3) Lymphocyte

Size: 7 – 10  $\mu$ m (They are smaller than the other kinds of WBCs)

Shape: Round

### Coloring by Giemsa stain

Nucleus: One compact and large nucleus occupying most of the cell.  
Chromatin is dark purple and dense.

Cytoplasm: Very little visible, pale blue without granules.

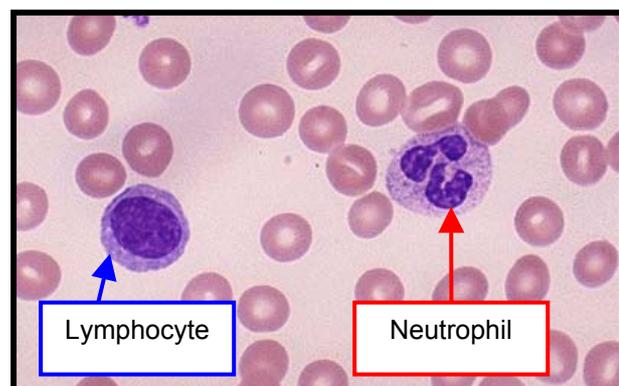
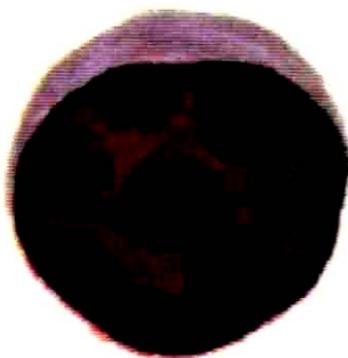


Fig 6. 4: Lymphocyte

## 2.4) Monocyte

Size: 15 – 25  $\mu\text{m}$  (They are the largest kinds of WBCs)

Shape: Irregular

### Coloring by Giemsa stain

Nucleus: Variable, often kidney – shaped.  
Chromatin arranged in strands, pale mauve.

Granules: fine, dust – like, blue.

Cytoplasm: blue – gray, sometimes vacuoles are present inside.

For patients suffering from severe malaria the cytoplasm may contain brownish – black masses. This is malaria pigment.



Fig 6. 5: Monocyte

## 3) THE PLATELETS (Plt.)

They are fragments of cells, which have an important function in the clotting of blood. Without platelets, bleeding from any scratches or cut wounds will not be stopped.

Size: 1 – 4  $\mu\text{m}$  (It is seen about 1 – 4  $\mu\text{m}$  by the x100)

Shape: various shapes (triangular, star – shape, oval, etc...)

Coloring by Giemsa stain: pink

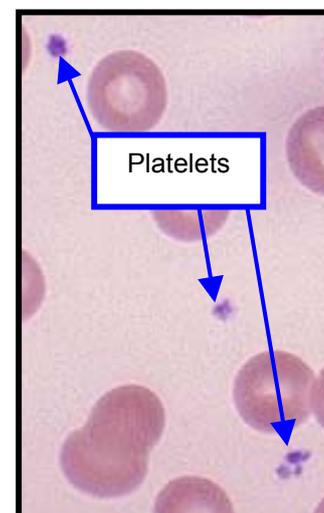
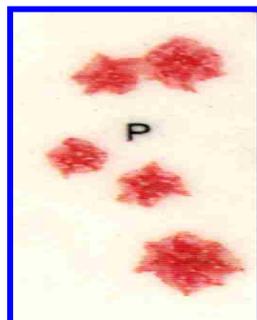


Fig 6. 6 Platelets

## 7. MALARIA

**Malaria** is a disease caused by a parasite, *Plasmodium*, which infects red blood cells. Malaria is spread by **mosquitoes**. Mosquitoes suck up the malaria parasite from the blood of an infected person and transfer parasites to another person when biting them.

### There are 4 kinds of Plasmodium:

- ❖ *Plasmodium falciparum* causes the severe malaria, which can cause coma and lead to death.
- ❖ *Plasmodium vivax*, *Plasmodium malariae* and *Plasmodium ovale* give mild malaria cases.

Along Thai – Myanmar border area, *Plasmodium falciparum* and *Plasmodium vivax* are often found and *Plasmodium malariae* is seldom found whereas *Plasmodium ovale* is rarely found.

### Symptoms and clinical signs:

A patient with malaria will complain of fever, chills, headache, and joint pain. During the clinical exam, the medic or doctor sometimes finds anaemia and big spleen (especially among children). In cases of severe malaria, the patient may also be unconscious.

### Diagnosis:

Microscopic examination of blood will show parasites inside the red blood cells. The name of the laboratory exam is **MS or M/S** (malaria smear).

- *Plasmodium falciparum* or PF are small and ring shaped. They are often found in great number in 4+ or +++++ cases, more than 4 % of RBCs are infected.
- *Plasmodium vivax* or PV and *Plasmodium ovale* or PO are bigger than PF, but usually are less numerous and less harmful.
- *Plasmodium malariae* or PM are also bigger than PF, found in very small quantities in the blood and they develop very slowly.

### Treatment:

- In this area, usually patients infected with PV, PO or PM are treated with **Chloroquine**.
- For patients with PF, the treatment might be done by Quinine, **Mefloquine**, **Artesunate** and their combinations with other medicines (refer to the updated S.M.R.U malaria handout).

### Prevention:

Malaria is spread by a mosquito. This mosquito is called **Anopheles** and it usually bites from sunset until dawn. To prevent malaria, it is necessary to avoid being bitten by the Anopheles mosquito. This can be done by:

- Covering up between sunset and dawn.
- Taking a bath before sunset or after dawn then use repellent.
- Sleeping under a mosquito bed net.
- Killing mosquitoes, reducing mosquito-breeding places.

***To prevent severe malaria from developing, have your blood checked for every fever.***

### 7.1 MALARIA LIFE CYCLE

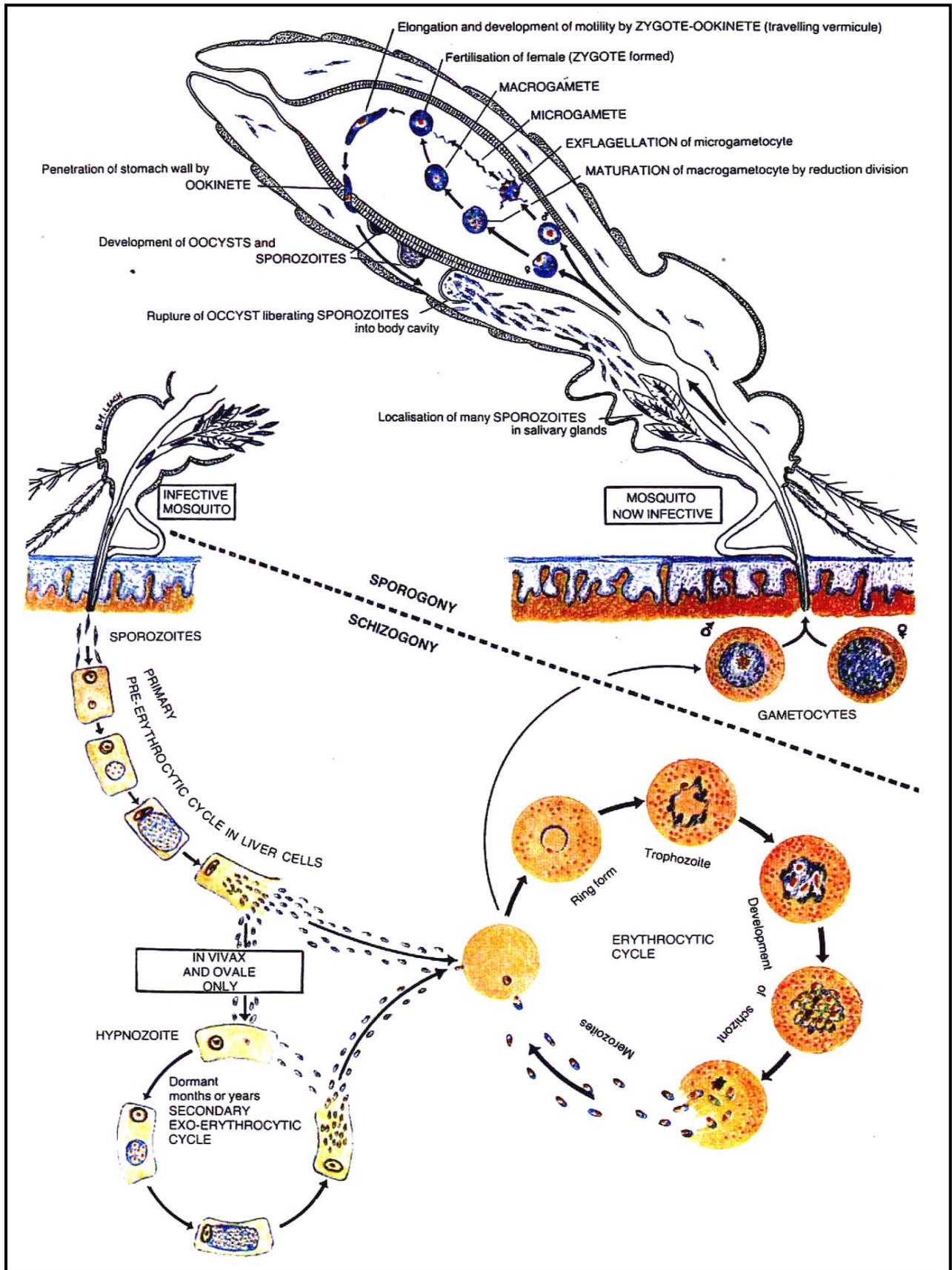


Fig 7. 1

Female anopheles mosquito needs to eat blood when it is pregnant. The anopheles infected with Plasmodium bites a person to suck his blood and at the same time, it releases **SPOROZOITES** in the blood of this person. **SPOROZOITES** are the infective form of Plasmodium (see Fig.7. 1)

#### Pre – Erythrocytic Cycle in Liver cells (Cycle in liver before RBC cycle):

1. After **sporozoites** were released to the blood stream, they reach the liver and enter the liver cells then develop. Each parasite grows and divides up to 30.000 new parasites, remaining in each liver cell, called **hepatic schizont**. Then liver cells burst, and release a great number of new parasites, called **MEROZOITES** into the blood stream.

For *Plasmodium vivax* and *Plasmodium ovale*, some parasites remain in liver cells, as **sleeping forms (hypnozoites)**. In the liver cells, these sleeping forms cannot be destroyed by chloroquine. They can wake up and produce new merozoites into blood stream again causing a new malaria episode, not caused by a new infection. This is called **relapse**, which may occur from time to time after initial infection, during 2 – 3 years.

#### Erythrocytic Cycle (Cycle in Red blood Cell):

2. After each merozoite was released into blood stream, it enters a RBC and develops. The merozoites that cannot enter a RBC within 5 minutes are destroyed. Once inside the RBC, **merozoite** becomes **TROPHOZOITE** (PVT = Plasmodium Vivax Trophozoite).
3. **The trophozoite** grows and divides itself. It becomes a **SCHIZONT**: each **schizont** can contain up to 32 new parasites depending on the type of Plasmodium, which are also called **merozoites**. Then the infected RBCs burst and release the new **merozoites** in the blood. **This provokes the peak of fever.**

|                                |       |            |
|--------------------------------|-------|------------|
| DESTRUCTION OF RBCs            | cause | ANAEMIA    |
| DEAD RBCs ELIMINATED BY SPLEEN | cause | BIG SPLEEN |

Development of the parasite in the RBCs takes 42 – 48 hours for PV and 36 – 48 hours for PF. Delay between infected mosquito bite and the first peak of fever is called "**incubation period**" and it takes about 2 weeks (12 – 17 days for PV and 9 –14 days for PF).

4. Each new **merozoite** will infect one new RBC and make a new cycle, causing the fever to go UP and DOWN. Chloroquine destroys **schizonts**, so it stops the cycle in the blood (but not in the liver: see 1. above).
5. After some cycles in the RBCs, instead of developing into schizonts, some **merozoites** develop in a different way. They become sexual reproductive form called **GAMETOCYTES**. There are **male gametocytes** and **female gametocytes**. Their presence in the blood of an infected person does not cause any disease and if a mosquito does not take them up, they die.

#### Infective form in the mosquito:

6. When the anopheles mosquito bites and sucks an infected person's blood, in the mosquito's stomach, all the **trophozoites** and **schizonts** die. Only **gametocytes** can survive. Fertilisation occurs between male and female Gametocytes and produces a **ZYGOTE** (egg). The **zygote** enters the stomach wall of the mosquito, and develops there. Each egg gives birth to a great number of new **sporozoites**, which is the infective form of malaria. **Sporozoites** then collect in the salivary glands of the mosquito.

Life cycle of each type of Plasmodium malaria are different in the number of merozoites contained in mature schizonts, the incubation period, the length of time of the parasite cycle in the RBCs and the presentation of relapse.

| <i>Characteristics</i>                                 | <i>P. Falciparum</i>                      | <i>P. Vivax</i>  | <i>P. Malariae</i>        | <i>P. Ovale</i>             |
|--|---|--|---------------------------|-----------------------------|
| <b>No. of merozoites in Each mature liver Schizont</b> | Up to 30,000                              | 10,000   | 15,000                    | 15,000                      |
| <b>No. of merozoites in each mature RBC schizont</b>   | 8 – 32<br>Rarely seen in Peripheral blood | Up to 24   | 10 - 12                   | 8 - 10                      |
| <b>A cycle duration of parasite in RBC</b>             | 36 – 48 hr.                               | 42 – 48 hrs  | 72 hrs                    | 48 - 50 hrs                 |
| <b>Incubation period</b>                               | 9 – 14 days<br>Exceptionally up To 1 year | 12 – 17 days   | 18 – 40 days              | 16 – 18 days                |
| <b>Time of relapse after initial infection</b>         | No relapse                                | 8 – 10 weeks, or<br>30 – 40 weeks, or<br>Up to 8 years | Up to 53 years            | No certain report           |
| <b>Entering of merozoites to RBCs</b>                  | Enter every stage Of RBCs                 | Particularly enter Young RBCs                          | Usually invade Older RBCs | Usually infect Younger RBCs |

### **RESISTANCE**

The appropriate treatment of malaria in each area depends on the susceptibility of malaria parasite to the anti malarial drugs. Presumptive treatment, wrong lab diagnosis, and the incorrect use of these drugs (e.g. self treatment) contribute to the parasites resistance, which means that the parasite can survive and/or multiply despite taking medicine.

The first anti malarial drugs were discovered about 3000 years ago, but since 1950 **resistance** appeared among *P. falciparum* against chloroquine and this parasite continues a great facility to become resistant to many other malaria drugs. The falciparum parasites on the Thai – Myanmar border are the most multidrug resistant in the world.

**The follow up of patient is very important in order to monitor the efficacy of the drugs.**

## 7.2 MALARIA SMEAR

It is a laboratory exam that finds out if a patient has malaria. The examination is done with blood, because malaria parasites infect Red Blood Cells.

### Method

- 1) On a tray, prepare:
  - Clean slides
  - Cotton
  - Savlon or rubbing alcohol
  - Gloves
  - Blood lancets
  - Little dustbin for dirty cotton
  - Glass container for used blood lancets
  - Pencil
- 2) Clean one of the slides, holding it by the edges:  
**!! Never touch the slide surface !!**
- 3) Put on the gloves.
- 4) Clean the third or the fourth finger of the patient with cotton soaked in rubbing alcohol or savlon (see figure 7. 2)

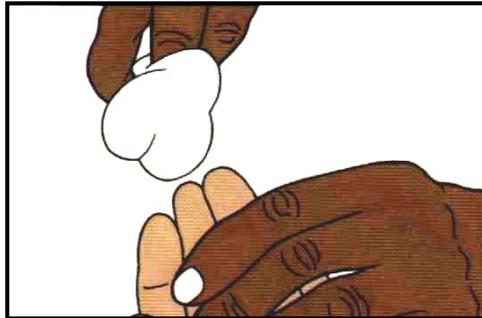


Fig 7. 2

*In baby under 6 months or very small children, it might be difficult and also dangerous to get the blood from the finger as the lancet may pierce finger bone and cause possible infections. You may try to take blood from the side of the heel (as shown by blue arrows in figure 7. 3) or from the side of big toe instead (as shown by red arrow in figure 7. 3)*

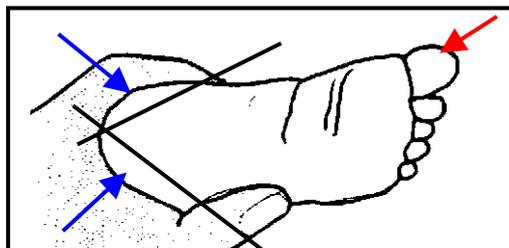


Fig 7. 3

- 5) Open the blood lancet paper, **from the side opposite to the sharp end.**
- 6) Remove the lancet from the paper and prick the finger or the site firmly and rapidly (see figure 7. 4).  
**!! Place the used lancet in the glass container or dispose of it properly !!**



Fig 7. 4

- 7) With a **DRY** piece of cotton or gauze, wipe away the first drop of blood.
- 8) Pressing the finger firmly, apply 3 drops of blood (size ) at one end of the slide for thick smear and about 1 cm away, apply 1 drop (this size ) on the slide for thin smear. ***When applying the blood on the slide, avoid the finger to touch the slide.*** (See figure 7. 5).

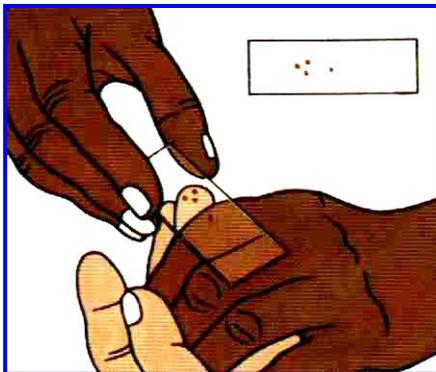
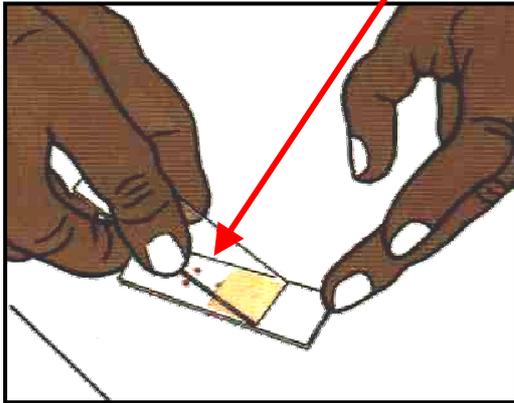


Fig 7. 5

**! The quantity of blood is very important for the thick smear (See figure 7. 5)!  
Not enough blood may lead to a WRONG NEGATIVE RESULT.  
A smear with too much blood cannot be stained properly and CANNOT BE EXAMINED**

- 9) Use a second slide (called spreader) to spread the big blood drop for thin smear. Do it with a **slow and regular movement** to make a good blood film.
  - Put the spreader on the first slide and draw it backward
  - Let the blood run across the edge of the spreader. The spreader must have a **smooth & cleaned edge** otherwise it is not possible to obtain a good thin smear.
  - Firmly push the second slide, keeping it at an angle of 45 degrees, with a **slow and regular movement** until it is off the first slide (see figure 7. 6).

Spreader with clean & smooth edges



OR

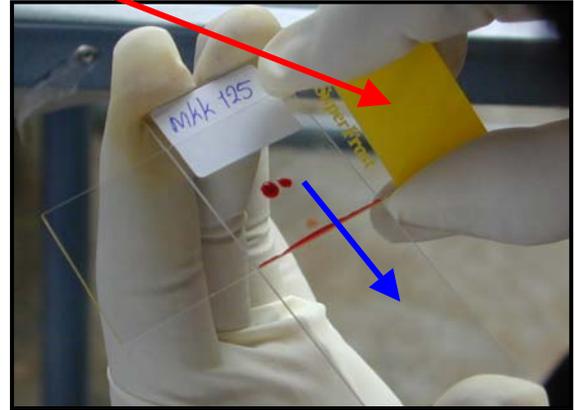


Fig 7. 6

- 10) If the smear is good prepare the thick film:  
Quickly join the 3 small drops of blood and mix them circularly not less than 10 times by using the corner of the spreader (see figure 7.7). **Then use an alcohol soaked cotton to wipe the blood off the spreader.**

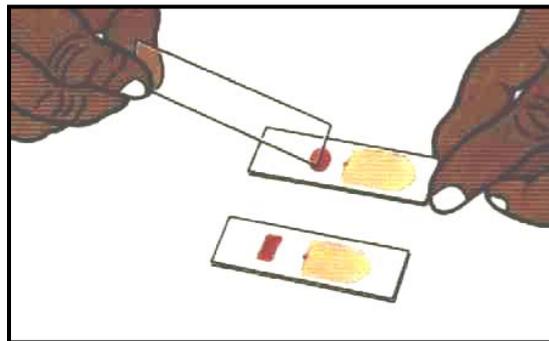


Fig 7. 7

- 11) **Let the slide dry on a plane surface.**  
- Label the slide with the patient's number and date: use a PENCIL.

