

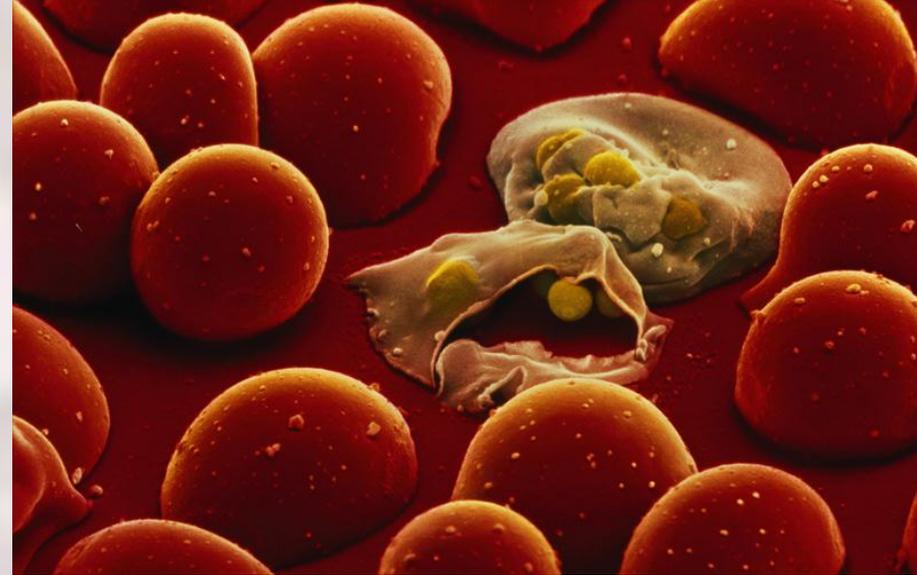
Malaria

Diagnosis: A Brief Review

Dr. Rafiei, PhD assistant professor of medical parasitology

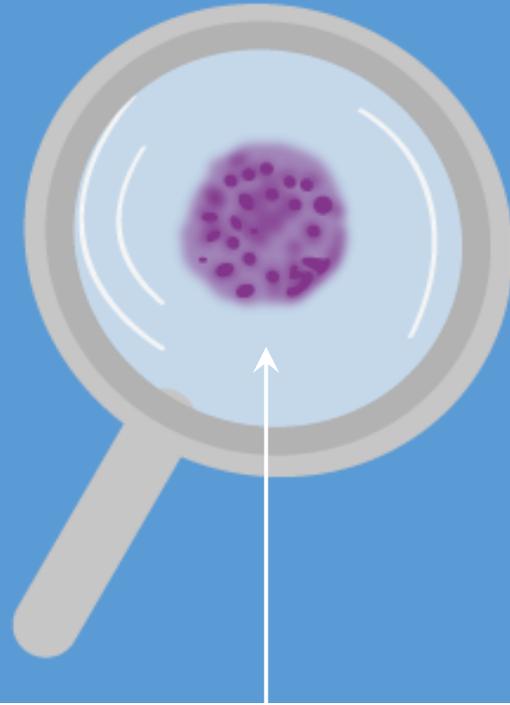
Department of parasitology and mycology, School of medicine ,Iran university of
medical science, Tehran,Iran

Email: Rafiei.r@iums.ac.ir

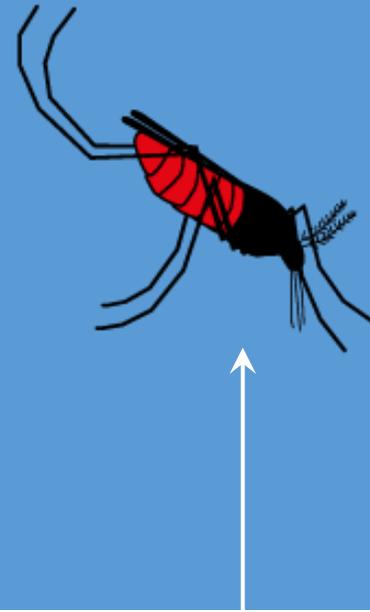


We know the main culprits *parasites & mosquitoes...*

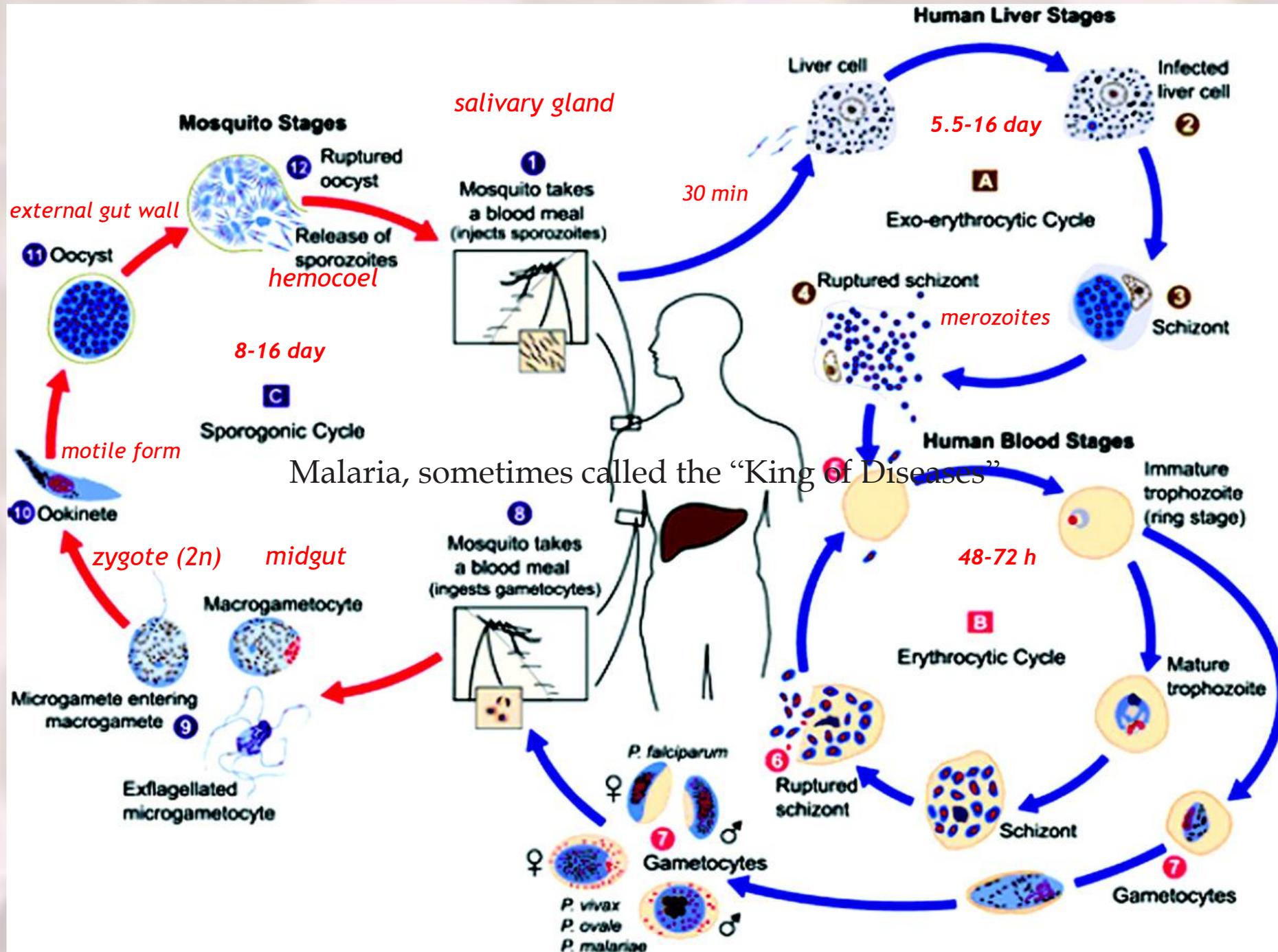
Malaria, sometimes called the “King of Diseases”



Malaria parasites
(genre: *Plasmodium*)