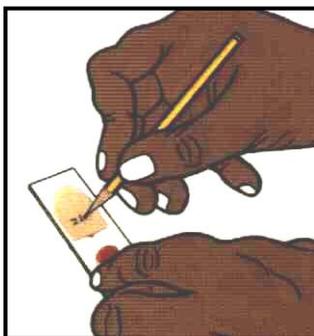


Fig 7. 8

## 7.3 FIXING AND STAINING

### A. FIXING

#### 1) **Materials needed:**

- Absolute methanol
- Slide-rack

#### 2) **Method:** After the blood smear **completely dries:**

- Fix only the thin smear by dipping it QUICKLY in methanol 3 – 5 seconds. Be careful because the vapour of methanol may fix the thick smear.
- Let the slide dry on the slide-rack completely before staining.

### B. STAINING

#### 1) **Materials needed:**

- Pure GIEMSA stain
- Clean filtered water with pH at or near 7.2
- Wash-bottle
- Graduated cylinder (100 ml)
- Graduated pipette (10ml)
- Slide-rack
- Staining tray

*\*You may need to filter the pure Giemsa stain before using it (depend on the quality of stain). This should be prepared by shaking the original bottle of pure Giemsa stain first then filter the stain using a filter paper into another dry clean bottle **and tighten the lid** (Giemsa contains methanol)*

*\* We cannot stain the smear with pure Giemsa **BEFORE STAINING WE HAVE TO DILUTE GIEMSA WITH FILTERED WATER** in order to obtain a 10 % Giemsa solution.*

*\* The percentage of Giemsa in the solution may vary. It depends on the quality of the water, the pure Giemsa stain and the use of filtered or non-filtered pure Giemsa stain. You must be able to recognise good stain from bad stain in order to adjust the appropriate dilution.*

#### 2) **How to dilute Giemsa stain for 10 % dilution (for 20 – 30 slides):**

- Fill about half of 100 ml cylinder with filtered water.
- Add exactly 10 ml of filtered Giemsa into the water by using dry pipette.
- Complete exactly the cylinder to the mark 100 ml with filtered water.
- Pour into separate clean and dry wash bottle.
- This 100 ml of the solution is enough for 20 – 30 slides.
- Clean your equipment after use.

*\* Staining one slide needs about 3 to 5 ml of Giemsa solution. The quantity of solution prepared depends on the amount of slides.*

#### 3) **How to stain:**

- Set the timer for 20 minutes and cover the first slide with the Giemsa solution
- Then cover the 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup> ... slides.
- Let the solution remain on the slides for 20 minutes.
- Wash slides with filtered water following the same order as for the staining; 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup> ... slides by pouring the water gently on the slides from the thin smear-end and then tip them up.
- Let the slides dry on the slide-rack.

**7.4 PRINCIPLE of SMEARING, FIXING and STAINING****A. SMEARING**

**Thin smear:** A thin smear is prepared by spreading a small drop of blood evenly on a slide so that it forms only one layer of cells (see figures 7. 9 & 7. 10).

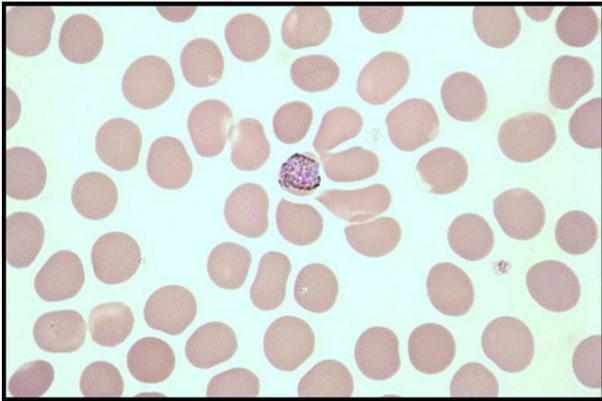


Fig 7. 9: *P.malariae* gametocyte on the thin smear.

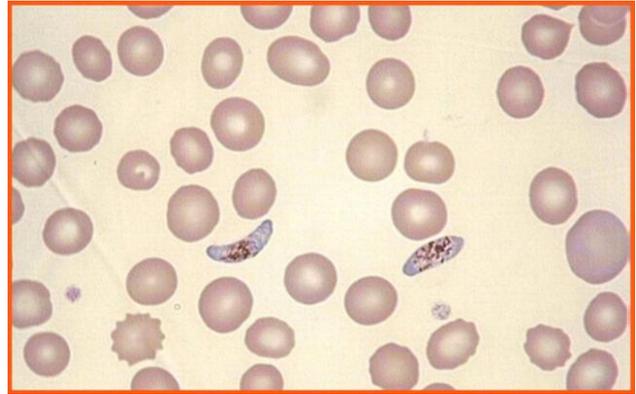


Fig 7. 10: *P.falciparum* gametocytes on the thin smear.

**Thick smear:** A big amount of blood on a small surface makes it possible to find parasites more quickly and easily when only a few parasites are present (see figure 7. 11).

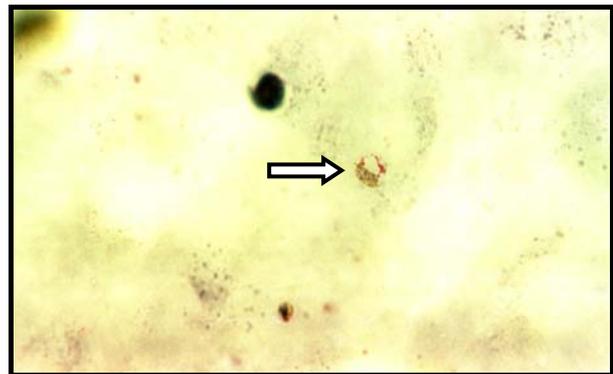
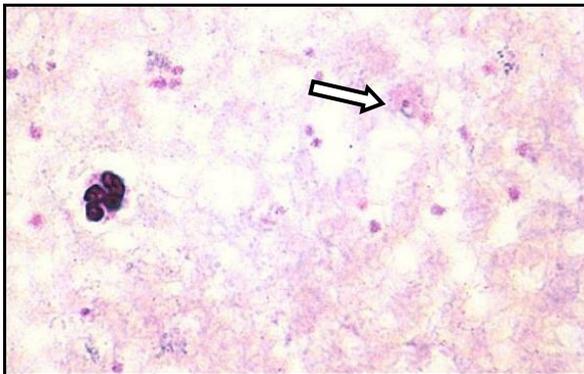


Fig 7. 11: Plasmodium on thick smear

## B. FIXING

Thin smear: To ensure that RBCs are not destroyed by water (from the staining solution) and to attach the film securely to the slide. Methanol preserves and protects cells.

**!!! Water destroys the cells, so**

- Be careful not to have water mixed with methanol, close the bottle of methanol properly.
- During the rainy season, change the methanol more often.

Thick smear: **Must not be fixed.**

**!!! Methanol fixes cell membrane, so**

- Do not let methanol touch the thick smear.
- Fixing time must be as short as possible (about 3 – 5 seconds).  
If it takes too long vapours of methanol may fix the thick smear.

## C. STAINING

Thin smear: To colour blood cells and parasites inside blood cells.

Thick smear: During staining, Red Blood Cell membranes are broken by water in the staining solution (see figure 7. 12).

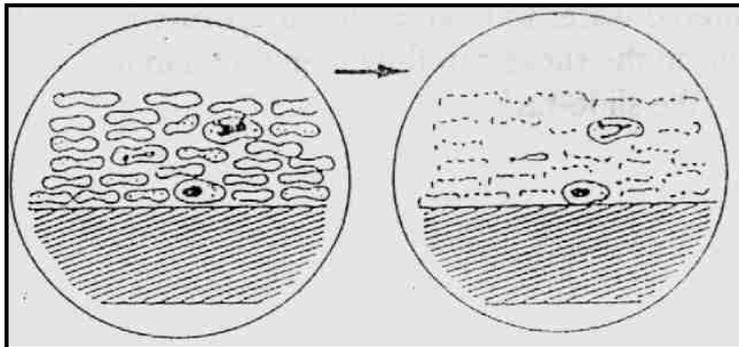


Fig 7. 12

## 7.5 HOW TO RECOGNIZE A GOOD STAIN

A good stain is necessary in order to have a good result. A bad stain will cause a bad result and an incorrect treatment. So you must be able to manage your stain quality.

### A. CRITERIA FOR A GOOD STAIN

Colour of the blood cells and malaria parasites after Giemsa staining:

|              |                |   |
|--------------|----------------|---|
| Thick smear: | WBC nucleus:   | <b>deep purple</b>  |
|              | WBC cytoplasm: | should not be seen  |
|              | RBC cytoplasm: | should not be seen (if you can see it, it means That the thick smear was fixed with methanol) |
|              | Platelets:     | <b>pink</b>   |
|              | Background:    | pale grey (derived from lysed RBCs) and free from dust.                                       |
|              |                | <b>Blue background means that the stain is bad.</b>   |

|                                |                  |
|--------------------------------|------------------|
| Nucleus of malaria parasite:   | <b>deep red</b>  |
| Cytoplasm of malaria parasite: | <b>deep blue</b> |

|             |                                |                            |
|-------------|--------------------------------|----------------------------|
| Thin smear: | WBC nucleus:                   | <b>deep purple</b>         |
|             | WBC cytoplasm:                 | blue or pale pink          |
|             | RBC cytoplasm:                 | grey – pink to pale purple |
|             | Platelets:                     | <b>pink</b>                |
|             | Back ground:                   | free from dust and clear   |
|             | Nucleus of malaria parasite:   | <b>red</b>                 |
|             | Cytoplasm of malaria parasite: | <b>blue</b>                |

### B. CAUTION for HAVING A GOOD STAIN

**All the technical steps from smearing to staining are very important.**

#### 1) Blood smear:

- \* Use well cleaned slides.
- \* A standardized thick smear is needed: thick smears with too much blood cannot be stained properly.
- \* Thin smears should be spread evenly by using a good spreader (smooth edged slide) and a proper quantity of blood.
- \* You must allow the thick smear to **dry thoroughly**; otherwise it will not stain properly.

#### 2) Fixing:

- \* Allow the blood smear to dry completely **on a plane surface** before fixing
- \* Fix only the thin smear by dipping it quickly in methanol.
- \* Let methanol dry completely before staining.

#### 3) Preparing the Giemsa dilution:

- \* Filter the pure Giemsa before using it. Shake it well before filtering.
- \* Use clean and dry instruments for preparing dilution (cylinders, pipettes, etc.).
- \* Make an accurate dilution.
- \* **Do not use this dilution if it is more than 2 hours since preparation.**
- \* Rinse the instruments very well with filtered water after washing them:  
**Never mix soap with reagents or filtered water.**

**4) Staining:**

- \* Stain out of direct sunlight.
- \* Stain for exactly the amount of time needed.
- \* For washing the stained slides, pour clean water gently on the slide before draining the stain from it. This will protect the smear from Giemsa precipitation. Do not pour water directly on the thick smear side (the blood might be removed from the slide).
- \* The proportion of Giemsa dilution and staining time should be adjusted before using any new batch of stain or new manufacturer.

**5) Quality of water:**

- \* To have a good stain, you need neutral water with pH about 7.2 (neither too acid nor too alkaline).
- \* The water must be clean and must be filtered.

*Note: pH is the symbol for the degree of acidity or alkalinity of the solution; pH values from 0 to 7 indicate acidity and from 7 to 14 indicate alkalinity. The pH can be found by using pH indicator paper (litmus paper)*

**C. WHAT TO DO IF YOU HAVE A BAD STAIN**

- First, check that every technical step are done properly:

- Blood smear
- Fixing
- Giemsa dilution
- Staining
- The washing of staining instruments and storage

- If they are properly done and you still have a bad quality stain, it might be due to bad water quality (pH not adequate) or a bad batch of Giemsa. So try to change the water or use a new batch of Giemsa.
- Sometimes you only have few bad slides in a day. In this case, repeat the smear. If not possible, you might re – stain those slides by washing them with methanol. Remove the old stain then let those slides completely dry and carefully stain them again.

**7.6 EXAMINATION of BLOOD FILMS for MALARIA PARASITES****A. 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 especially from the thin blood film as shown in figure 7. 13.

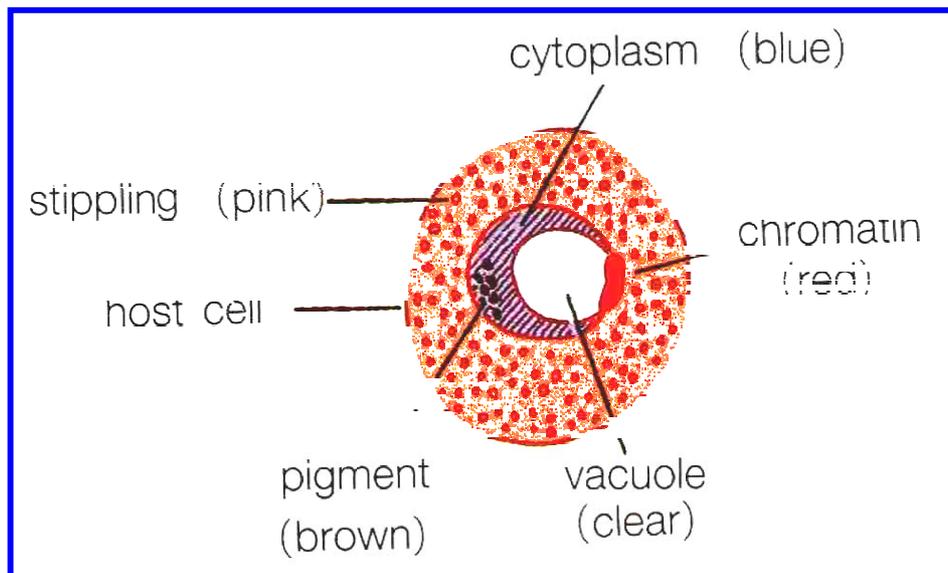


Fig 7. 13: Parts of a malaria parasite inside a red blood cell

Malaria parasites develop through many stages; however, in all stages the same parts of the parasite will stain the same color:

**Chromatin** (part of the parasite nucleus) is usually round in shape and stains deep red.

**Cytoplasm** is formed in various shapes from a ring shape to an irregular shape. It always stains blue.

## B. Stages of the malaria parasite

### I The Trophozoite stage

This stage is the most commonly seen; often called the ring stage form even sometimes it is an incomplete ring (see figure 7. 14).

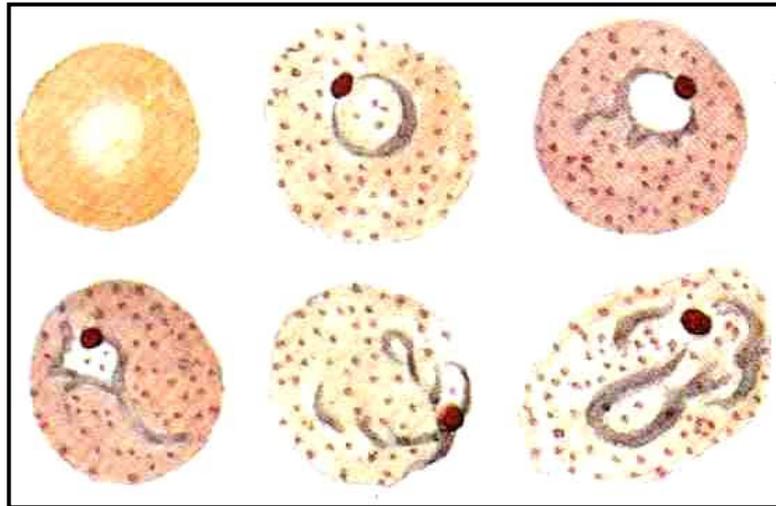


Fig 7. 14: The trophozoite stage of *P.vivax*

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

### II The Schizont stage

At this stage, the parasite reproduces itself by simple division so its chromatin may be seen from two pieces to a certain number of chromatin dots as shown in figure 7. 15



Fig 7. 15: The schizont stage of *P.vivax*

### III The Gametocyte Stage

Gametocytes may be either round or banana – shaped, depending on the species (see figure 7. 16).

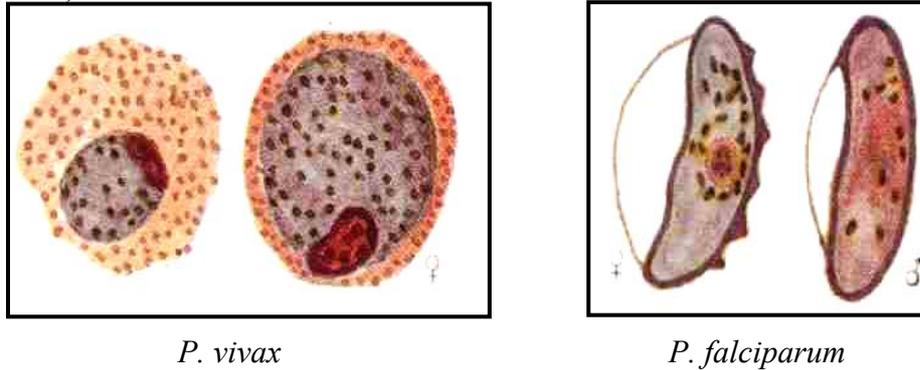


Fig 7. 16: Male and female gametocytes

#### C. Species of malaria parasite

##### Appearance of parasite species in thin blood films

After knowing how to recognize malaria parasite and their stages in thin blood films, you need to distinguish the species of malaria. The simplest guide is the effect the parasite has on infected red blood cells. These are:

##### SIZE of the RBC:

- \* No change for PF infections.
- \* No change or smaller for PM infections.
- \* Enlarged for PV infections.
- \* 20 to 30 % of parasitised RBC becomes slightly enlarged and oval in shape, with fimbriated (ragged) ends for PO infections.

##### COLOR of the RBC:

- \* No change for PF infections.
- \* No change or darker for PM infections.
- \* More pale for PV and PO infections.

##### DOTS (in RBC cytoplasm):

- \* No dots for PM infections.
- \* Sometimes few coarse and irregular purple – red dots called "**Maurer's dots**" in PF infections.
- \* Usually numerous small pink round dots scattered all over the RBC cytoplasm for PV infections. These dots are called "**Schuffner's dots**".
- \* Usually numerous dark dots called "**James's dots**" in PO infections. They appear brighter and larger than "Schuffner's dots".

You should consult the 4 color plates of "*Appearance of Plasmodium parasite stages in Giemsa – stained thin and thick blood films*" produced by WHO (see Figure 7.17a to figure 7.17d). The left hand side of each plate shows the various stages of each malaria parasite species as they appear in thin blood films, and the right hand side shows their appearance in thick blood films.

## Appearance of parasite species in thick blood films

Not only the appearance of both red and white blood cells differs in thin and thick blood films; there are also differences in the appearance of malaria parasites. These are:

- No red blood cells seen in thick blood film but the ghosts of red cells can sometimes be seen surrounding parasites in the thinner parts of the films.
- Like the WBCs, the malaria parasites appear to be smaller in thick blood films than in thin blood films.
- The fine rings of cytoplasm of the trophozoites may appear incomplete or broken.
- The schuffner's dots sometimes are seen in thick blood films.
- The Maurer's dots of PF sometimes are seen in thick blood films.

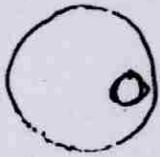
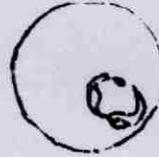
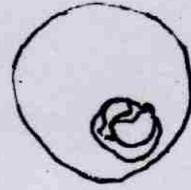
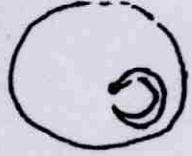
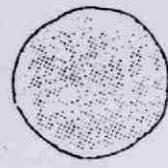
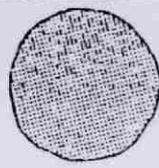
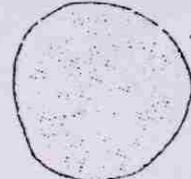
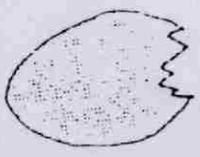
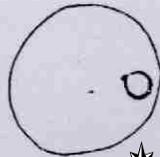
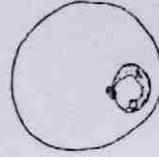
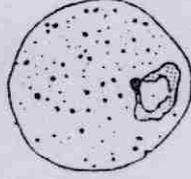
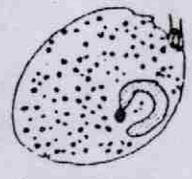
By looking at the thick films, you can find out if the slide **is negative** or **positive**.

You can also estimate the amount or density of malaria parasites by reporting in the number of plus signs: 1+, 2+, 3+ or 4+.

## COMPARISON OF INFECTED RED BLOOD CELLS IN THE THIN BLOOD FILMS

|   | <i>P. falciparum</i>   | <i>P. malariae</i>  | <i>P. vivax</i>   | <i>P. ovale</i>                                       |
|---|--|---|---|---|
| Size of <b>young</b> trophozoite in comparison with diameter of red blood cell (at the same stage of development) | <i>1/5 to 1/3 of RBC diameter</i>  | <i>1/4 to 2/3 of RBC diameter</i>   | <i>1 / 4 to 2/3 of RBC diameter</i>                     | <i>1 /4 to 2/3 of RBC diameter</i>                    |
| APPEARANCE: of infected red cell  | <i>Remains unchanged</i>   | <i>Remains unchanged or becomes smaller and sometimes more deeply colored</i> | <i>Enlarged and often pale staining</i>                 | <i>Slightly enlarged, oval with jagged edges</i>      |
| DOTS: in the infected red cell  | <i><b>Big and few</b> blue-mauve Maurer's dots in older trophozoites</i>         | <i>None</i>   | <i><b>Small and numerous</b> pink, Schuffner's dots</i> | <i><b>Big and numerous</b> deep brown James' dots</i> |
| STAGES found:   | <i>Trophozoites and/or gametocytes. Schizonts rarely seen in simple malaria.</i> | <i>All forms might be found in the same film</i>                              | <i>All forms might be found in the same film</i>        | <i>All forms might be found in the same film</i>      |
| PARASITAEMIA  | <i>Up to 50 % of RBCs infected</i>   | <i>Rarely more than 1 % of RBCs infected</i>                                  | <i>Rarely more than 2 % of RBCs infected</i>            | <i>Rarely more than 2 % of RBCs infected.</i>         |

COMPARISON OF INFECTED RED BLOOD CELLS IN THE THIN BLOOD FILMS

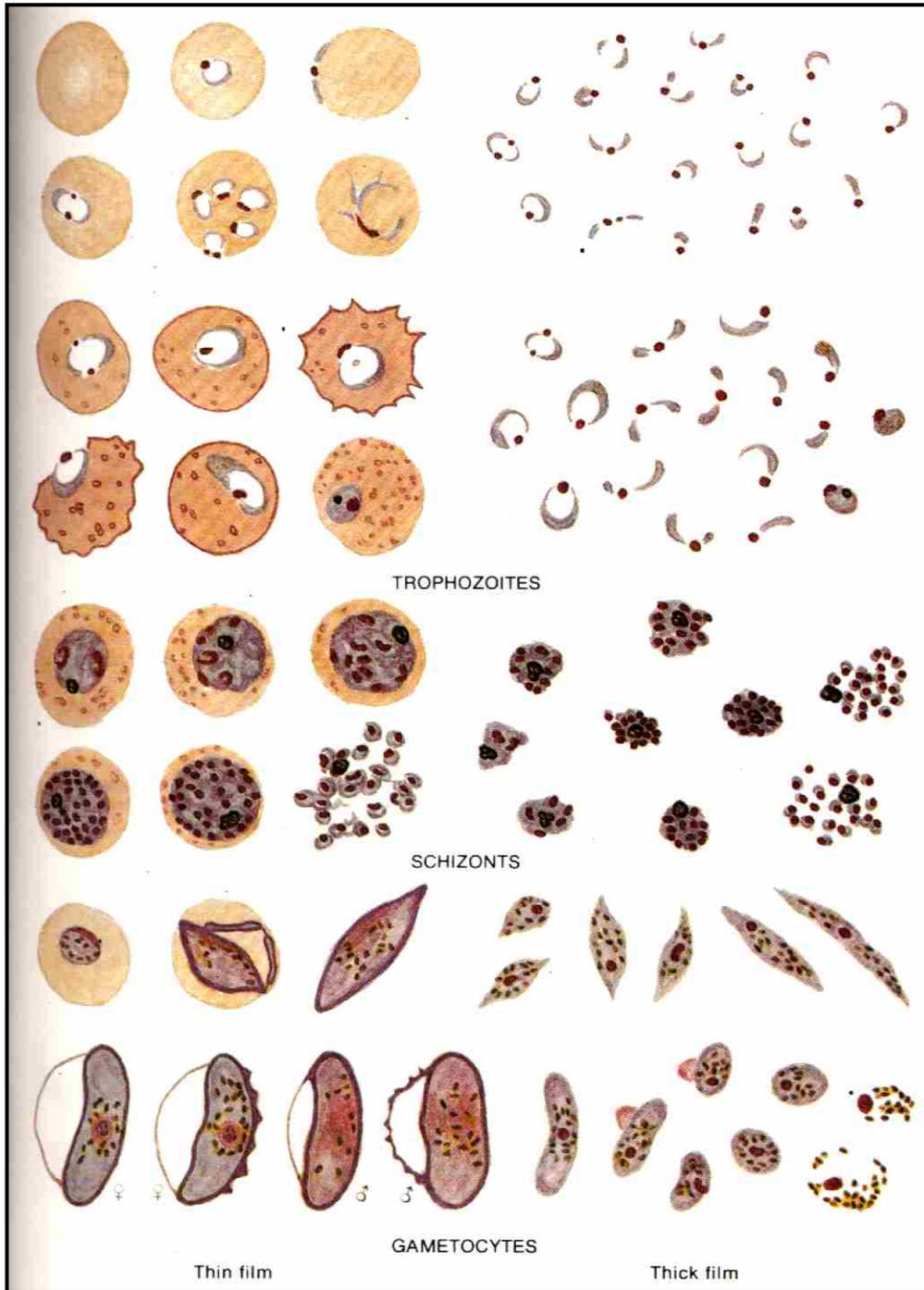
|  | <i>P. falciparum</i>  | <i>P. malariae</i>   | <i>P. vivax</i>   | <i>P. ovale</i>   |
|--|---|--|---|---|
| SIZE of young trophozoite in comparison with diameter of red cell (at the same stage of development) | <br>1/5 to 1/3 of diameter   | <br>1/4 to 2/3 of diameter, but often band form seen                        | <br>1/4 to 2/3 of diameter           | <br>1/4 to 2/3 of diameter                 |
| APPEARANCE of infected red cell  | <br>Remains unchanged  | <br>Remains unchanged or becomes smaller and sometimes more deeply coloured | <br>Enlarged and often pale-staining | <br>Enlarged, oval, with torn jagged edges |
| DOTS in the infected red cell  | <br>Often none  | <br>None  | <br>Small pink, Schüffner dots     | <br>Large James dots always present      |
| STAGES found   | Trophozoites or gametocytes or both together; many trophozoites can be found in one cell  | All forms found in the same film   | All forms found in the same film  | All forms found in the same film  |

 Note: Keep in mind there is Maurer's dots in older trophozoites of *P. falciparum*.

COMPARISON OF EACH TYPE OF MALARIA PARASITES IN THIN BLOOD FILMS

|              | <i>P. falciparum</i> | <i>P. vivax</i> | <i>P. malariae</i> | <i>P. ovale</i> |  |
|--------------|----------------------|-----------------|--------------------|-----------------|--|
| Trophozoites | Young                |                 |                    |                 |  |
|              | Old                  |                 |                    |                 |  |
| Schizonts    | Immature             |                 |                    |                 |  |
|              | Mature               |                 |                    |                 |  |
| Gametocytes  | Male                 |                 |                    |                 |  |
|              | Female               |                 |                    |                 |  |

**COMPARISON OF EACH TYPE OF MALARIA PARASITES IN BOTH THICK AND THIN BLOOD FILMS.**



**Fig 7. 17a: *Plasmodium falciparum***

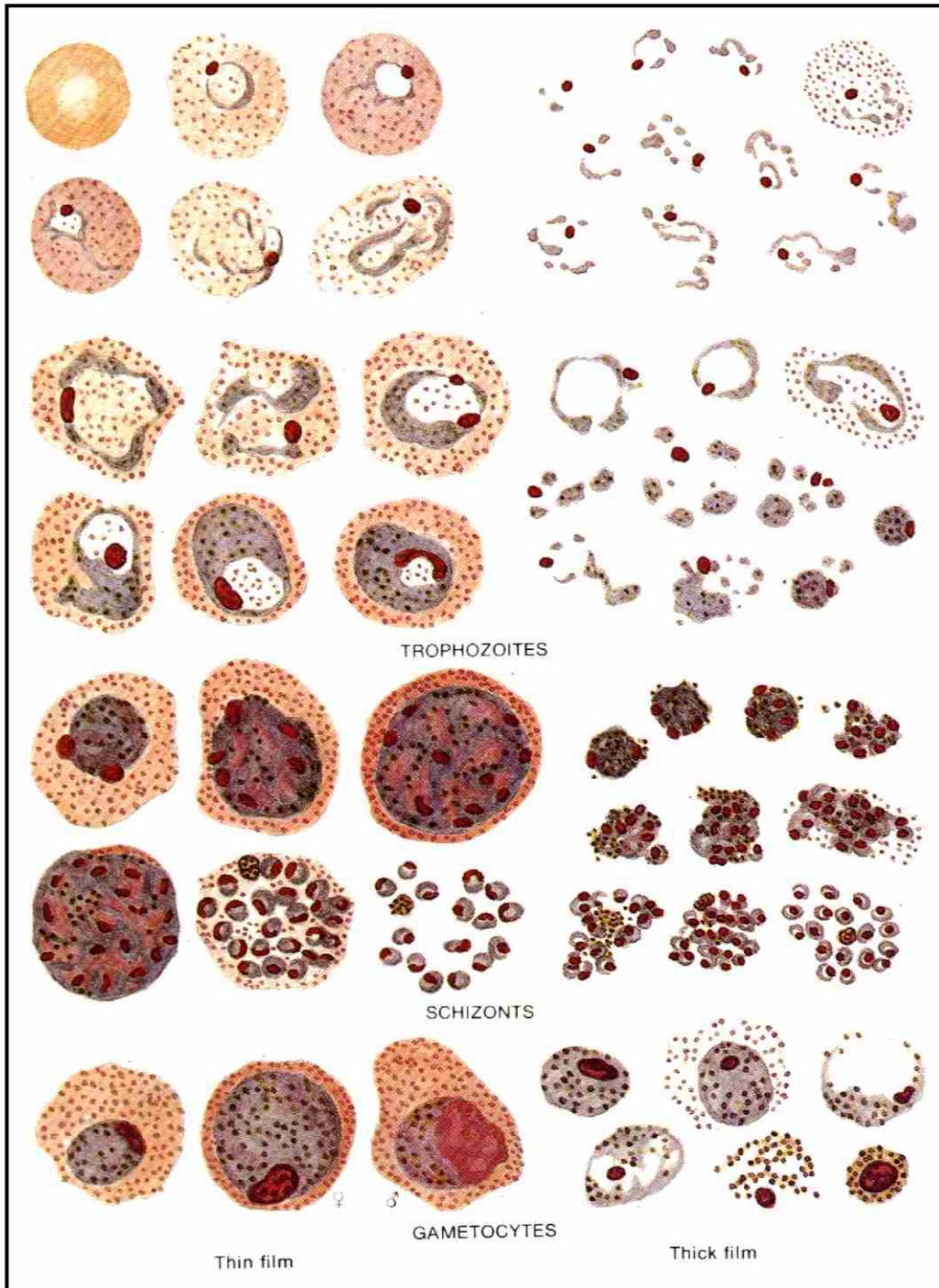


Fig 7. 17b: *Plasmodium vivax*



Fig 7. 17c: *Plasmodium malariae*

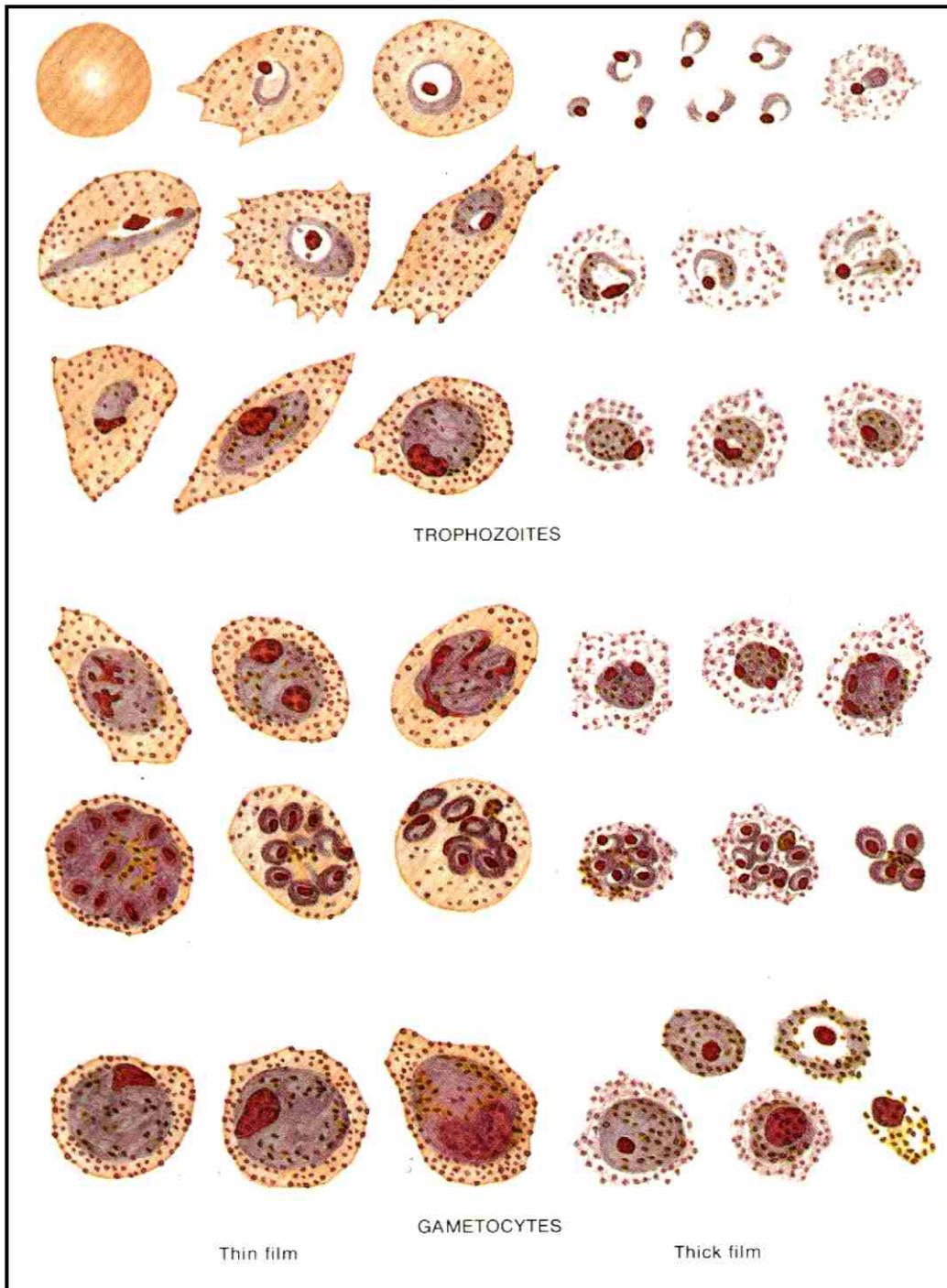


Fig 7. 17d: *Plasmodium ovale*

## 7.7 ROUTINE EXAMINATION OF BLOOD FILMS FOR MALARIA PARASITES

### A. Examining the thin film:

Since it takes 20 to 30 times as long to examine a thin film as to examine a thick film, routine examination of thin films is not recommended except in these following circumstances:

- When it is necessary to confirm the identification of a specie.
- When estimation of the percentage of RBC infected is needed (see lesson 7.8).
- When the thick smear is invalid and you cannot repeat the smear. The thick smear might not be possible to examine because it is too small, or it has become fixed, or it is wiped out **but it is preferable to repeat the smear.**

**NEVER GIVE A NEGATIVE RESULT BY LOOKING AT THIN SMEAR ONLY!**

### B. Examining the thick film:

Routine examination for malaria parasites is done on the thick blood films. However, sometimes it is difficult to differentiate some stages or species of malaria parasites so it needs to be confirmed by examination of the thin blood film.

The examination of each blood film should be done in a systematic way as follows:

1. Use X100 objective (with oil immersion).
2. Start looking from the edge of the middle of the film (as shown in figure 7.18).
3. Examine the blood film, following the pattern of movement shown one by one field systematically.
4. Continue the examination **for 100 good fields** to determine whether the blood film is positive or negative for malaria.
5. If doubtful diagnosis, more fields should be examined, or a second smear requested.



Fig 7.18: Examination of a thick and thin blood films

6. At the end of the examination, record the result on the lab request form and in the record book. The result must include parasite specie(s), stage(s) and density.

## 7.8 MALARIA PARASITE DENSITY

It is necessary to know the density of parasites in blood film because of these following reasons:

- To know how severe the malaria is.
- To know if the patient will need a blood transfusion.
- To know whether the parasites are responding to the antimalarial treatment being given.
- To know the severity of malaria infections being seen in the camp.

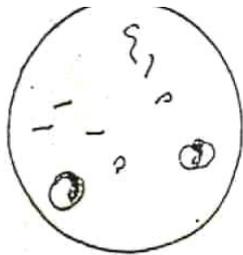
### REPORT:

Parasites density is estimated on the **thick smear** by counting parasites in each field and then averaging the number and reporting as:

|                          |            |
|--------------------------|------------|
| < 1 parasite / field     | rare       |
| 1 – 2 parasites / field  | + or 1+    |
| 3 – 25parasites / field  | ++ or 2+   |
| 26 – 60parasites / field | +++ or 3+  |
| > 60parasites / field    | ++++ or 4+ |

In order to get an accurate result; the number of parasites should be counted only from the standardized thick smear (see figure 7. 19) in which WBCs are regularly scattered.

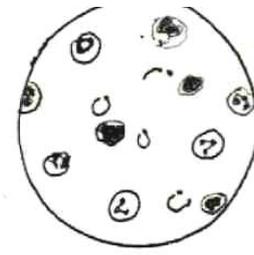
**ON A GOOD SMEAR, YOU SHOULD FIND ABOUT 10 WBCs PER FIELD**



Bad smear  
(not enough blood)



Fig 7. 19:



Good smear  
(enough blood)

Sometimes the smear looks good with proper amount of blood but it is not spread regularly. Therefore some parts are thinner than others. In this case, you should count only in the good parts (see about 10 WBCs / field).

If the smear is too thick (too much blood), Giemsa stain is not able to enter the blood cells. The colour will be bad (blue) and you cannot see the parasites. In this case a new smear should be prepared.

## PERCENTAGE OF INFECTED RBCs

This percentage is estimated on the thin smear.

**By definition a PFT 4+ is when 4 % or over 4% of the RBCs are infected.**

### **IN ALL PATIENTS WITH PFT 3+ OR PFT 4+, THE PERCENTAGE OF RBCs PARASITISED MUST BE ASSESSED on the thin smear because:**

- \* Treatment for patients with PFT 3+ or PFT 4+ might be different.
- \* Sometimes it is not easy to determine on the thick smear whether it is a PFT3+ or a PFT 4+, the percentage of RBCs parasitised will give you the accurate result (see definition of PFT 4+ above).
- \* In a PFT 4+ case, it is important for the medic to know if the patient has 5 %, 10 %, 20 % or 40 % of RBC parasitised. The management of the patient will be partially based on this result.

#### HOW TO DO:

1. Have a GOOD thin smear where RBCs are regularly spread. If not the case repeat a couple of thin smear.
2. Check first the thin smear with objective x 40 (without immersion oil) and find a place where **RBCs are regularly spread**: RBCs not overlapping or not too far away from each other. This is important for the accuracy of the result.

**IN ONE HIGH POWER FIELD (HPF: with objective x 100) WHERE RBCs ARE REGULARLY SPREAD, THERE IS APPROXIMATELY 200 RBCs.**

**Important remark:** This is valid for the Olympus microscopes (models CHD, CH-2, CHS and their respective components) widely used on this border.  
If others brands or models of microscope are to be used the average number of RBCs per HPF has to be assessed in the first place.

3. When the place is found, shift on the x 100 objective with immersion oil.
4. Count RBC with parasites (\*) in 10 fields MINIMUM (\*\*).

\* **Note:** One RBC infected with 3 parasites will be counted as 1 (not 3)

\*\* **Note:** If the percentage is high (many parasites) counting 10 fields is enough to provide a good estimation. If the percentage is low (between 2 and 10%) there is fewer RBC with parasites to count. It is therefore recommended to count in 20 fields for a more accurate result.

## 5. Result

If N is the number of RBCs with parasites counted in 10 fields.

Then N is also the number of RBCs with parasites for 2000RBCs (1 field = 200RBCs)

But we need a percentage, which is the number of RBCs with parasites for 100 RBCs.

N Divided by 2 will be the number of RBCs with parasites for 1000 RBC, and then you need to divide again by 10 to find the number of RBCs with parasites for 100 RBCs.

Note: you do not need a calculator to divide a number by 2 and then by 10.

Example:

You counted 252 RBCs with parasites in 10 fields (for 2000 RBCs)

So you have 126 RBCs with parasites for 1000 RBCs.

The result will be PFT 4+ with 12.6 % of RBC parasitised

### Laboratory indicators of severity:

- \* **Parasites 4+ or  $\geq 4\%$**
- \* **Schizonts presence**
- \* **Malaria pigment in WBCs (monocytes and/or neutrophils): see figure 7. 20.**

**!!! These should be reported to the medic !!!**

Example of good results:

PFTS 4+; 12% of RBCs parasitised; presence of malaria pigment in WBCs.

PFTG 4+; 16% of RBCs parasitised.

Examples of bad results:

PFTS 4+

PFT4+; 3% of RBCs parasitised.

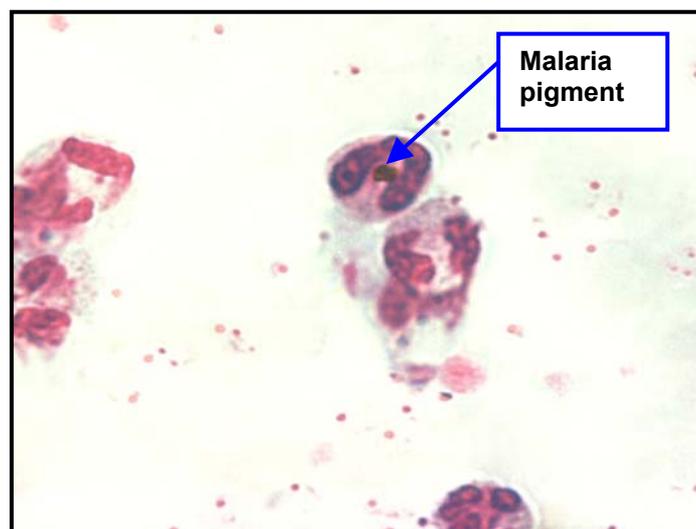


Fig 7. 20: Malaria pigment inside a neutrophil (blue arrow).