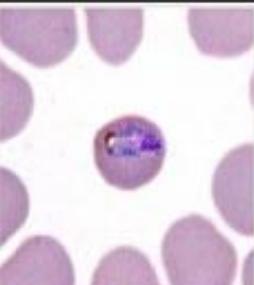
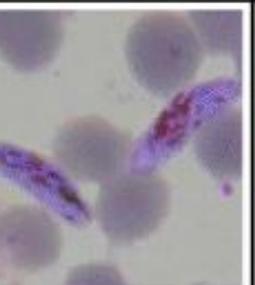
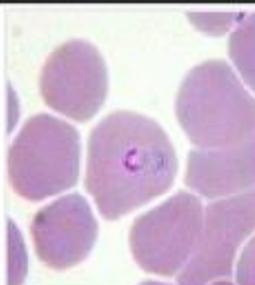
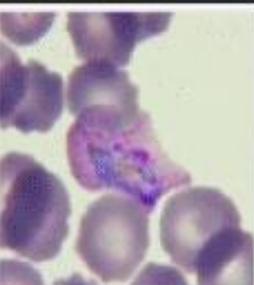
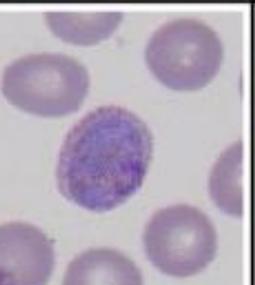
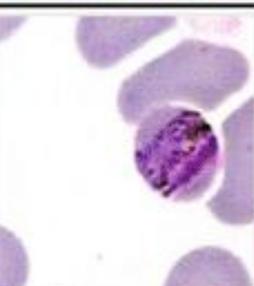
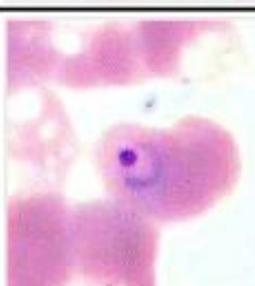
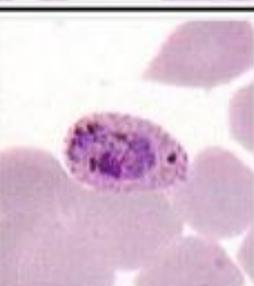
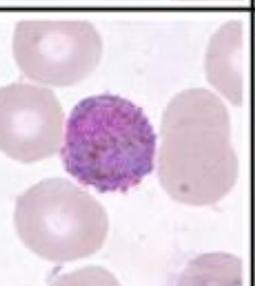


Female mosquitoes
(genre: *Anopheles*)



Malaria, sometimes called the “King of Diseases”

Human Malaria

Stages Species	Ring	Trophozoite	Schizont	Gametocyte	
<i>P. falciparum</i>					<ul style="list-style-type: none"> Parasitised red cells (pRBCs) not enlarged. RBCs containing mature trophozoites sequestered in deep vessels. Total parasite biomass = circulating parasites + sequestered parasites.
<i>P. vivax</i>					<ul style="list-style-type: none"> Parasites prefer young red cells pRBCs enlarged. Trophozoites are amoeboid in shape. All stages present in peripheral blood.
<i>P. malariae</i>					<ul style="list-style-type: none"> Parasites prefer old red cells. pRBCs not enlarged. Trophozoites tend to have a band shape. All stages present in peripheral blood
<i>P. ovale</i>					<ul style="list-style-type: none"> pRBCs slightly enlarged and have an oval shape, with tufted ends. All stages present in peripheral blood.

4 گونه از جنس پلاسمودیوم
عامل مالاریای انسان:

1. فالسیپاروم عامل مالاریای سه یک بدخیم
2. ویواکس عامل مالاریای سه یک خوش خیم
3. مالاریه عامل مالاریای چهار یک
4. اوواله عامل مالاریای سه یک خوش خیم

نشانه ها:

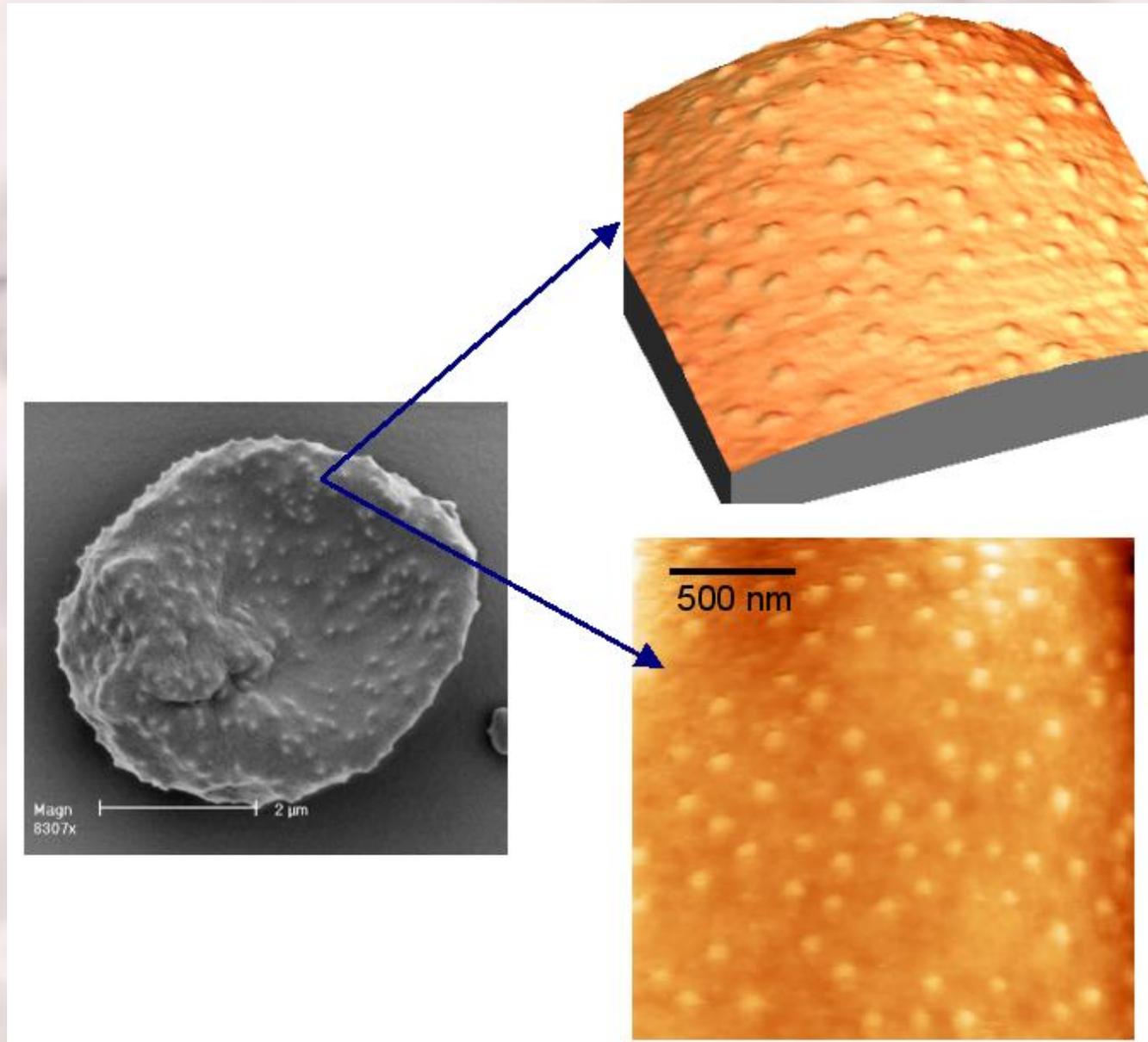
- high fever
- headache
- severe chills
- general body pains.

In some cases the following symptoms are also present:

- vomiting
- diarrhoea.

- دوره نهفته 8-40 روز
- نشانه های اولیه غیر اختصاصی شبیه انفلوآنزا
- حمله مالاریایی (پاروکسیسم): همزمان با منظم شدن پارگی گلوبول قرمز هر 48 یا 72 ساعت یکبار رخ می دهد و شامل:
- لرز 10-15 دقیقه cold stage
- تب بالا 2-6 ساعت hot stage
- تعریق چند ساعت sweating stage
- در فاصله حملات مالاریایی بیمار کاملا سر حال است
- بزرگی طحال
- سیر مالاریای فالسیپاروم سریع تر از بقیه می تواند باعث مرگ شود