## 7.9 MALARIA RECORD

### FOR POSITIVE RESULT:

- You must write:
- The name of malaria parasite types (PF, PV, PM, or PO)
  - The stage of the development
    - T** = Trophozoite
    - S** = Schizonts
    - G** = Gametocyte
  - The parasite density (+, ++, +++, +++++ and/or percentage infected RBCs)
  - Malaria pigment in WBCs

- Example of malaria report
- PFT 1+
  - PVTS 2+
  - PFT 1+; PVT2 +
  - PVT 2+; PMTS 1+

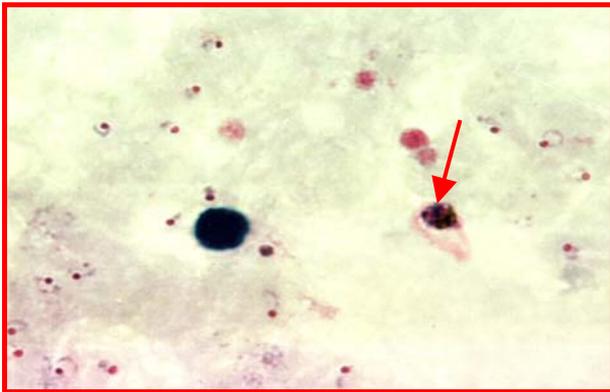
### FOR NEGATIVE RESULT:

- You must write: - **NF** (Not found) or **Neg** (Negative)

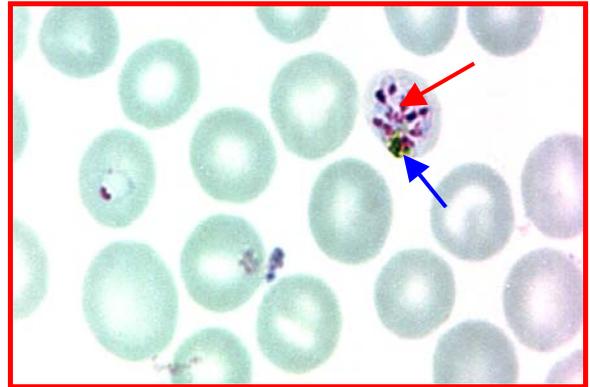
**All of the following data must be written on the request form as well as in the malaria register book:**

- Date
- Slide number
- Patient's name
- Patient's age
- Patient's sex
- Address of the patient
- Age group (A, B or A, B, C or whatever on your medical data system)
- Request test (MS or M/S)
- Examination result
- Signature.

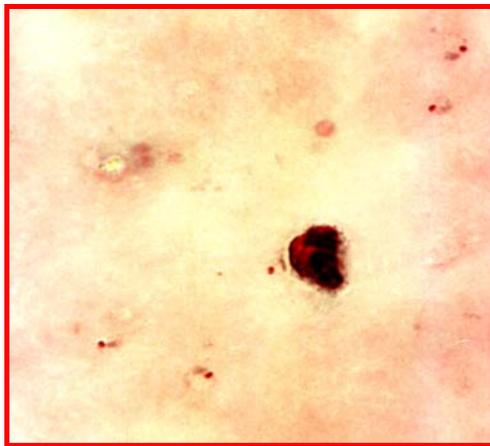
Pictures of *Plasmodium falciparum*



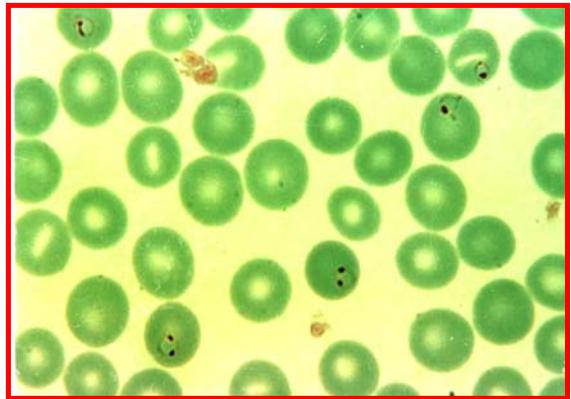
*P.falciparum* rings and schizont (arrow) on thick blood film.



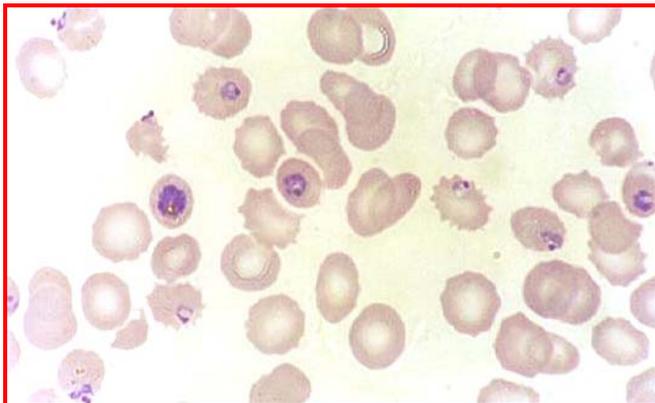
*P.falciparum* schizont (right) with merozoites (red arrow) and malaria pigment (blue arrow). One ring form on the left.



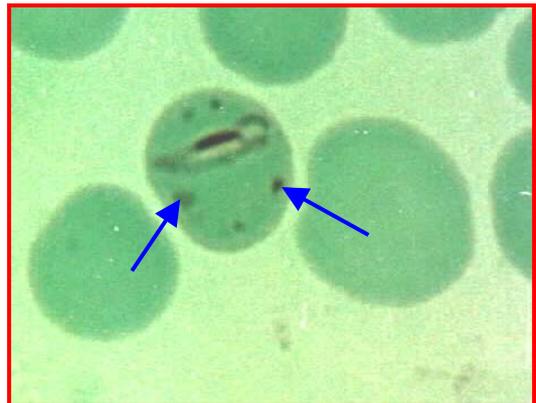
*P.falciparum* trophozoites on thick film.



*P.falciparum* tiny rings on the thin film. Note the size of the ring compare to the RBC size.

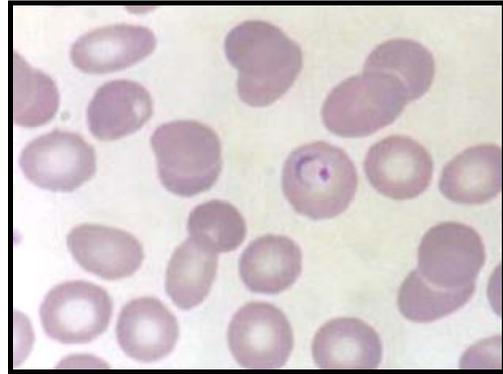
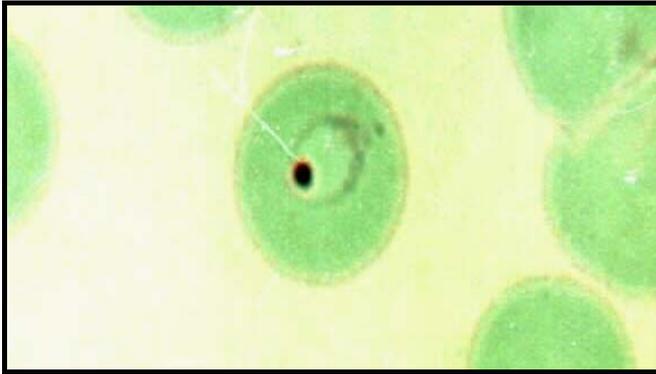


*P.falciparum* old trophozoites on thin film. Note the parasite cytoplasm is thicker and the RBC size remains unchanged.

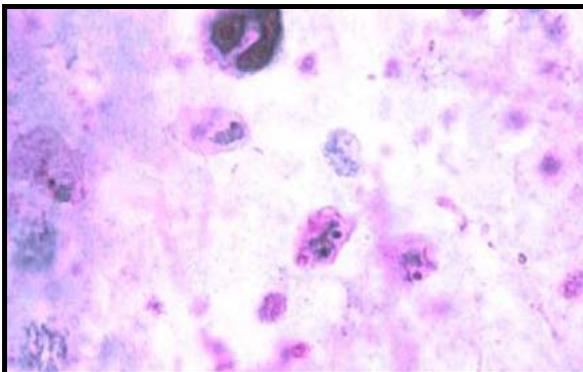


*P.falciparum* old trophozoites with 5 Maurer's dots in RBC cytoplasm (blue arrows).

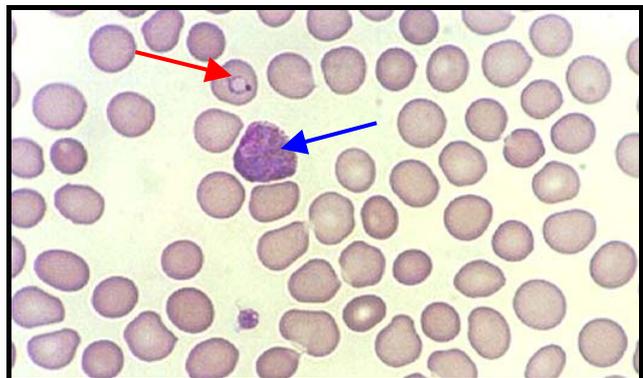
**Pictures of *Plasmodium vivax***



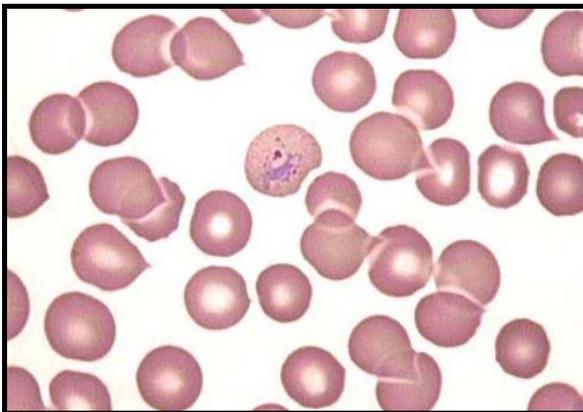
*P. vivax* young trophozoites on the thin film: infected RBCs are just very slightly enlarged but mostly; what makes the diagnosis is the size of the ring.



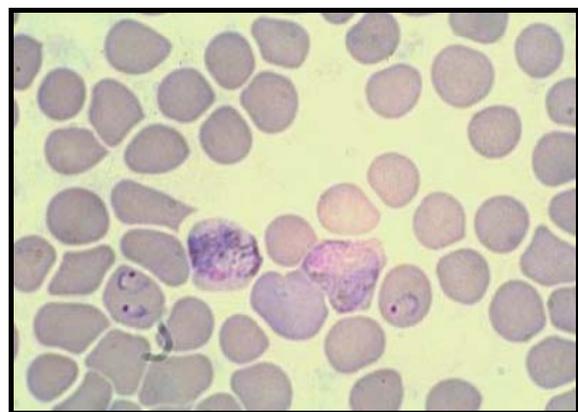
*P. vivax* old trophozoites on thick film: big rings with irregular cytoplasm.



*P. vivax* young ring (red arrow) and *P. vivax* gametocyte (blue arrow).



*P. vivax* old trophozoite with Schuffner's dots in RBC cytoplasm.

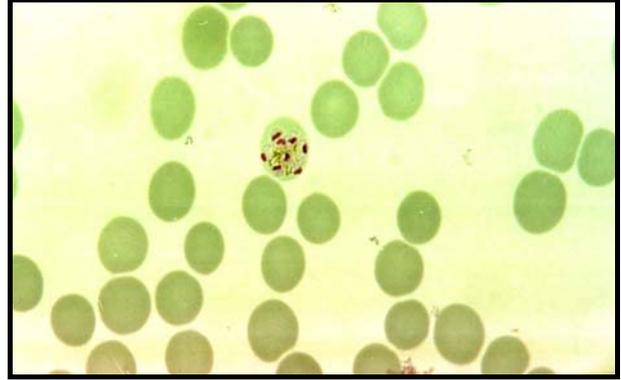


*P. vivax* young rings (RBC on the left is infected with 2 rings) and gametocytes.

**Pictures of *Plasmodium malariae***



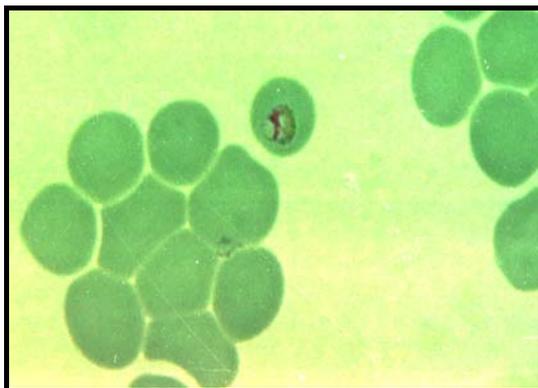
*P. malariae* schizonts on the thick film. Note the characteristic yellow-brown pigment.



*P. malariae* schizont with 8 neatly arranged merozoites (rosette formation). RBC not enlarged and yellow-brown pigment



2 trophozoites of *P. malariae* on thick film. Characteristic dense and compact cytoplasm close to the nucleus

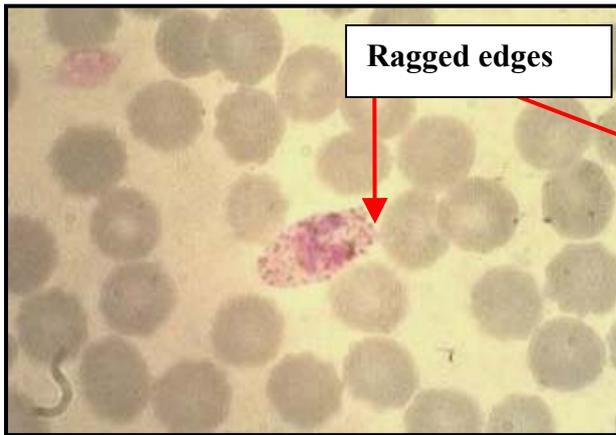


*P. malariae* on thin film. Note the RBC size is clearly smaller here.



*P. malariae* trophozoite: typical "band form" across the RBC.

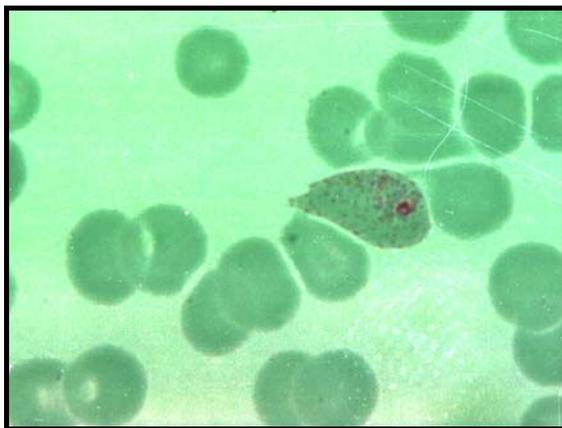
**Pictures of *Plasmodium ovale***



*P. ovale* old trophozoite on thin film. Note the oval shaped RBC with ragged (fimbriated) edges. RBC slightly enlarged compared to *P. vivax*. James' s dots are bigger and darker than Schuffner's dots.



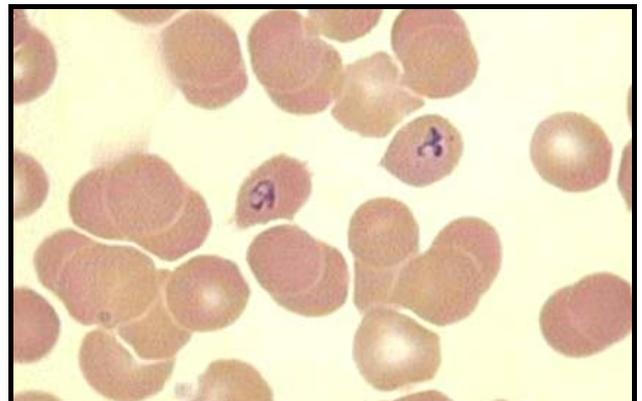
*P. ovale* old trophozoite (up) and gametocyte (down). The gametocyte is smaller than a *P. vivax* gametocyte and has ragged edges. The upper infected RBC is slightly enlarged but has a normal shape.



*P. ovale* old trophozoites on thin film. Both infected RBCs are oval shaped with ragged edges.

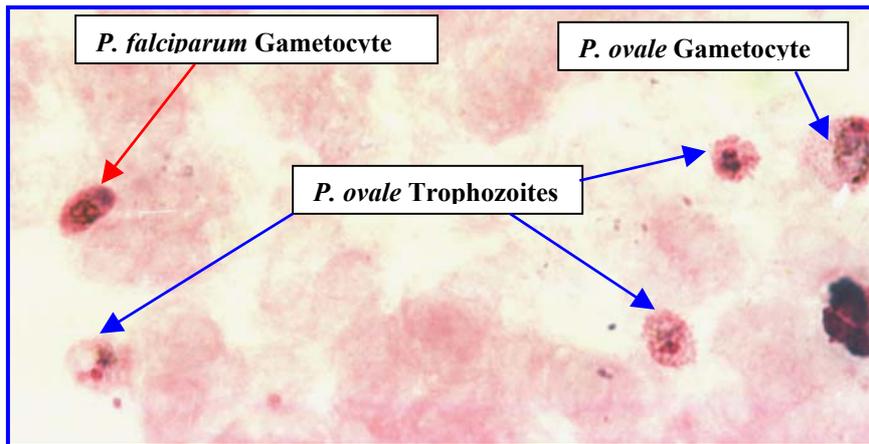


*P. ovale* young trophozoite (small ring). RBC is slightly enlarged and oval shaped. Note the very nice James's dots.

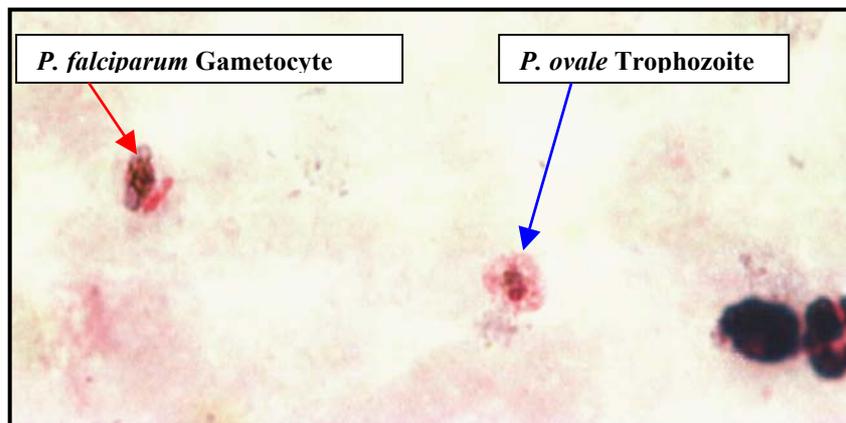


*P. ovale* young trophozoites. James's dots are invisible in the left RBC and barely visible in the right RBC because of the stain quality.

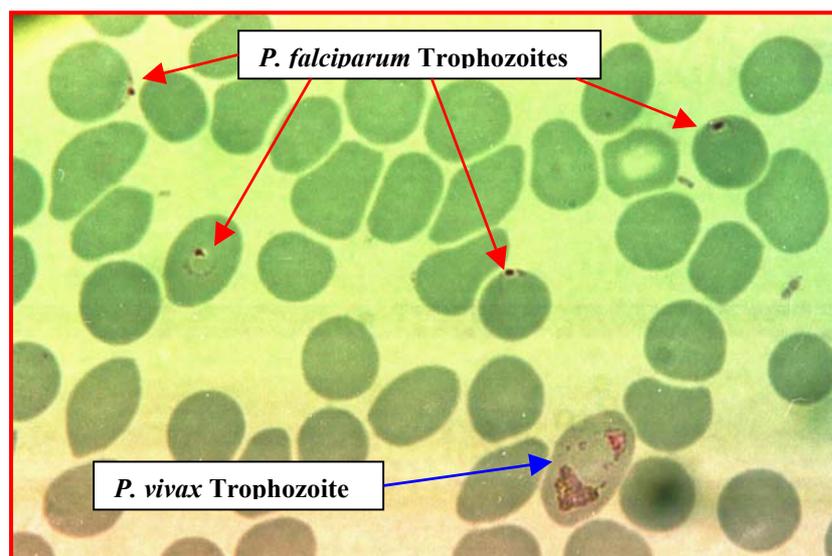
## Pictures of mixed infections



*P. ovale* trophozoites on thick film are smaller than *P. vivax*. They also appear; like *P. malariae*, with dense and compact cytoplasm close to the nucleus: *P. ovale* needs to be confirmed on the thin film.



Same patient as above: *P. falciparum* trophozoites were not found. The result was POTG ++ and PFG Rare. Note that the *P. falciparum* gametocyte is more easily identifiable here.



*P. falciparum* trophozoites are young: small rings.

*P. vivax* trophozoites is old: infected RBC enlarged, parasite with big nucleus and irregular cytoplasm. Note that Schuffner's dots are present at that stage but are not visible on the picture.

## 8. FILARIASIS

**Filariasis** is a disease caused by parasites called **filarial worms**. Adult forms of these filarial worms live in the lymphatic tissues. Their young larvae live in blood circulation so we examine them in the same way as we do a malaria examination.

### TRANSMISSION:

Transmission of these filarial worms is similar to malaria transmission by 3 types of mosquitoes including *Anopheles*.

### SYMPTOMS AND CLINICAL SIGNS:

Only some infected persons develop clinical symptoms such as recurrent fever painful inflamed lymphatics.

The infection can cause thickened and rough skin on genital organs, breasts and limbs.

The well-know sign is the appearance of the big lower leg (or foot) called "**Elephant-foot**" or "**Elephantiasis**"

### DIAGNOSIS:

Filarial infections can be diagnosed by examining the **blood**. The young larvae called "microfilariae " are present in the blood and occasionally in **urine**. A high percentage of **eosinophils** can also be found.

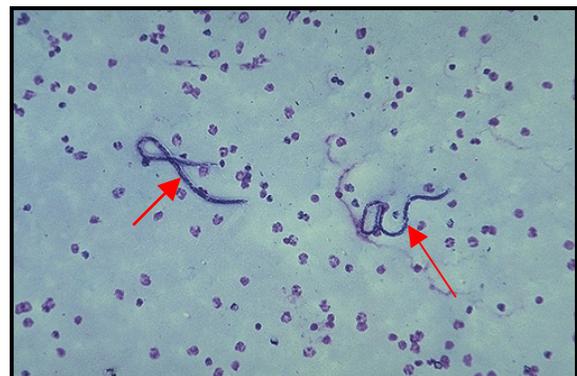
The **optimal time** for collecting the blood sample is between 10:00 PM - 4:00 AM when the greatest number of microfilariae is present in the blood. Sometimes you may also find microfilariae during a regular malaria examination.

**Thick blood smears** stained with Giemsa are recommended for this exam, preferably using a bigger drop of blood than regularly used in the examination for malaria.

With a **microscope**, look for microfilariae which are:

- Very big compared with a malaria parasite: 200 to 300 um long large according to the species.
- They do not live inside the blood cells.
- They are big enough to be detected with X10 objective (see figure 8. 1), and then confirmed with X40 or X100 objective (see figures 8. 3 & 8. 4).

Fig 8. 1: Microfilariae as seen on a Giemsa stained blood smear with X10 objective.



Special characteristics of microfilariae are:

- 1 A colorless or pale sheath is often seen at both ends.
- 2 Nuclei are seen along the whole parasite body.

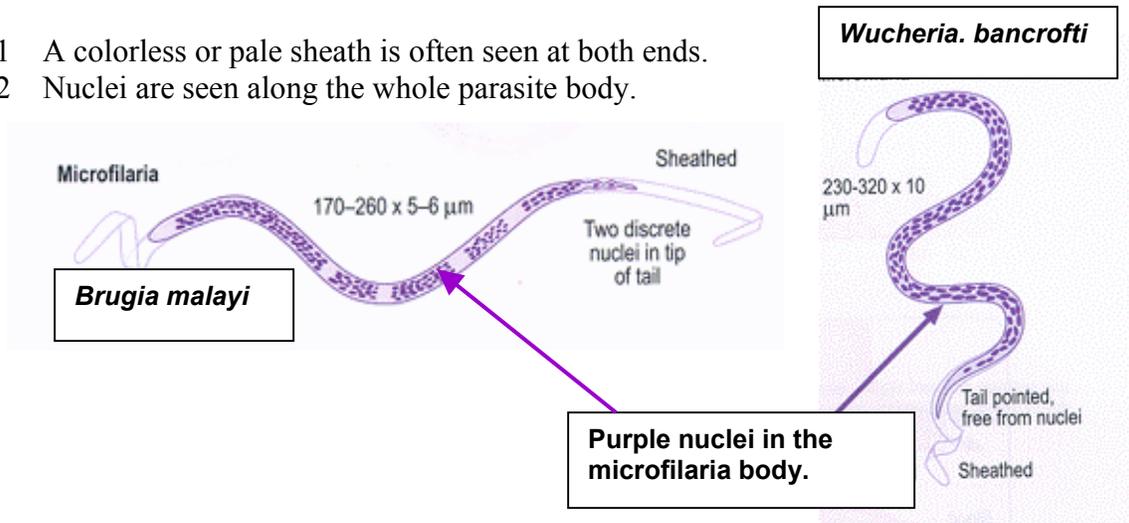


Fig 8. 2: Main morphological differences between the two species encountered in S.E Asia.

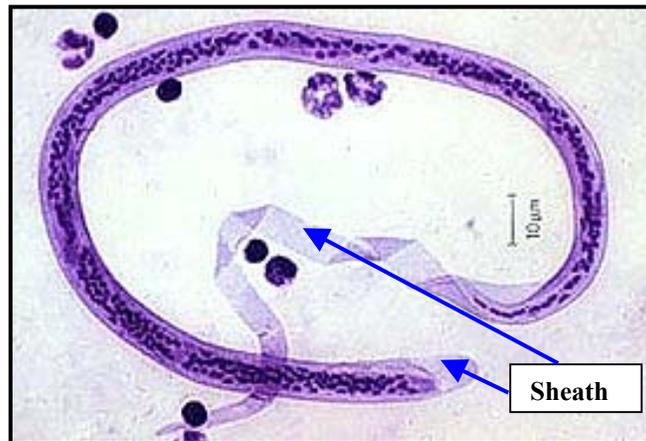


Fig 8. 3: *Wucheria bancrofti* microfilaria as seen with X40 objective. Note the pale sheath and the absence of terminal nuclei.

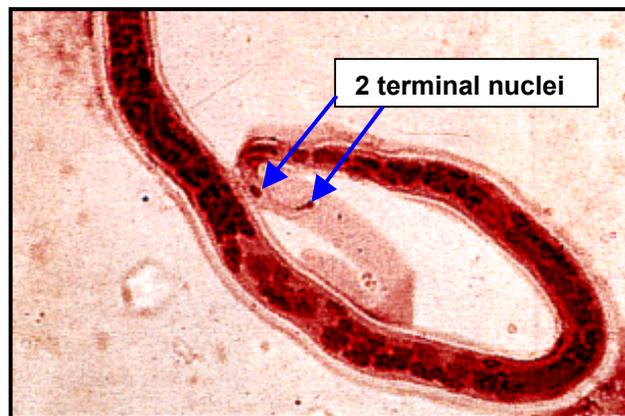
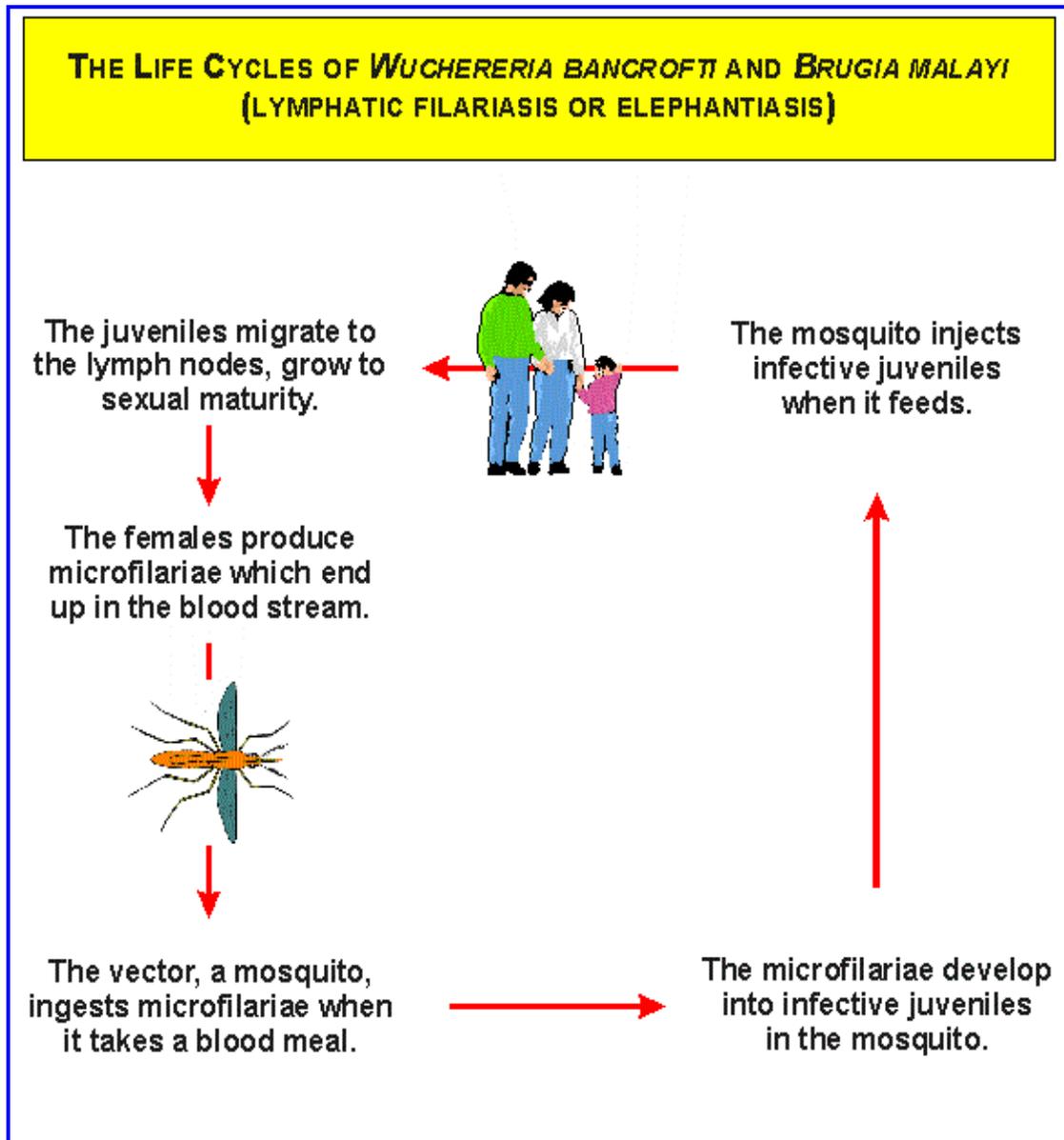


Fig 8. 4: *Brugia malayi* microfilaria as seen with X100 objective. Note the two terminal nuclei in the tail.

## LIFE CYCLE OF FILARIA PARASITE



**TREATMENT:** with diethylcarbamazine (DEC)

*People in endemic areas should check their blood for microfilariae once or twice yearly.*

## 9. DETERMINING HAEMOGLOBIN AND HAEMATOCRIT

### 9.1 DETERMINING OF HAEMOGLOBIN LEVEL (USING UNDILUTED BLOOD)

**Haemoglobin** (Hb) is the part of the red blood cell that carries oxygen from the lungs to all cells of the body. If haemoglobin is low (lacking in our body), there will not be enough Hb to carry oxygen to the tissues. When this happens, the patient is said to be **anaemic**.

#### Principle of the method:

Blood obtained by pricking the finger, is run directly into a special slide.  
The color is compared with a series of colored glasses in a Lovibond comparator.

#### Materials needed:

- \* Lovibond reader.
- \* Disc A covers the number 20, 24, 28, 32, 36, 40, 46, 52, 58.
- \* Disc B covers the number 64, 70, 76, 84, 92, 100, 110, 120.
- \* Special 0.004 slide.
- \* Gloves / Blood lancets / Cotton.
- \* Savlon / water / tissue paper / dustbin

#### Method:

1. **Clean** the 2 slides thoroughly, first with water and then with savlon; rinse Well, and dry with clean tissue paper.  
If the cleaning is not correctly done, air bubbles form when the blood is run into the space between the 2 slides, leading to incorrect result.
2. **Place** the 2 slides in opposition and clip them together (figure 9. 1 A and B).

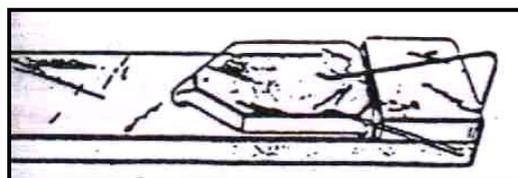
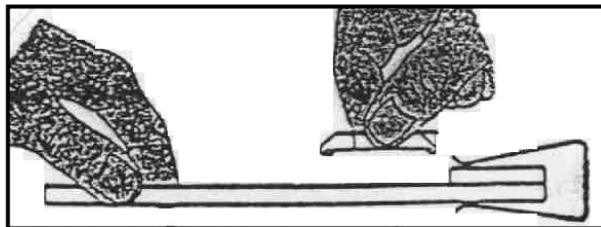


Fig 9.1 (A, B)

3. **Fill** the space between the 2 slides, from the side, with capillary blood (figure 9. 1C)

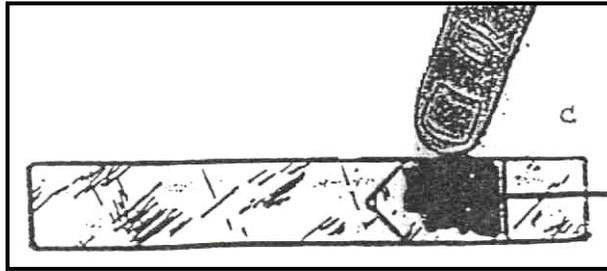


Fig 9. 1 C

4. **Remove** the clip, as the 2 slides will stick together by capillarity.
5. **Place** the slides carefully into the right-hand holder of the comparator.
- The blood film must completely fill the right-hand field of view and be free from air bubbles.
6. **Hold** the comparator with your arm straight and face a white light (direct sunlight or incorrect artificial light gives incorrect result).
7. **Move** the disc until the glass color corresponds to the color of the blood. The reading should be done **AS SOON AS POSSIBLE** after filling the cell (within one minute).
8. **Use** the conversion table to translate the number written on the disc in g/100 ml.
9. **Wash** the 2 slides as soon as possible after use (as in step 1).

**Attention:**

**Please handle the glass slides with good care, as their cost is quite expensive.**

**CONVERSION TABLE**

| Reading on disc A or B | g /100ml |
|------------------------|----------|
| 20                     | 3.3      |
| 24                     | 4.0      |
| 28                     | 4.7      |
| 32                     | 5.3      |
| 36                     | 6.0      |
| 40                     | 6.7      |
| 46                     | 7.3      |
| 52                     | 8.7      |
| 58                     | 9.7      |
| 64                     | 10.7     |
| 70                     | 11.7     |
| 76                     | 12.7     |
| 84                     | 14.7     |
| 92                     | 15.3     |
| 100                    | 16.7     |
| 110                    | 18.3     |
| 120                    | 20.0     |
| 130                    | 21.7     |

## 9.2 DETERMINING OF HAEMATOCRIT

**Haematocrit** (Hct) is the proportion of packed red cells volume per whole blood volume and is presented in percentage as follows:

$$\text{Haematocrit} = \frac{\text{total volume of packed red cell} \times 100}{\text{Volume of whole blood}}$$

*Example:* 10 ml of one patient's whole blood has 4ml of packed RBC, so % Hct of this patient is:  
$$\frac{4}{10} \times 100 = 40\%$$

The haematocrit indicates the proportion volume of RBC in order to know if the patient has anaemia.

### Haematocrit measurement

#### Principle of the method:

Blood is collected into a small capillary tube and is spun at high speed. The blood will be separated firmly into 3 parts: upper part is plasma, middle part is a small volume of white blood cells and the lower is packed red cells. The fraction of packed red cells volume to the entire blood volume is measured as the percent haematocrit.

#### Materials needed:

Heparinised capillary tube: It contains dried heparin as anticoagulant inside so the blood will not clot.

Clay sealer

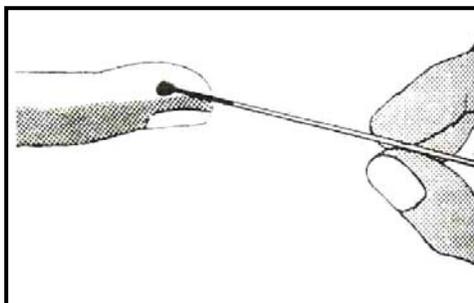
Microhaematocrit centrifuge (electricity needed)

Specially designed scale for reading the result.

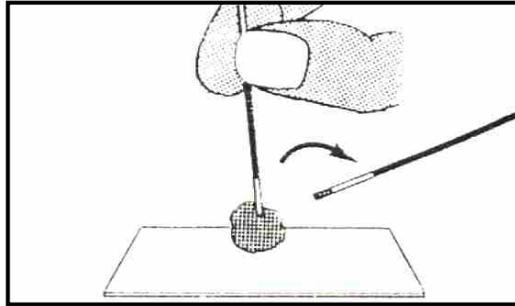
Rubbing alcohol or savlon / Gloves / Blood lancets / Cotton / Container for disposal

#### Method:

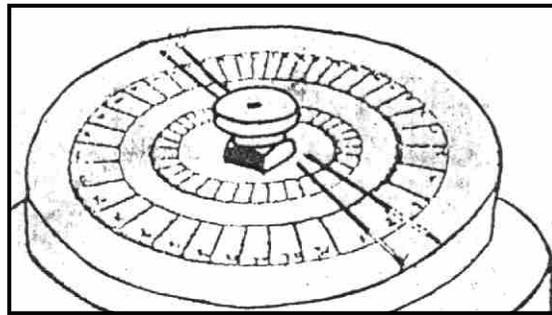
1. Collect blood by using blood lancet as the safety method. Fill 3/4 of the capillary tube by applying the blood to the side **opposite** to the red coloured end.



2. Plug the red coded end of the tube into the clay completely (depth of about 2 mm).



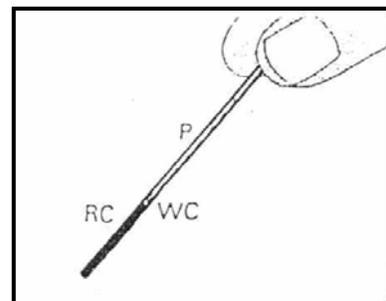
3. Place the tube in the numbered slots in centrifuge head. The sealed end of the tube should point outward, away from the center. Don't forget to balance the tubes.



4. Centrifuge at high speed (10,000 to 12,000 rounds per minutes) for 5 minutes. After 5 minutes, allow the spinner head to stop.

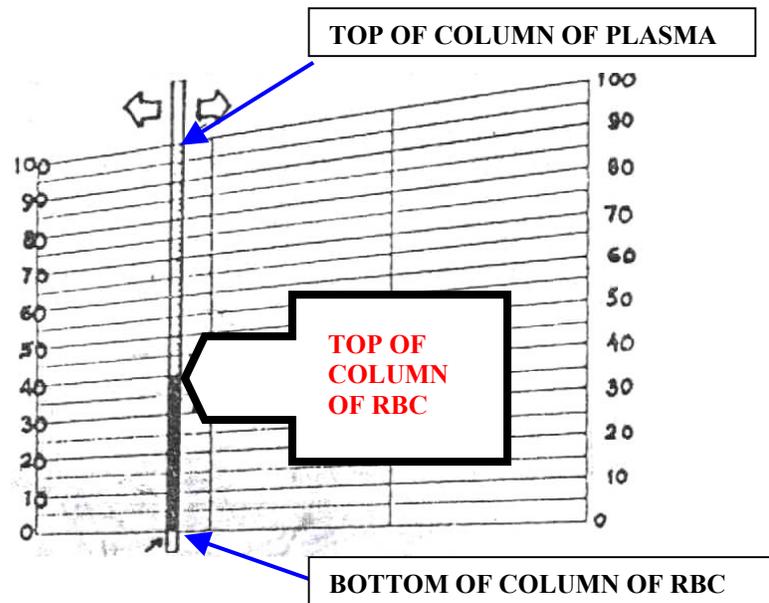
5. The tube shows 3 layers of blood.

- At the top, a column of plasma (P)
- In the middle, a very thin layer of WBCs (WC)
- At the bottom, a column of RBCs (RC)



The packed RBC volume reading is made exactly at the top of the RBC column.

6. Read the layers by using the scales:
- Hold the tube against the scale.
  - The bottom of RBC column (not the bottom of the tube) should be aligned with the horizontal zero line.
  - Move the tube across the scale until the top of the plasma column reaches 100% mark line.
  - Make sure that the bottom of RBC column is still on the line 0 (zero) and the tube is always vertical.



7. The line that passed through the top of the RBC column gives packed red cell volume fraction (40% in the figure above).