Knobs on malaria-infected RBC



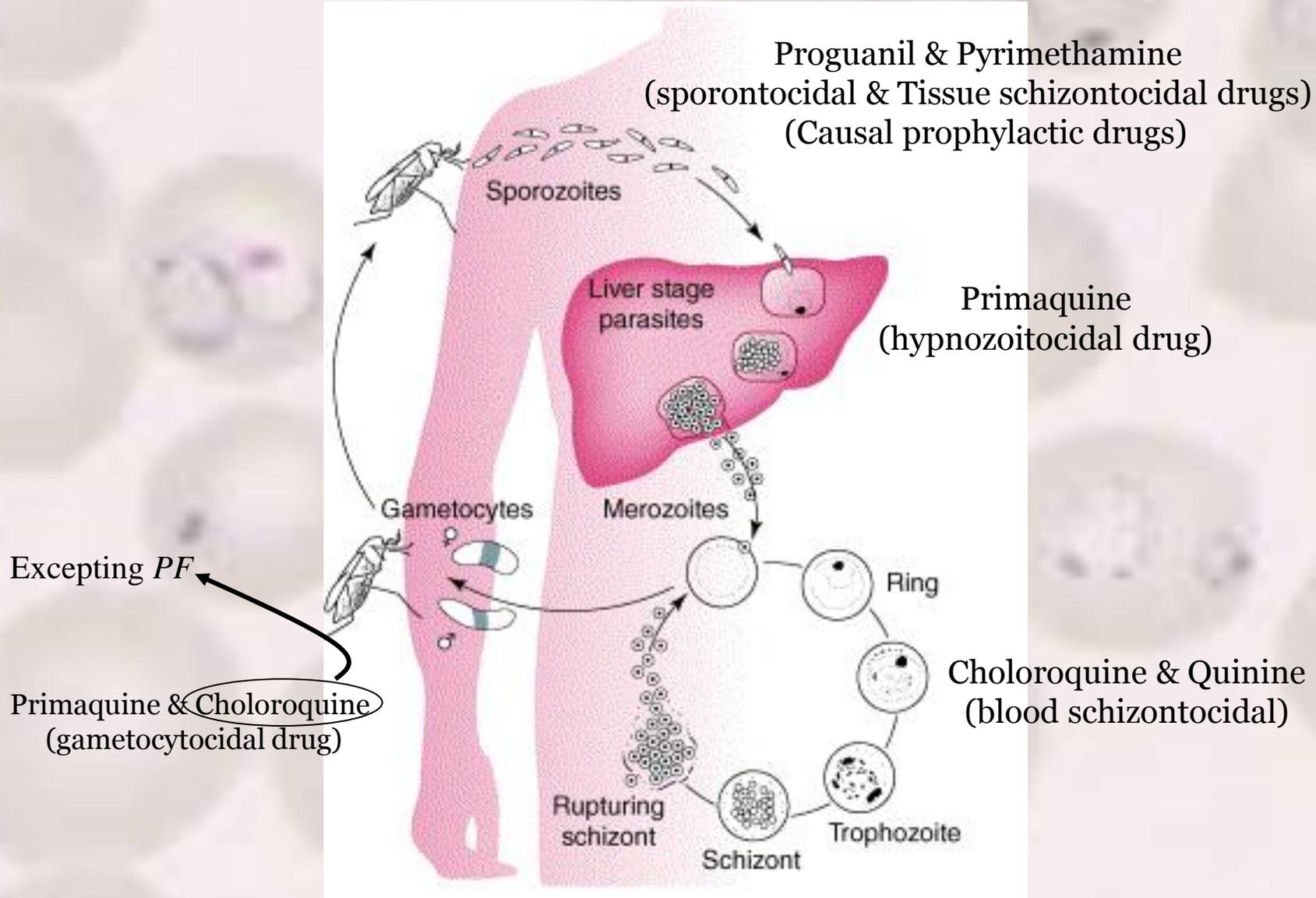
عوارض:

- در ویواکس، اواله و مالاریه نادر ولی در فالسیپاروم شایع ، شامل:
- انسداد عروق
- مالاریای مغزی cerebral malaria اغلب موارد عامل مرگ و میر مالاریا
- کم خونی
- تب پیشاب سیاه black water fever
- سندروم نفروتیک
- عوارض کلیوی
- مالاریای سرد algid malaria
- سندروم بزرگی طحال گرمسیری tropical splenomegaly syndrome
- هیپوگلیسمی hypoglycemia
- هایپر پارازیتمی hyperparasitemia
- Dysentric malaria

راه های انتقال:

- نیش پشه آنوفل آلوده به انگل
- استفاده از سرنگ مشترک در معتادان تزریقی
- انتقال خون
- به ندرت مادر زادی