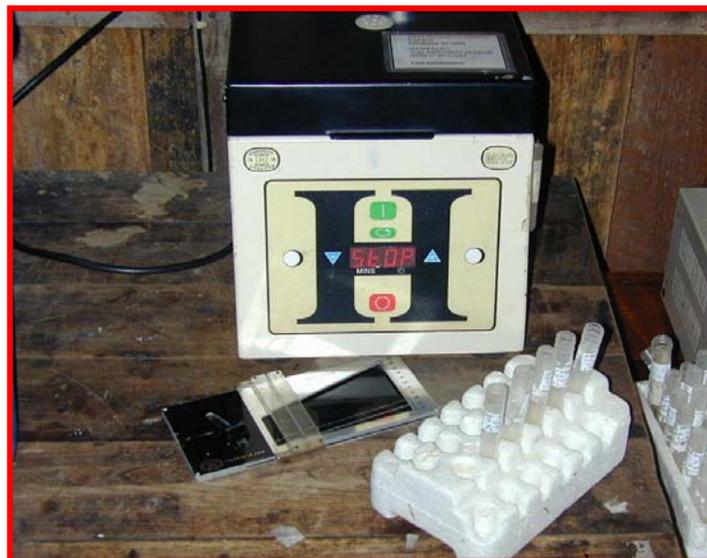
| Normal values                          | Haematocrit |
|--|-------------|
| Men (>15 years old)                    | 40-50%      |
| Women (>15 years old)                  | 37-43%      |
| Children (>2-15 years old)             | 38-44%      |
| Baby infants (10 days old -2 year old) | 35-49%      |
| Newborn infants (1 day old-9 days old) | 44-64%      |

**Lower values of % Hct** are found in patients suffering from anaemia. For example: lower than 40% in men and lower than 37% in women.

**High values of % Hct** are found in cases where the patient is suffering from a loss of plasma: severe burns, dehydration, infant diarrhea, dengue hemorrhagic fever, etc.



**A scale for the haematocrit measurement**



**Microhaematocrit centrifuge**

## 10. TUBERCULOSIS

**Tuberculosis (TB)** is a disease caused by infection with BACTERIA, *Mycobacterium tuberculosis* or TB bacilli (bacilli = rod shape bacteria). Tuberculosis usually attacks the LUNGS, causing pulmonary TB (see figure 10. 1). The bacilli cause holes to form in the lung. TB can also affect the spine, hips, lymph nodes, kidneys and many other parts of the body.

### Transmission:

- Tuberculosis is spread by people, **NOT** by insects or blood transfusion.
- Like the common cold, TB is spread through the air.
- Only people who are sick with pulmonary TB can spread the disease. When they cough, sneeze, talk, sing or spit; the TB bacilli inside their lungs come out to the air.
- A person can get these bacilli by breathing them into the lungs.
- Most infected people never develop the disease because their bodies have systems to fight against TB bacteria.

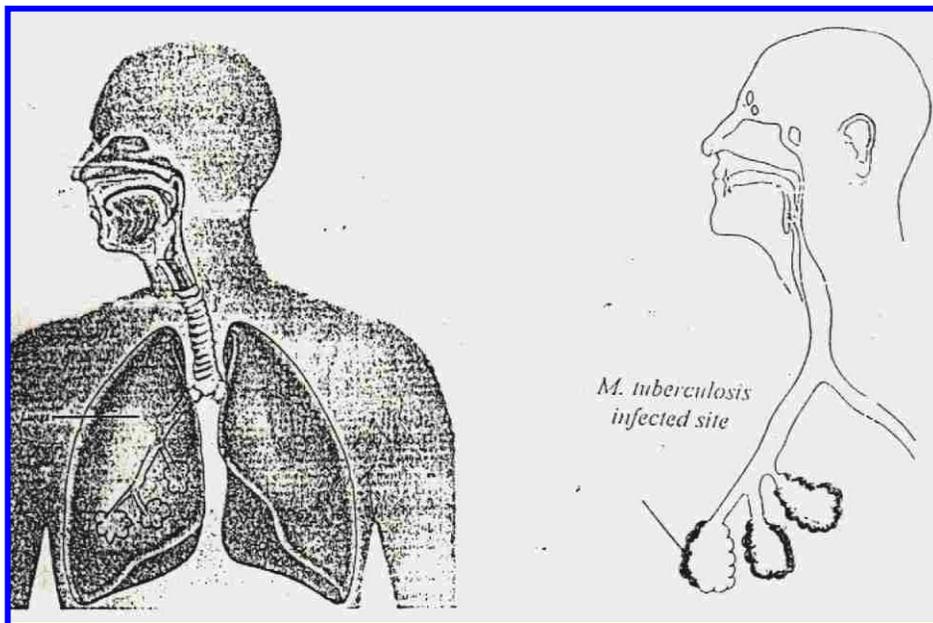


Fig 10. 1

**Signs and symptoms:**

- Chronic cough for more than 3 months.
- Loss of weight.
- Loss of appetite.
- Slight fever and sweating at night.
- Chest pain.
- Sometimes difficulty to breathe.
- Sometimes cough sputum with blood.

**Diagnosis:**

- To confirm an active case of pulmonary TB, microscopic examination of **SPUTUM** should be done to see whether it contains **TB bacilli** (Acid Fast Bacilli = AFB). **Acid Fast Bacilli** are small and irregular shaped bacilli.
- The request test for the laboratory exam is called "**Sputum for AFB**".
- If this test is not conclusive, a chest x-ray in the hospital can confirm whether there are any small holes in the lungs.

**Treatment:**

- TB bacilli very easily become **DRUG RESISTANT**; so many drugs are used at the same time.
- Treatment is long and must be taken on a regular basis, for at least 6 months.

## 10.1 SAFETY PRECAUTIONS

W.H.O (World Health Organization) has classified microorganisms into 4 groups according to the level of danger they represent to the laboratory technician and the community.

*Mycobacterium tuberculosis* is classified in risk group III.

Risk group III is defined as a group containing organisms that present a **high risk** to the laboratory worker.

In order to avoid being infected in the lab; follow the safety rules listed below.

### 1. GENERAL SAFETY RULES

- Eating in the lab is not permitted.
- Keep children out of the lab.
- The laboratory must be kept neat, clean and free of materials not useful to the work.
- A special place, separated if possible, should be kept exclusively for the smearing and fixing of sputum. This place should be well ventilated (but not windy) and with as much light as possible.
- This TB working area should have a bench with all the material required and a closed dustbin.

**No material should leave this place.**

**Materials used in other departments should not be brought to this place.**

- While working, only **one** lab technician should be in the room.
- The bench must be decontaminated every day after smearing with alcohol or with chlorine or bleach solution.
- The content of the bin must be disposed of daily and incinerated. Someone has to be responsible for that.
- Specimen should be collected in a clean (need not to be sterile), dry, **wide-necked** and **leak proof** container. Plastic bags are not recommended.

### 11. SAFETY RULES APPLYING TO THE WORKER

- Wearing a “**duck**” **mask with micro-pore filtration < 140 μm** is recommended. Surgical masks and cotton masks give a false impression of safety.
- Wear gloves while smearing and fixing. Remove and dispose of them before leaving the working area.
- Always wash your hands with soap when work is completed.
- **Follow exactly the technical procedure for smearing and fixing.**

## 10.2 SPUTUM COLLECTION

### I PREPARE THE SPUTUM CONTAINER

Give clean containers to the patient.

On the side of container (not on the lid), the following information should be written (see figure 10. 2):

- The patient's name.
- The date of each sputum collection.
- The sputum number.

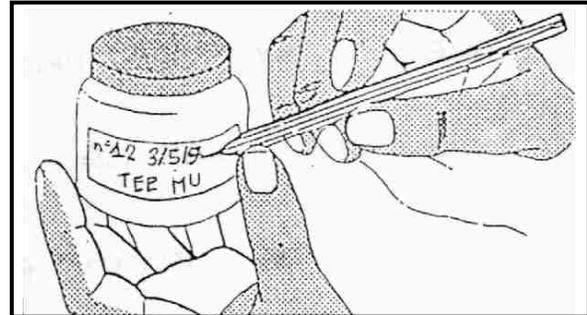


Fig 10. 2

### II SPUTUM COLLECTION

This is the responsibility of medics, nurses or lab technicians: patients should be carefully instructed about sputum collection.

This **MUST NOT** be done in the lab. It should be done in special well-aerated room or outside with nobody around.

Tell the patient to spit **in the morning**:

- 1) The patient should be standing if possible.
- 2) **Before eating** anything, just when he gets up, he should wash his mouth by **water** first.  
This will avoid the bacteria and cells from the mouth.
- 3) Take a very deep breath, filling his lung.
- 4) He should empty his lung in one breath; cough as hard and deeply as he can.
- 5) Spit into the container.

**The patient must spit every morning during 3 consecutive days.**

### III SPUTUM SPECIMEN CONTROL

The patients should bring the sample with the request from correctly filled to the laboratory technician.

You must check the quality and quantity of the sputum specimen.

#### The quality of the sputum:

**Good sputum** contains:

- Particle of pus (white, yellow or green), thread of fibrin, thick mucus and occasionally brownish streaks of blood.

**Bad sputum** is a liquid secretion and transparent (only saliva) coming from the mouth and Should not be processed.

#### The quantity of the sputum:

The quantity of the sputum should be **at least 2 ml**.

### 10.3 SPUTUM SMEARING

#### Materials (see figure 10. 3):

- New frosted-end slides
- Pencil
- Examination gloves
- “Duck” mask
- Bamboo sticks
- Wide mouth pot with sand immersed in alcohol
- Spirit lamp, matches
- Dustbin



Fig 10. 3

#### Method:

**FOLLOW THE ORDER FOR SAFETY REASON**

1. Clean the slides
2. Write on each slide (see fig 10. 4):
  - The patient’s name
  - The number of the sputum
  - The date written on the container
3. Put the gloves and mask on.
4. Light the flame of the spirit lamp.

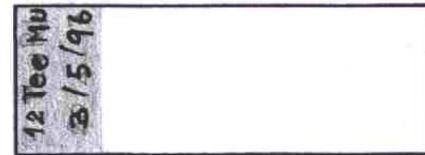


Fig 10. 4

**FOR THE NEXT STEPS, ALWAYS KEEP THE LIGHTED LAMP BETWEEN YOU AND THE SPECIMEN.  
(see figure 10. 5)**



Fig 10. 5: How to work safely

5. Carefully open the specimen container **POINTING AWAY FROM YOU** (see figure 10. 5).

6. With two bamboo sticks take a solid part of sputum (pus) and put it on the slide.
7. With the point of the sticks, smear the sputum as regularly as possible, with a circular movement. The smear should be in an oval shape of about 3 cm across.  
(see figure 10. 6).

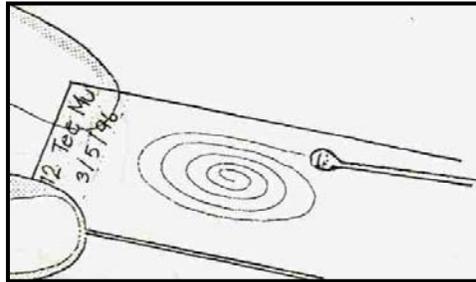


Fig 10. 6

8. Rub the bamboo sticks into the wide-mouth pot containing sand and alcohol in order to remove all of the sputum particles from the sticks.
9. Burn the sticks up to the middle. Throw them in the dustbin.
10. Close the specimen container. Do not discard yet; if anything happens, you may still need the specimen.
11. Let the smear air-dry in a safe place protected from flies, ants and dust.
12. Remove your mask and gloves, and dispose of in the bin.
13. Wash your hands.
14. When the smear is completely dry, fix it by passing the slide **over** the flame 3 times (smear on the upper side). **Don't burn it**. It must be possible to lay the slide on the back of your hand without feeling uncomfortably hot. Allow the smear to cool down before staining

## 10.4 AFB STAIN OF SPUTUM SMEAR

There are 2 methods for staining a sputum smear for AFB in order to read the smear with the ordinary microscope:

**I) Cold staining method**

**II) Hot staining method**

### STAINING METHOD:

1. **Stain with carbol fuchsin.**

**5 minutes**

#### **I) Cold staining method:**

Cover the cool side completely with the filtered, undiluted carbol fuchsin stain and leave it for 5 minutes.(see figure 10. 7)

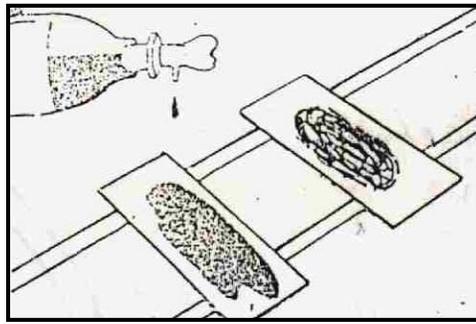


Fig 10. 7: Cold staining method.

#### **II) Hot staining method (see figure 10. 8):**

*With this method, concentration of carbol fuchsin solution used is lower than the one for the cold method.*

- Cover the smear completely with filtered, undiluted carbol fuchsin.
- Heat under the slide with a small flame unit vapour just begins to come off, then take the flame away. **Do not boil the stain.**
- Allow the stain to remain on slide for 5 minutes.

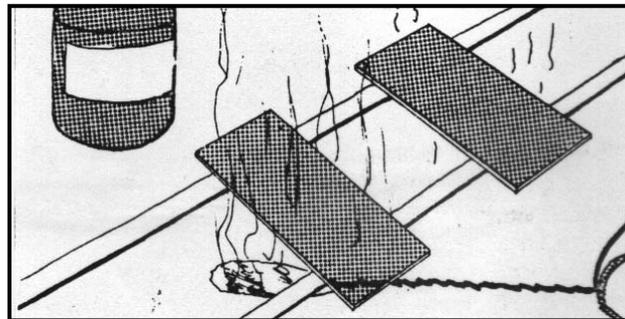


Fig 10. 8: Hot staining method

**CARBOL FUCHSIN STAINS ALL THE ORGANISMS IN THE SPUTUM RED**

2. Wash the slide gently with **WATER** and drain it well.

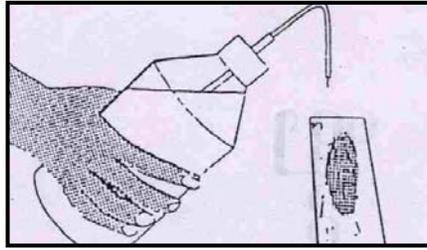


Fig 10. 9

3. **Decolourise with 3% acid-alcohol.**

|                  |
|------------------|
| <b>2 minutes</b> |
|------------------|

- Cover the slides completely with 3% acid alcohol.
- Leave it for 2 minutes or until no more colour appears in the washing (see figure 10. 10).

**Note:**

3% acid-alcohol is made by carefully mixing 3 ml of hydrochloric acid into 97 ml of ethanol.

*DO NOT MOUTH PIPETTE THESE SOLUTIONS!*

|  |
|--|
| <b>ACID-ALCOHOL WILL REMOVE THE COLOUR FROM ALL ORGANISMS EXCEPT ACID – FAST BACILLI (AFB)</b> |
|--|

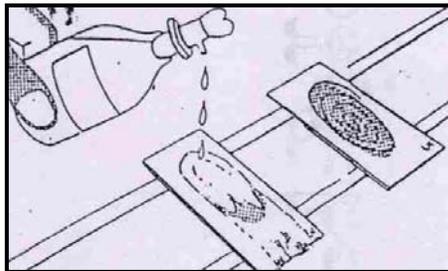


Fig 10.10

4. Wash gently with **WATER** and drain well.

5. **Stain with methylene blue.**

|                   |
|-------------------|
| <b>30 seconds</b> |
|-------------------|

Cover the slide completely with filtered undiluted methylene blue for 30 seconds.

|   |
|---|
| <b>METHYLENE BLUE STAINS ALL THE ORGANISMS DECOLOURED BY THE 3% ACID-ALCOHOL IN BLUE.</b> |
|---|

|  |
|--|
| <b>THE ACID – FAST BACILLI REMAIN RED.</b> |
|--|

6. Wash gently with **WATER** and drain well.

7. Dry the slide in the air.

## 10.5 TB SLIDE EXAMINATION

### I) Characteristic of tubercle bacilli from AFB stain.

- Red on a blue background.
- Straight or slightly curved.
- Quite short and thin.
- Often seen with red granules or spots on the bacilli, because they are often not uniformly stained.
- They can appear singly, arranged in small groups of 3-10 bacilli close together and in branches (like Chinese letters) or in big packs (less frequent). See figure 10. 11.

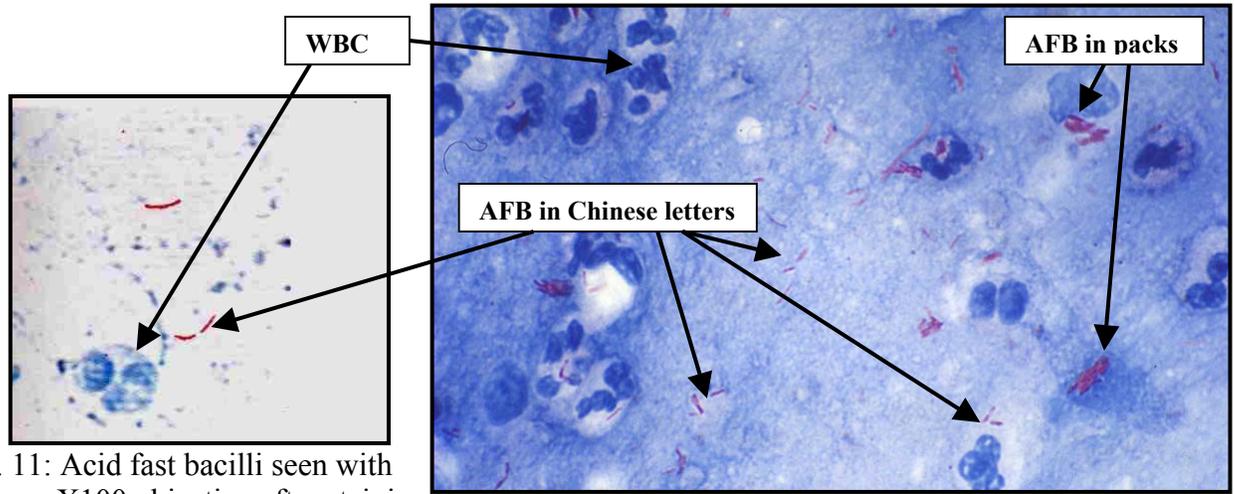


Fig 10. 11: Acid fast bacilli seen with X100 objective after staining.

### II) Microscopic examination for AFB smear

The examination of each sputum smear should be done in a systematic way as follow:

1. Use  $\times 100$  objective (with oil immersion).
2. Start looking from the upper edge of the smear.
3. Examine the smear for AFB, following the pattern of movement shown in the figure bellow, one by one field systematically.

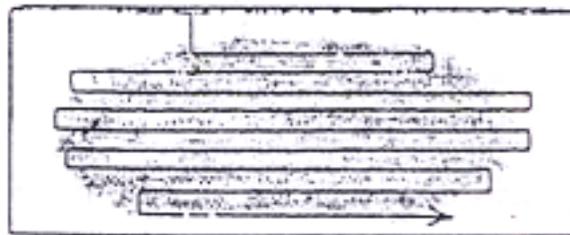


Fig 10. 12: How to examine an AFB smear.

4. Continue the examination for approximately **300 fields** to determine whether the smear is positive or negative for AFB. **This should take at least 15 minutes.**
5. If doubtful diagnosis, more fields should be examined.

**10.6 TB RECORD****1) TB REGISTER BOOK**

All the follow data should be written in the book:

- The patient's number
- The date
- The patient's name
- Sex
- Age
- Address
- TB results
- Lab technician signature

**2) TB RESULTS****\*NEGATIVE SLIDE**

Examine the whole smear thoroughly at least 15 minutes

**\*POSITIVE SLIDE**

If AFB is seen on the sputum smear, the TB result must be registered as follows:

| <b>NUMBER OF AFB FOUND:</b> | <b>REPORT</b> |
|-----------------------------|---------------|
| 0 bacilli / 300 fields      | NO AFB SEEN   |
| 1-2 bacilli / 300 fields    | AFB RARE      |
| 1-9 bacilli / 100 fields    | AFB +         |
| 1-9 bacilli / 10 fields     | AFB ++        |
| 1-9 bacilli / field         | AFB +++       |
| >1-9 bacilli / field        | AFB ++++.     |

## 11. PARAGONIMIASIS

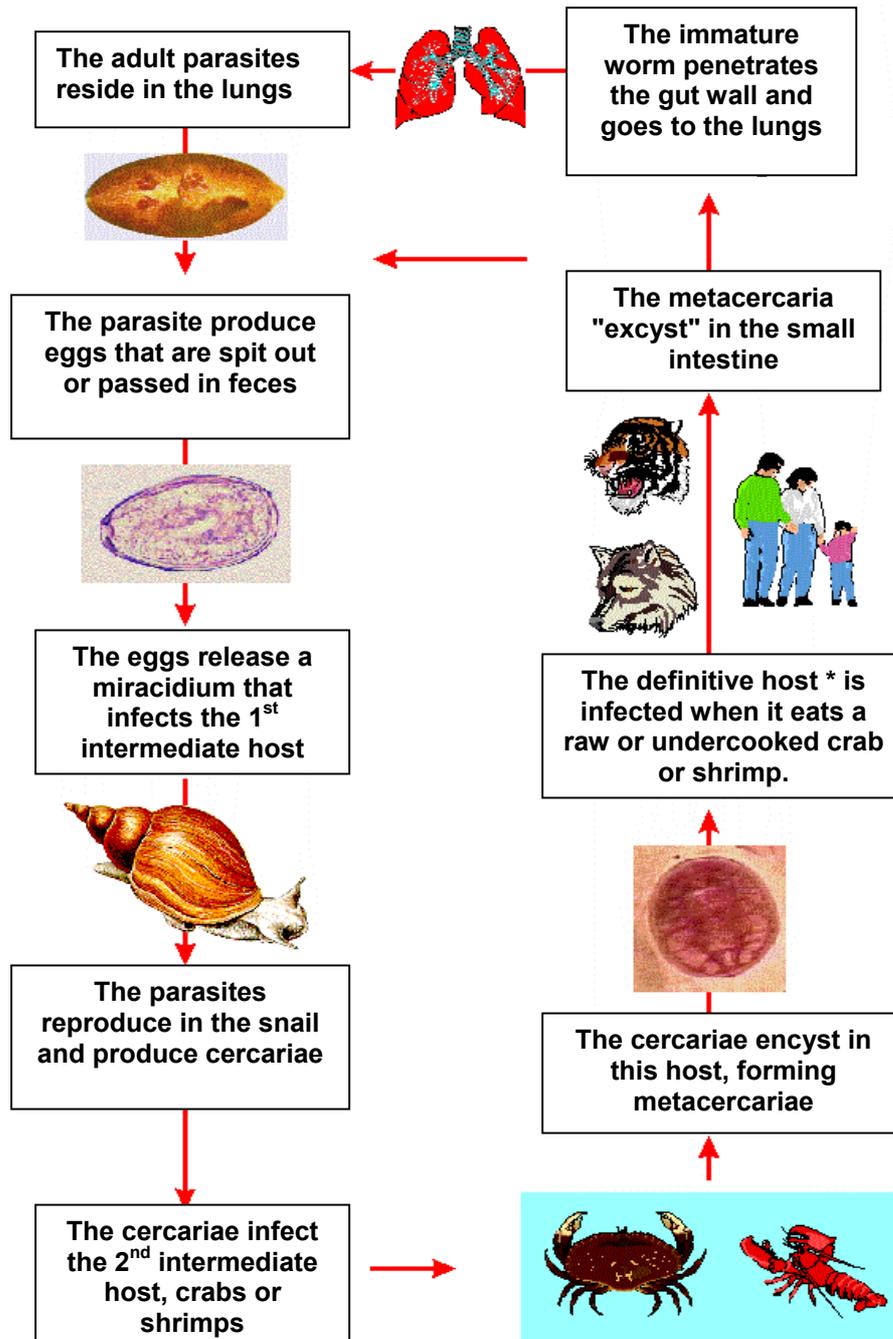
Paragonimiasis is a disease of the LUNGS caused by a PARASITE: *Paragonimus westermani*.