Treatment



We know how to control it:

prevention

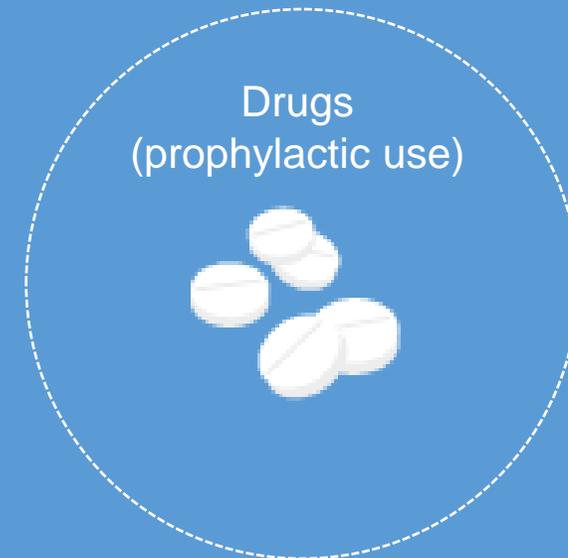
vs mosquitoes



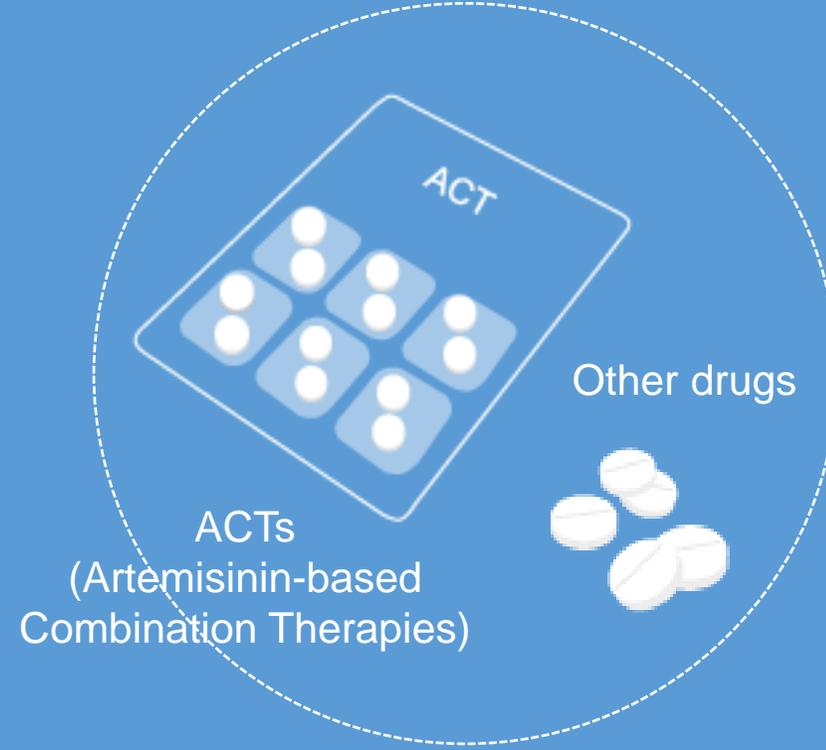
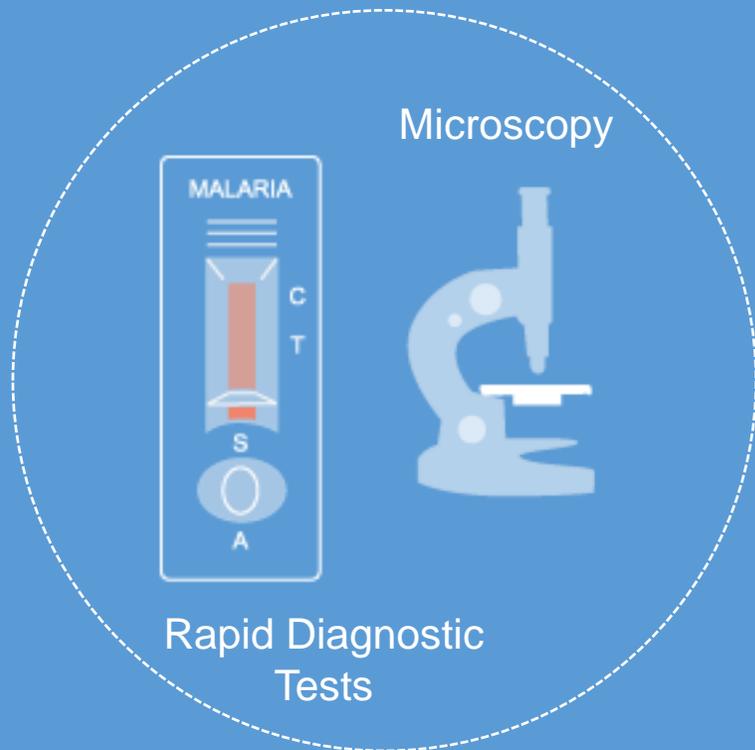
vs vector-human contact