**SYMPTOMS:** Similar to Tuberculosis symptoms:

Difficulty to breath, sweating at night, loss of weight.

Patients have a cough **with** a characteristic **rust coloured** sputum.

**TRANSMISSION AND LIFE CYCLE OF *Paragonimus westermani*.**



**Note:** the definitive hosts are humans and animals like cats, dogs, pigs...etc

**Prevention:**

People get Paragonimiasis by eating contaminated crabs or shrimp.  
**So, avoid eating crabs or shrimps that are NOT WELL COOKED.**

**Diagnosis:**

A direct microscopic examination of sputum or stool will show the Paragonimus eggs produced by the adult worms living in the lungs.

**11.1 SPUTUM PREPARATION**

**WORK WITH THE SAFETY PRECAUTIONS (*Lesson 10.1*) AND FOLLOW THE ORDER DESCRIBED FOR SPUTUM SMEARING (*Lesson 10.3*).**

1. Wear gloves and a “duck” mask.
2. With 2 bamboo sticks take a thick part of sputum and put it on a clean slide.
3. Cover it with a clean cover slip as shown in figure 11. 1.

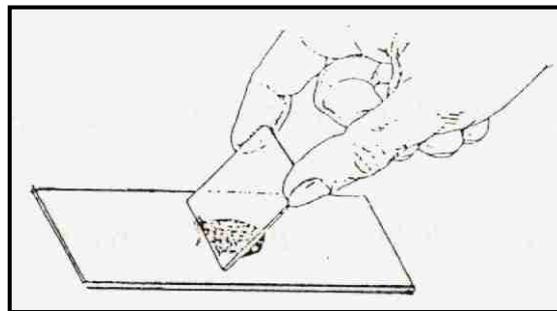


Fig 11. 1

4. Remove the sputum from the bamboo sticks by dipping them in a bottle containing sand and alcohol.
5. Burn the bamboo sticks and throws them away with sputum, which has been soaked in disinfectant.
6. **WASH YOUR HANDS.**

## 11.2 MICROSCOPIC EXAMINATION

1. Examine the whole preparation systemically **with X10 objective** (condenser down, diaphragm closed) as shown in figure 11. 2.

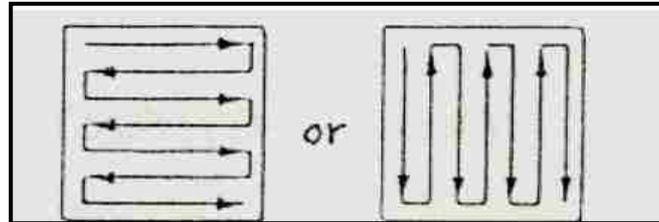


Fig 11. 2: Examination of a wet preparation.

2. If you see Paragonimus eggs, **confirm with X40 objective** (condenser half way, diaphragm slightly open).
3. Paragonimus eggs are:

- Yellow-brown or brown.
- It measures 70 to 100  $\mu\text{m}$  long and 50 to 65  $\mu\text{m}$  wide
- **Asymmetrical** shape with one side slightly flattened.
- There is a flat operculum (lid) on one side (not always well visible).
- The thickness of the shell (wall) varies but it is thicker at the bottom side.

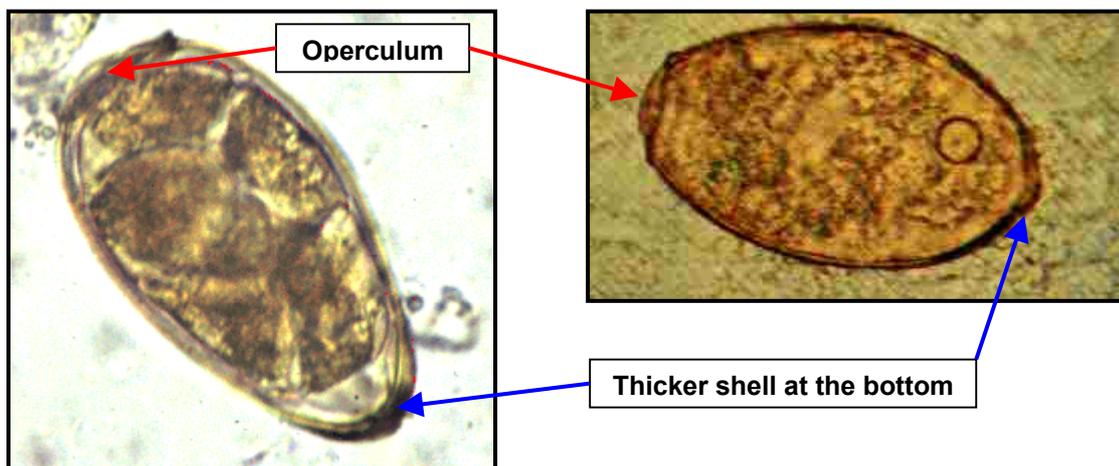


Fig 11. 3: *Paragonimus westermani* eggs

### RESULT:

The Paragonimus examination result should be written on the TB book as:

**For POSITIVE RESULT:**  
**For POSITIVE RESULT:**

**Paragonimus (+) or Paragonimus positive**  
**Paragonimus NF or No Paragonimus seen**

## 12. HEPATITIS B

**Hepatitis** means the infection of the liver.

Hepatitis is caused by a VIRUS called Hepatitis virus.

There are many types of Hepatitis viruses e.g. Hepatitis A or Hep-A, Hepatitis B or Hep-B, Hep-C and Hep-E; which are transmitted by different ways, and present different stage of illness.

We are most concerned with Hep-B, which is transmitted by blood and secretions the same ways as HIV.

### **SIGNS AND SYMPTOMS:**

Abdominal pain, jaundice, weight loss and weakness

Chronic infection of Hepatitis B virus shows high possibility of liver cancer.

Some people are infected with this virus in their body without any illness or symptoms present but they still can transmit the virus to others. These people are called **carriers**.

### **PREVENTION:**

Same way as for HIV infection.

### **LABORATORY EXAMINATION FOR HEPATITIS B INFECTION**

We test for Hep-BsAg or Hepatitis B surface antigen, which is a kind of protein on the surface of this virus. This antigen can be found in the blood several weeks before any signs of illness and then for months after. In some people the antigen is found for the rest of their life.

### **HEP-BSAG TESTS**

There are different tests being used in the field but all use the same principle to find the antigen. Blood sample is needed for the testing.

### 13. BLOOD TRANSFUSION TESTING

A blood transfusion is the taking of a small amount of blood from a donor and giving it to a patient.

Transfusion testing is the group of tests we do on a donor when a patient is in need of a blood transfusion.

These tests include:

- Haemoglobin or Haematocrit
- Blood group
- Malaria smear
- Hepatitis B test
- HIV test

Transfusion is done only when the patient present low haemoglobin or low haematocrit and the medic decide that it will be life threatening unless the patient receives blood transfusion.

Transfusion is serious and can be dangerous. A patient receiving the wrong blood group can become severely ill or even die. A patient receiving blood positive for malaria, Hepatitis B or HIV will develop these diseases.

**It is important to follow the blood transfusion chart closely when a transfusion is required.**

#### REGISTRATION:

All tests performed for transfusion are registered on a blood transfusion request card and in a blood transfusion register book.

The following information should be included:

**Patient information:**

- Date
- Name
- Age
- Sex
- Blood group
- Haemoglobin or haematocrit
- Diagnosis

**Donor information:**

- Date
- Number
- Age
- Sex
- Haemoglobin or haematocrit
- Blood group
- Malaria smear result
- Agreement of transfusion (Yes or No)

*\* Note that the donor's name does not appear on the register.*

## CONFIDENTIALITY

Donor confidentiality is very important!!!

Because of the social problems attached to the diagnosis of HIV it is unfair to disclose information to anyone.

The tests used are very sensitive: there are no false negative results. Therefore they are suitable for blood donor testing because we are sure of a negative result and that someone's blood is safe for donation.

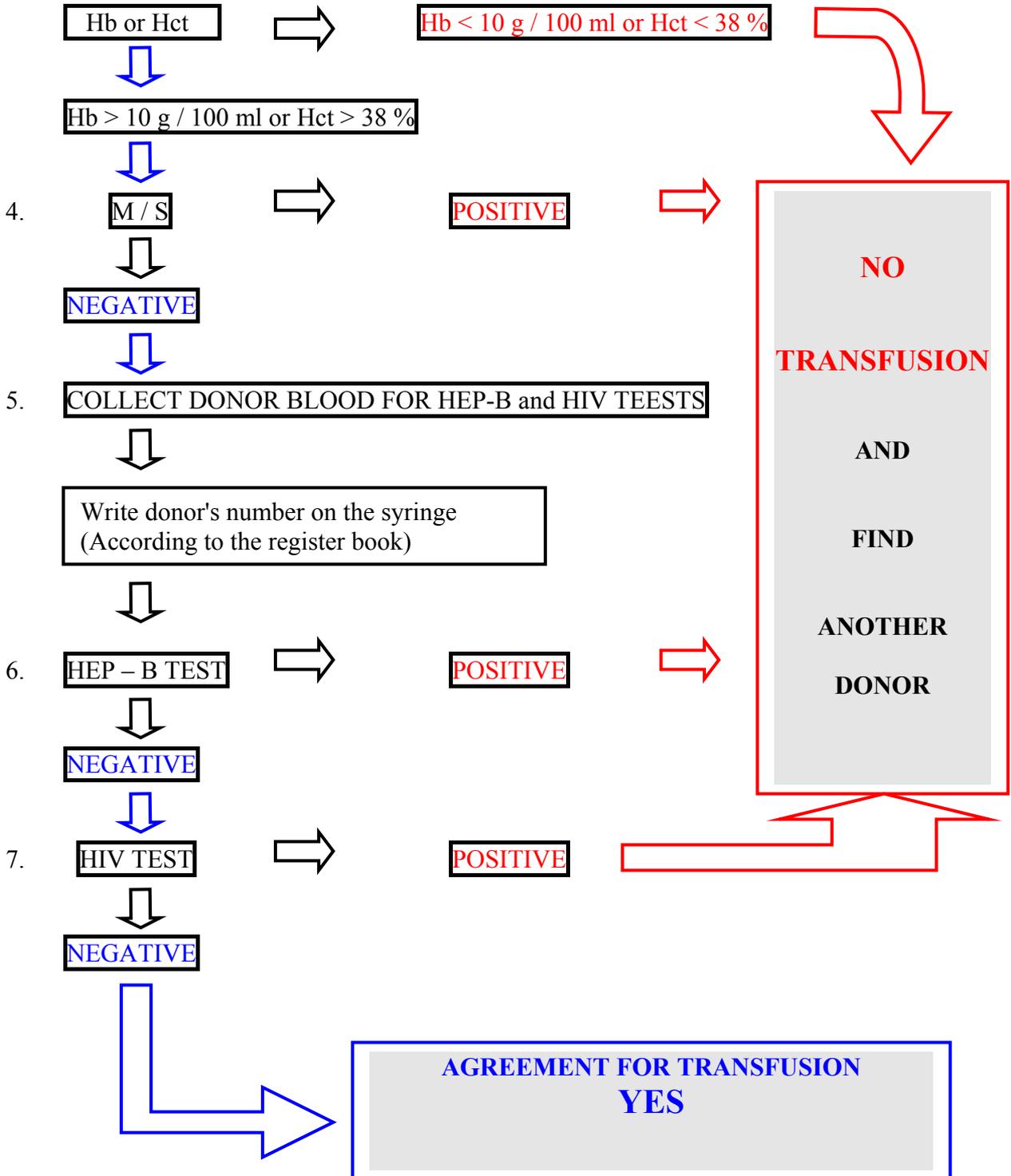
On the other hand, the tests used are poorly specific: false positive results are common. **Therefore these tests are not suitable for diagnosis** and a positive result always needs to be confirmed by a more specific test.

NEVER ALLOW ANYONE INTO THE LAB WHILE MAKING THE HEP-B OR HIV TESTS.  
NEVER DISCUSS OR RECORD THE RESULT OF THESE TESTS EVEN WITH THE  
MEDIC!!!

\*\* The medic or nurse must never ask for the Hep-B or HIV result.

**PROCESS OF BLOOD TRANSFUSION TEST:**

1. Fill information of the patient and donor in the register book and blood transfusion request card.
2. Test for blood group of the patient and donor.
3. If donor has the same blood group as the patient, test for Hb or Hct.



## BLOOD GROUP:

Before a blood transfusion you have to check the patient's blood group and the donor's blood group to know if they are compatible.

What is a blood group?

It is a chemical substance on the membrane of a red blood cell, which is called ANTIGEN. Each person has his own type of antigen since they were born. Blood is divided into 4 groups by the presence of its antigen and we call them as group A, B, AB and O. Each blood group antigen has a different character.

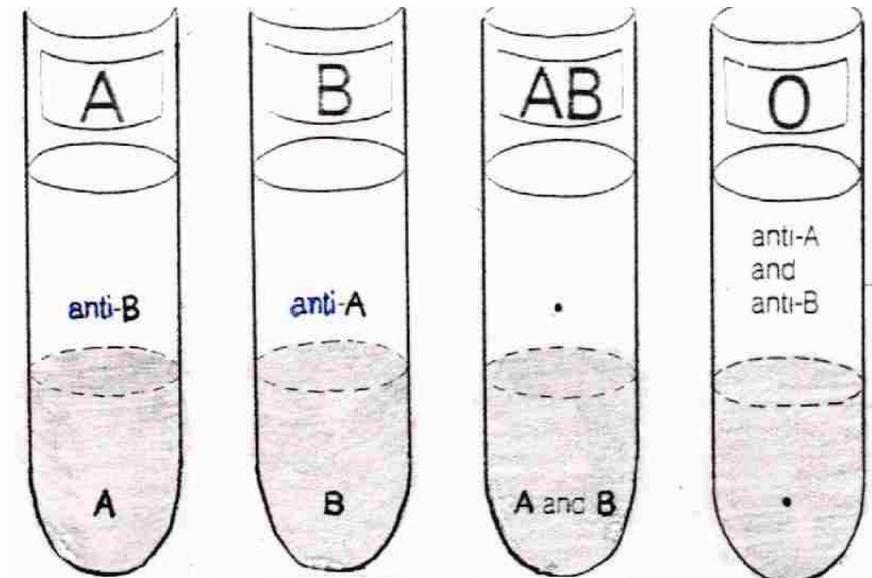
In the plasma, there are some substances called ANTIBODIES, which act as the protectors of your body. They attack and kill bacteria, virus and any antigen, which does not belong to your body.

Each blood group antigen has a corresponding antibody. Antibodies are specific for each antigen. You are born with the antibody for the group you don't have

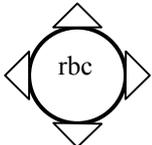
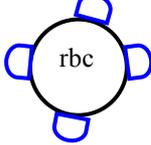
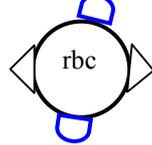
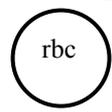
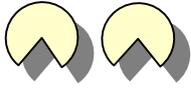
PATIENT'S  
BLOOD GROUP

ANTIBODIES  
*In the plasma*

ANTIGENS  
*On the red blood cells*



The following drawings in this table propose the idea of each group antigen and antibody.

| Blood Group | A  | B  | AB  | O  |
|-------------|--|--|---|--|
| Antigens    | A = <br> | B = <br> | AB =  <br> | No antigen<br>          |
| Antibodies  | Anti - B<br>  | Anti - A<br>  | No antibodies   | Anti - A and Anti B<br> |

This is why you cannot just give anybody's blood without checking the group before transfusion.

If blood group A patient received blood group B from the donor, anti B in the patient's blood will attack RBCs from donor because they have antigen B on. This fighting reaction will cause the patient to die quickly.

You don't need to always give the exact same blood group to a patient as long as you give the group that the patient doesn't have the antibodies for.

However, always try to transfuse donor blood with the same group as the patient's group.

|                 |    | Donor Group |    |    |    |
|-----------------|----|-------------|----|----|----|
|                 |    | A           | B  | AB | O  |
| Patient's Group | A  | OK          | X  | X  | OK |
|                 | B  | X           | OK | X  | OK |
|                 | AB | OK          | OK | OK | OK |
|                 | O  | X           | X  | X  | OK |

## LABORATORY EXAMINATION FOR BLOOD GROUPING

To know the blood group of a person, we test their red blood cells for the antigen. The test can be done by using anti – A and anti – B sera.

### Principle:

A sample of blood from the donor or patient will be mixed with the known antibodies and the clumping of its reaction will be observed. We call this reaction "agglutination". The agglutination seen means that the antigen and the antibody has corresponded and stuck together.

| Reaction with Anti - A | Reaction with Anti - B | Blood Group |
|------------------------|------------------------|-------------|
| +                      | -                      | <b>A</b>    |
| -                      | +                      | <b>B</b>    |
| +                      | +                      | <b>AB</b>   |
| -                      | -                      | <b>O</b>    |

### Materials:

- Gloves, blood lancet, savlon or rubbing alcohol, cotton
- 2 microscopic slides
- Antiserum-A and antiserum-B
- Disinfectant solution

### Method:

1. Put the gloves on.
2. Check the antiserum whether it has expired or not. If it has expired, check for a new bottle in the fridge. Check that the antiserum solution is not cloudy. If so, it has gone bad (wipe condensation away first), check for a new bottle.
3. Collect blood specimen from the finger as described in lesson 7.2.
4. Handle a clean slide only by the edges.
5. Place two drops of blood on the slide at least 2 cm apart.
6. Carefully and **without touching the end of the dropper to the blood**, add one drop of antiserum A to one drop of blood and add one drop of antiserum B to the other drop of blood.
7. With 2 **different corners** of the other slide, mix each drop of blood with the added antiserum separately.
8. Pick the slide up and gently rock back and forth looking for clumping RBC (agglutination) for 2 minutes

9. Read the result as follows:

| Anti-A  | Anti-B  |                  |
|---|---|------------------|
|    |    | → Blood Group O  |
|    |    | → Blood Group A  |
|    |    | → Blood Group B  |
|  |  | → Blood Group AB |

## 14. LABORATORY RECORDING

### LAB RECORDING

- I. Lab request
- II. Registration
- III. Statistics

#### I. LAB REQUEST:

How to request the lab test?

After the medic examines the patient, if he/she needs other examination from the laboratory how can he/she do? Using the lab request card is the suitable way that medic can ask lab technician for the test he/she needs. The result from the lab will also return to the medic through this request card.

A lab request card should provide sufficient information of the patient especially name, age, sex and address to keep in record and to prevent confusion of the specimens. The card must also state the type of specimen and the test required by the medic. Some other information of patients such as their suspected diseases and their clinical signs might be useful for the lab.

The lab request card is accompanied either with the specimen or the patient himself to the lab. Numbering of each specimen by laboratory is the way to make the lab – work run easier than using the patient's name. So provide some room for labeling.

The medics who request the test should also sign their name on the card: if any problem occurs the lab technician knows who to refer to. Also the lab technicians should sign on the card, as they are responsible for their results.

*Example of lab request card:*

| Front side   | Back side                                  |
|--|--|
| <p><b>LAB REQUEST FORM</b></p> <p><b>Date</b>..26/08/95... <b>Pt. No</b>...213...<b>Lab No</b>...304...</p> <p><b>Name</b>...<i>Say Moo</i>....<b>Age</b>...18 ys....<b>Sex</b>...F.....</p> <p><b>Address</b>...<i>Camp 3</i>...<b>Specimen/Test</b>...M / S..</p> <p><b>Information</b>.....<i>fever and chills</i>.....</p> <p><b>Result</b>...<i>PFT 3+</i>...; ...<i>3 % of RBC</i> parasitised.</p> <p><b>Request by</b>....<i>Luka</i>...<b>Report by</b>....<i>Pel Reh</i>...</p> <p style="text-align: center;">(Medic)                      (Lab tech)</p> | <p><b>Further Information / Result</b></p> |

Note: **Pt. No** = Patient Number.

**Lab No** = Lab Number.

**Age**: yr = year / ys = years / ms = months / ds = days, **or** you also can use the proportion as 1/12 = 1 month, 1/30 = 1 day (example: 2 years 4 months = 2 4/12)

**Sex**: F = female, M = male or ♀=female ♂= male

**Test**: M/S = malaria smear, UA urine analysis.

## II. REGISTRATION:

After each lab examination is done, it is necessary to register all information with lab result to the register book. Then the card will be returned to the medic. Whenever we need to recheck any result or look back to any information, we can find it from this register book. The same tests should be registered in the same register book.

### *II a. Example of a malaria registration:*

In the register book, you should have these following information:

| Date    | No | Name    | Age | Sex | Address | C/O | Group | Result           | Sign |
|---------|----|---------|-----|-----|---------|-----|-------|------------------|------|
| 28.7.97 | 22 | KoKo    | 20y | M   | Camp #1 | C   | B     | NF               | PK   |
|         | 23 | Saw Paw | 10m | F   | MC      | O   | A     | PVT (++)         | KN   |
|         | 24 | Cho Aye | 7y  | M   | KD      | O   | B     | PVT (++) G,<br>S | KN   |

C/O: C = Camp / O = Other (outside the camp)

Group: A = < 5 years old

B = ≥ 5 years old

*Note: The classification of aging group depends on NGO's policy.*

### *II b. Example of a sputum examination registration:*

In the register book, you should have this following information:

| No. | Name    | Age | Sex | C/O | Date                          | TB Result               | Paragonimus Result |
|-----|---------|-----|-----|-----|-------------------------------|-------------------------|--------------------|
| 1   | Mg Mg   | 23y | M   | C   | 15/5/95<br>16/5/95<br>17/5/95 | NF<br>NF<br>NF          | NF<br>NF<br>NF     |
| 2   | Htee Oo | 30y | M   | O   | 20/5/95<br>21/5/95<br>22/5/95 | AFB +<br>AFB++<br>AFB++ | NF<br>NF<br>NF     |

**Every patient should have 3 sputum samples checked**

**You must do 1 slide per 1 sputum sample**

**1 PATIENT = 3 SLIDES**

**III. STATISTICS:**

From the register book, you may check the quantity of each lab test done in a certain time; such as the number of positive malaria slides found last month or the number of AFB tests done in camp # 3 last year. In general, all the numbers are collected on a monthly basis. These figures should be continuously kept in record. We call this process **statistics**

Statistics are useful in the control and prevention of diseases. Lab statistics also can give you the amount of test done in each period around the year and the number of positive cases found from all suspected cases. It also helps you to estimate the quantities of each test in the future and this also helps you in ordering supplies.

Every month you must give a report of the number of examinations you made during the month.

**Statistic Form:**

Daily or weekly check the register book and count the number in each group. At the end of each month, you must fill in the following statistic form **correctly**.

**Month / Year:** \_\_\_\_\_ **Camp:** \_\_\_\_\_

**MALARIA**

|                | <b>A</b><br><b>&lt; 5 years old</b> |               | <b>B</b><br><b>≥ 5 years old</b> |               | <b>A + B</b><br><b>TOTAL</b> |               | <b>PW (*)</b> |
|----------------|-------------------------------------|---------------|----------------------------------|---------------|------------------------------|---------------|---------------|
|                | <b>Camp</b>                         | <b>Others</b> | <b>Camp</b>                      | <b>Others</b> | <b>Camp</b>                  | <b>Others</b> |               |
| <b>PF</b>      |                                     |               |                                  |               |                              |               |               |
| <b>PV</b>      |                                     |               |                                  |               |                              |               |               |
| <b>PF + PV</b> |                                     |               |                                  |               |                              |               |               |
| <b>PFG</b>     |                                     |               |                                  |               |                              |               |               |

|                  |  |  |  |  |  |  |  |
|------------------|--|--|--|--|--|--|--|
| <b>Total Pos</b> |  |  |  |  |  |  |  |
| <b>Total Neg</b> |  |  |  |  |  |  |  |

|                     |  |  |  |  |  |  |  |
|---------------------|--|--|--|--|--|--|--|
| <b>Total Slides</b> |  |  |  |  |  |  |  |
|---------------------|--|--|--|--|--|--|--|

(\*): PW = Pregnant women

### SPUTUM

| Origin      | Total Slides |   |        |   | Total Positive Slides |   |        |   | Total Patients |   |        |   | Total Positive Patients |   |        |   |
|-------------|--------------|---|--------|---|-----------------------|---|--------|---|----------------|---|--------|---|-------------------------|---|--------|---|
|             | Camp         |   | Others |   | Camp                  |   | Others |   | Camp           |   | Others |   | Camp                    |   | Others |   |
| Group       | A            | B | A      | B | A                     | B | A      | B | A              | B | A      | B | A                       | B | A      | B |
| AFB         |              |   |        |   |                       |   |        |   |                |   |        |   |                         |   |        |   |
| Paragonimus |              |   |        |   |                       |   |        |   |                |   |        |   |                         |   |        |   |

A: less than 5 years old (< 5 ys)

B: more than or equal to 5 years old ( $\geq$  5 ys)

**In case there is a lot of work in your lab, doing daily statistic can help you to fill monthly statistic forms more easily and also can help you to see the trend in malaria situation day by day.**

If you classify more than 2 aging groups, you need another table to cover all data.

#### ***III a. Malaria statistics***

Count these numbers from the malaria register book and fill in the form:

- The total number of the slides.
- The number of positive slides.
- The number of negative slides.
- The number of PFT (Plasmodium falciparum trophozoite) slides.
- The number of PVT(\*) (Plasmodium vivax trophozoite) slides.
- The number of mixed (P. falciparum+P.vivax) slides.
- The number of PFG (Plasmodium falciparum gametocyte) slides.

(\*)Remark: It is not necessary to count P. M and P.O separately. They can be added to the PV (as Non-PF malaria).

#### ***III b. Sputum examination statistics***

For AFB and Paragonimus tests, you need to count these numbers from the register book and fill in the form:

- The total number of slides.
- The total number of positive slides.
- The total number of patients.
- The total number of positive patients.

## 15. LABORATORY QUALITY CONTROL

**A good laboratory** is a great instrument in the health care community to tackle the diseases especially malaria problem in endemic area.

**It will allow the health leaders to:**

- Treat the patient on the basis of biological proof.
- Have a good epidemiological survey of the population they control.
- Check the efficiency of the health policy they set up.
- Check the efficiency of the treatment according to the evolution of resistance.
- If necessary, organise studies to find new means of dealing with malaria.

**On the other hand, a bad laboratory will do the population a disservice**

- With false negative results, patient that would have been treated on the simple basis of a clinical diagnosis will not receive a proper treatment and will get worse.
- With false positive results, patients will get the useless treatment and become drug carriers in the population. This increases contact between drugs and parasites then speeds up the resistance of the parasite.
- Finally, the epidemiological data will be false and will lead the health leader to an inaccurate policy and a loss of control over the situation.

**NEVER FORGET THAT:**

**A health structure without laboratory is better  
than one with an unreliable laboratory**

According to these points, it is necessary to follow the laboratory activities regularly.

**The only way to do so is to set up a regular quality control of lab results.**

### 15.1 LABORATORY QUALITY CONTROL SERVICE

by

**S.M.R.U (Shoklo Malaria Research Unit)**

S.M.R.U offers free malaria quality control to various organisations since a long time.

The slides you are sending should not be more than one month old.

Positive slides and negative slides have to be separated.

For the sample size to be taken follow the SMRU quality control protocol.

**For more information:**

Contact person: Stephane Proux at SMRU PO Box 46 Mae-Sot 63110 TAK

Tel: 055 545 021

Fax: 055. 545 020

E-mail: [steph@shoklo-unit.com](mailto:steph@shoklo-unit.com)

## 16. INVENTORY / ORDER

The INVENTORY SYSTEM is needed to control the amount of materials you are using in the laboratory. This system will help you to continue your work smoothly.

You should record all of materials you have in your inventory book.

There are 2 kinds of materials:       **1. Permanent material**  
   **2. Consumable material**

- Permanent material:** It is the material that can be use for its lifetime if it is not broken and you hardly need to order it.

### List of permanent materials used in this manual is

|   |                |
|---|----------------|
| Microscope                                      | Cylinders      |
| Pipettes  | Wash bottles   |
| Drop bottle                                     | Funnels        |
| Staining basins                                 | Staining racks |
| Slide drying racks                              | Buckets        |
| Basins  | Trays          |
| Burning lamps                                   | Timers         |
| Slides boxes                                    | Test tube      |
| Centrifuge                                      | Tube rack      |
| Haematocrit centrifuge and/or Haemoglobin meter |                |

If one of these things is missing or broken, request a new one when you make the laboratory order.

- Consumable material:** It is the material you consume for the lab tests and need to be ordered to ensure that you will have enough to work in the coming period.

### List of consumable materials for each laboratory examination:

| For Malaria Exam.         | For Sputum Exam.             | For Blood Transfusion     |
|---------------------------|------------------------------|---------------------------|
| Giemsa stain              | Carbol fuchsin               | Antiserum–A & antiserum B |
| Absolute Methanol         | Methylene blue               | Microscopic slides        |
| Microscopic slides        | Acid–alcohol 3 %             | Blood lancets             |
| Blood lancets             | Burning alcohol              | Cotton ball               |
| Xylene                    | TB slides (with frosted end) | Rubbing alcohol or savlon |
| Immersion oil             | Immersion oil                | HIV–test kit              |
| Lens paper                | Lens paper                   | Hep–B test kit            |
| Filter paper              | Filter paper                 | Sterile syringe           |
| Silica gel (desiccant)    | Silica gel (desiccant)       | Sterile 18 or 21 G needle |
| Tissue paper              | Tissue paper                 | Disinfectant              |
| Cotton ball               | Cover slips                  |                           |
| Rubbing alcohol or savlon | Specimen container           |                           |
|                           | Masks                        |                           |
|                           | Matches                      |                           |

**List of Consumable Materials for administration in the laboratory:**

- Statistic forms
- Lab Request forms
- Quality Control forms
- Register books
- Pens
- Pencils

**THE STOCK AND ORDER**

In order to maintain the inventory effectively, you need to do the **stock** and **order** of materials.

***What is the stock – order?***

- The **stock** is the list of the quantities of **consumable** materials and some **permanent** materials, which can easily be broken (pipettes, oil drop bottle, and timer..) that you have in the cupboard of your laboratory.
- The **stock** is the materials that you currently have and which had **NEVER** been **USED**.
- The **order** is the list of the quantities of materials and reagents you need for the next period.
- You register the stock and the orders in the **laboratory stock – order form** when you make your laboratory order and give it to the in charge.

***How to do a laboratory stock – order?***

1. Check the stock at the **END** of **EACH MONTH** or **AS ARRANGED** with your in charge.
2. **NEVER** wait until the amount become "0" before ordering.
3. **NEVER** order without checking what you have in the stock.
4. **ORDER ENOUGH** for the number of months decided including the minimum necessary stock for emergency situation.
5. **GIVE** the laboratory **ORDER** to the **IN CHARGE**.

**ADVICE FOR MAKING YOUR LABORATORY STOCK - ORDER**

Estimate the amount of each material needed for each test using the statistics of the test done either in previous month or in the same month of the previous year.

| Material               | Amount per pack  | Amount used per test             | Approximated No. of tests / pack | Remark                |
|------------------------|------------------|----------------------------------|----------------------------------|-----------------------|
| Immersion oil          | 500 ml / btl     | 0.15 ml / slide                  | 3000 slides / btl                |                       |
| Giemsa stain           | 450 ml / btl     | 0.5 ml / slide                   | 800 slides / btl                 | 10% dilution          |
| Abs. Methanol          | 2.5 liter / btl  | 100 ml / time & 1 time / week    | 25 weeks / btl                   | <b>Rainy season</b>   |
| Abs. Methanol          | 2.5 liter / btl  | 100 ml / time & 1 time / 2 weeks | 50 weeks / btl                   | <b>Dry Season</b>     |
| Carbol fuchsin         | 450 ml / btl     | 5 ml / slide                     | 80 slides / btl                  |                       |
| Methylene blue         | 450 ml / btl     | 5 ml / slide                     | 80 slides / btl                  |                       |
| Acid-alcohol           | 450 ml / btl     | 10 ml / slide                    | 80 slides / btl                  |                       |
| Burning alcohol        | 450 ml / btl     | 5 ml / slide                     | 80 slides / btl                  |                       |
| TB slides              | 72 slides / box  | 1 slide / test                   | 70 tests / box                   |                       |
| Tissue paper           | 1 roll           |                                  | 200 slides / roll                |                       |
| Filter paper           | 100 pieces / box | 50 slides / piece                | 5000 slides / box                | To filter the water   |
| Silica gel (Desiccant) | 1kg / bag        | 1 kg / microscope for 6 month    | 1bag / microscope                | For 6 months          |
| Blood lancet           | 200 pieces / box | 1 piece / test                   | 200 tests / box                  |                       |
| Malaria slides         | 72 slides / box  | 1 slide / test                   | 70 tests / box                   | Slides can be re-used |

Other suggestion note:

Absolute Methanol:

\* Don't throw away the old methanol. Place this methanol into a clean bottle with label "Old Methanol".

What could we do with the old methanol?

- After washing the malaria slides with soap powder and water, leave them to dry and clean them with a piece of gauze containing this old methanol.
- You can use this old methanol to wash the glass pipettes or any old bottle containing stain in order to remove the stain that remains.
- You can also use it to renew from time to time the wide-mouth pot containing sand & alcohol in the TB room.

**Blood Lancets:**

For each malaria slide done, you need 1 blood lancet.

If 600 malaria slides done during 3 months correspond with the use of 3 boxes of blood lancets.

If you do the determination of 60 haemoglobin or haematocrit per month in your lab you need to add 1 box of lancets to perform this exam for 3 months.

The total number of boxes of blood lancets should be:

$(3+1) = 4$  boxes of blood lancets for 3 months.

**The number of blood lancets you need to order should be multiply by 1.5 in order to have a safety margin.**

As the above example the number of the boxes of blood lancets should be:

$(4 \times 1.5) = 6$  boxes of blood lancet for the next 3 months.

**Malaria Slides:**

The quantity of boxes of malaria slides ordered should be the same as the quantity of boxes of malaria slides used each month.

**Laboratory Request Forms:**

The number of the lab request forms needed correspond to the number of the request forms used during the past months.

**APPENDIX 1: LEARNING MATERIALS**

Color plates used in this training manual can be purchased at the following addresses:

1. Microscopic Diagnosis:

Bench Aid Series      No.1 Malaria  
                                 No.3 Filariasis  
                                 No.5 Meningitis – AIDS – Gonorrhoea – Syphilis – TB - Leprosy  
                                 No.7 Blood: Normal – Anaemias – Infections - Leukaemias

Available from Tropical Health Technology, 14 Bevills Close, Doddington, March,  
Cambridgeshire, PE15 OTT, UK.

2. Bench Aids for the Diagnosis of Malaria, Set No. 1 plates 1-4 and Set No. 2 plates 5-8.  
Illustrations by Yap Loy Fong. These teaching Aids can be obtained from:

World Health Organization  
Distribution and Sales Service  
1211 Geneva 27  
Switzerland

or from

HMSO  
P.O.Box 276  
London SW8 5DT  
UK

3. Plasmodium parasites pictures were provided by SMRU and by the Royal Perth  
hospital web site: <http://www.rph.wa.gov.au>

Note:

World Health Organization, Thailand  
Dr. E.B. Doberstyn  
WHO Representative,  
WHO, c/o Ministry of Public Health  
Samsen Road, Bangkok 10200  
Tel: (02) 5918198 fax: (02) 0 5918199

Distributor for WHO publications  
Suksit Saim Co. Ltd.  
113, 115 Fuang Nakorn Road,  
Opposite Wat Rajborpith,  
Bangkok 10200

## APPENDIX 2: REAGENTS AND EQUIPMENT

The quantities of laboratory supplies available should be sufficient to maintain the lab-work for the period of time between each supply distribution. The recording of all supplies remaining and received should be done regularly, at least before ordering the new supplies.

**Reagents** used in this manual are:

| Reagent                                | Use  |
|--|--|
| Burning alcohol                        | for spirit lamp and other flaming          |
| Carbol fuchsin                         | AFB staining                               |
| Concentrated Hydrochloric acid (HCL)   | making of acid-alcohol                     |
| Ethanol 95%                            | making of acid-alcohol                     |
| Giemsa stain                           | blood staining                             |
| Immersion oil                          | examination with X100 objective            |
| Hypochlorite                           | disinfectant for waste specimen and others |
| KOH solution 3% (diluted from 30% KOH) | dissolves the sputum                       |
| Methanol absolute                      | fixing thin blood films                    |
| Methylene blue                         | AFB staining                               |
| Rubbing alcohol                        | cleaning and disinfecting of skin          |

**Equipment** used in the examinations described in the manual:

|                                       |   |
|---------------------------------------|---|
| Alarm clock (in minute)               | timing for staining                       |
| Beaker 50 cc, 100 cc                  | for mixing of the stain                   |
| Lancet                                | for taking capillary blood                |
| Brush, soft hair                      | for microscope cleaning                   |
| Clay sealer                           | for plugging of microhaematocrit tube     |
| Cleaning gloves                       | for washing                               |
| Conical tube 12–15 cc                 | for specimen centrifugation               |
| Cotton wool                           | for skin cleaning and making swabs        |
| Square coverslips                     | for wet preparation                       |
| Cylinder 100 cc                       | for measuring of reagents                 |
| Dropper bottle, brown glass, 40–65 cc | for containing of reagents                |
| Filter paper 24 cm diameter           | for filtration of the water               |
| Filter 12.5 or 15.0 cm diameter       | for filtration of the stain               |
| Forceps                               | for holding of slide                      |
| Glass lid bottle, 60 – 100cc          | for containing of Giemsa stain            |
| Glass rods                            | for hanging of the slides during staining |
| Hand centrifuge                       | for sedimentation                         |
| Label sticker                         | for specimen labeling                     |
| Lab request form                      | for examination request                   |
| Lens paper                            | for cleaning of microscope lens           |
| Matches                               | for burning                               |
| Microhaematocrit tubes, heparinised   | for collection of capillary blood         |
| Microscope slides                     | for general microscopic examination       |
| Microscope slides, frosted ended      | for sputum examination                    |

|  |   |
|--|---|
| Needles 20 – 24 G                                  | for taking Giemsa stain from the bottle and for venous blood collection |
| Pipette, graduated 10cc                            | for measuring Giemsa  |
| Rubber bulb for pipette                            | for sucking of reagent by pipette                                       |
| Silica gel   | for absorption of the moist   |
| Syringe 5 cc, 10 cc                                | for preparing in Giemsa staining and for venous blood collection        |
| Slide box  | for keeping the examined slides   |
| Slide drying rack                                  | for hanging slide to be dry   |
| Specimen container, wide-mouth pots with screw cap | for specimen collection   |
| Spirit lamp  | for burning and flaming   |
| Staining tray                                      | for staining  |
| Tube brush   | for washing tubes   |

**APPENDIX 3: LABORATORY EQUIPMENT SUPPLIERS IN THAILAND**

- Microscope:
- 1: Rushmore Ltd., partnership (OLYMPUS)  
11 Tonglor Soi5, Sukhumvit 55, Bangkok 10110  
Tel: (02) 391 6122, (02) 391 6535, (02) 391 6727  
Fax: (02) 381 2862
  - 2: Hollywood International Ltd. (NIKON)  
501/4-7 Phetburi Road, Rajtaywee, Bangkok 10400  
Tel: (02) 2516032-7, (02) 255 3870-5  
Fax:(02) 253 9485, (02) 653 8267

## Other Laboratory Equipment:

1. E for L International Co., Ltd.  
224/5 - 8 Rajvithee Road, Bang Yee Khan,  
Bangplad, Bangkok 10700  
Tel: (02) 434 9487-8, (02) 435 1331-2  
Fax: (02) 433 8695
2. Qualify Group Co., Ltd.  
19/9 Moo 6 Soi Sompratthana, Sukhapiban 5 Road,  
Bangkhen, Bangkok 10220  
Tel: (02) 519 8962, (02) 519 8966  
Fax: (02) 519 8962
3. Biotechnical Co., Ltd.  
1895/25 Paholyothin Road, Jatujuk, Bangkok 10900  
Tel: (02) 579 5490, 579 7599  
Fax: (02) 561 1761
4. Abbott laboratories Ltd.  
2/4 Nai lert tower, 9<sup>th</sup> floor, Wireless Road, Lumpini,  
Pputumwan, Bangkok 10330  
Tel: (02) 267 9048-50, (02) 267 9060-4  
Fax: (02) 267 9043
5. Bio Med Group Co., Ltd.  
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