vs the parasite



We know how to control it: *diagnosis & treatment*



We know how far we've come...



1900

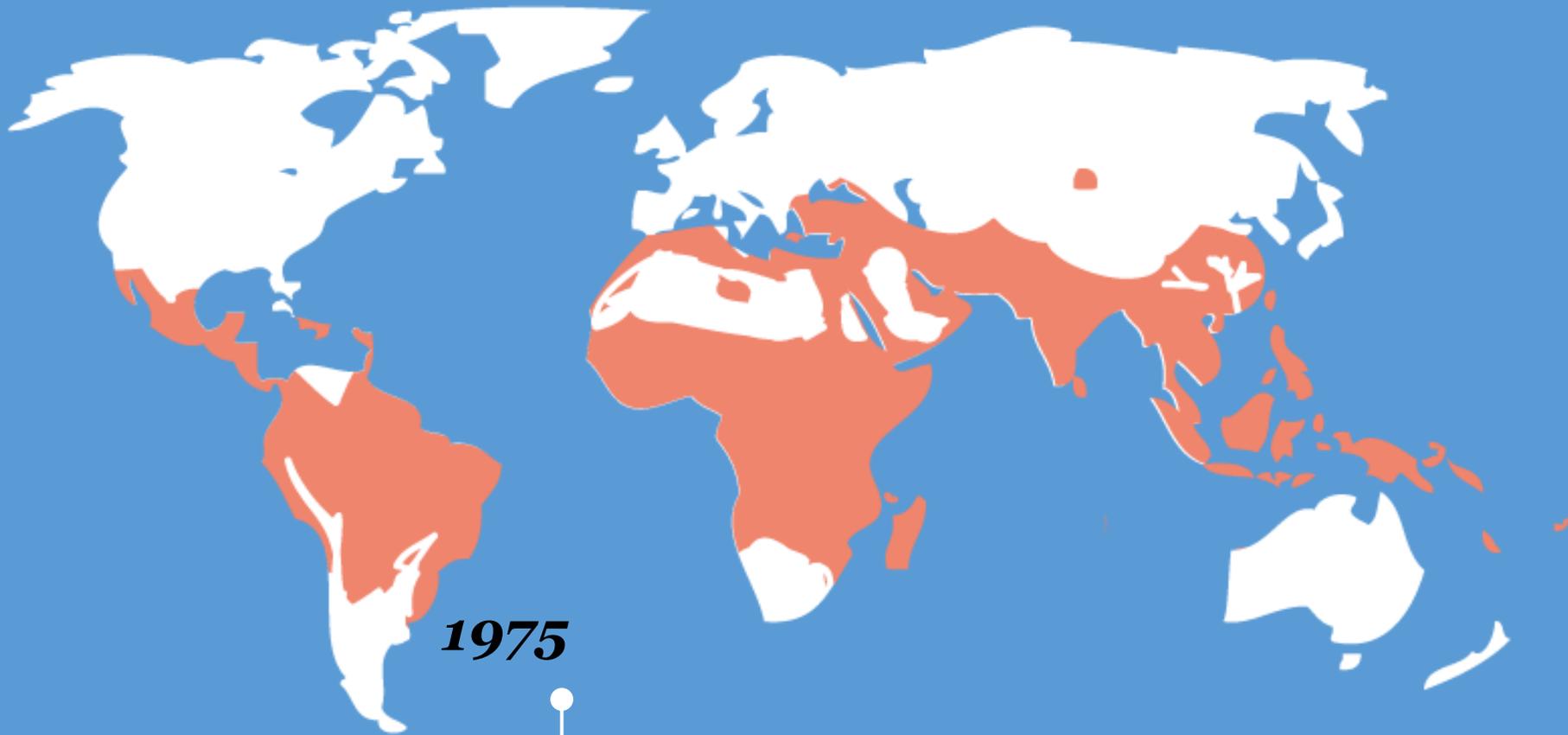


Malaria endemic areas



1946

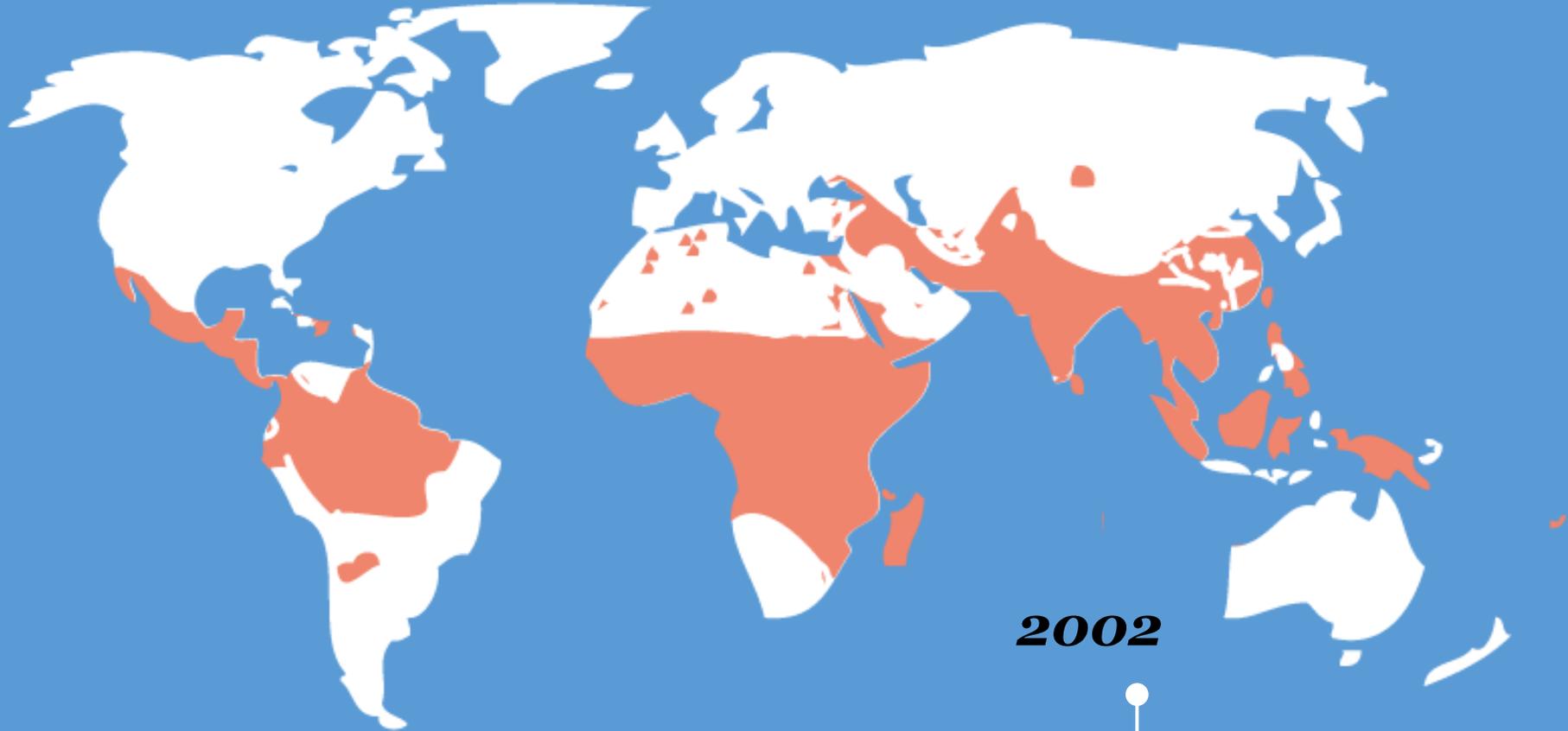
 *Malaria endemic areas*



1975

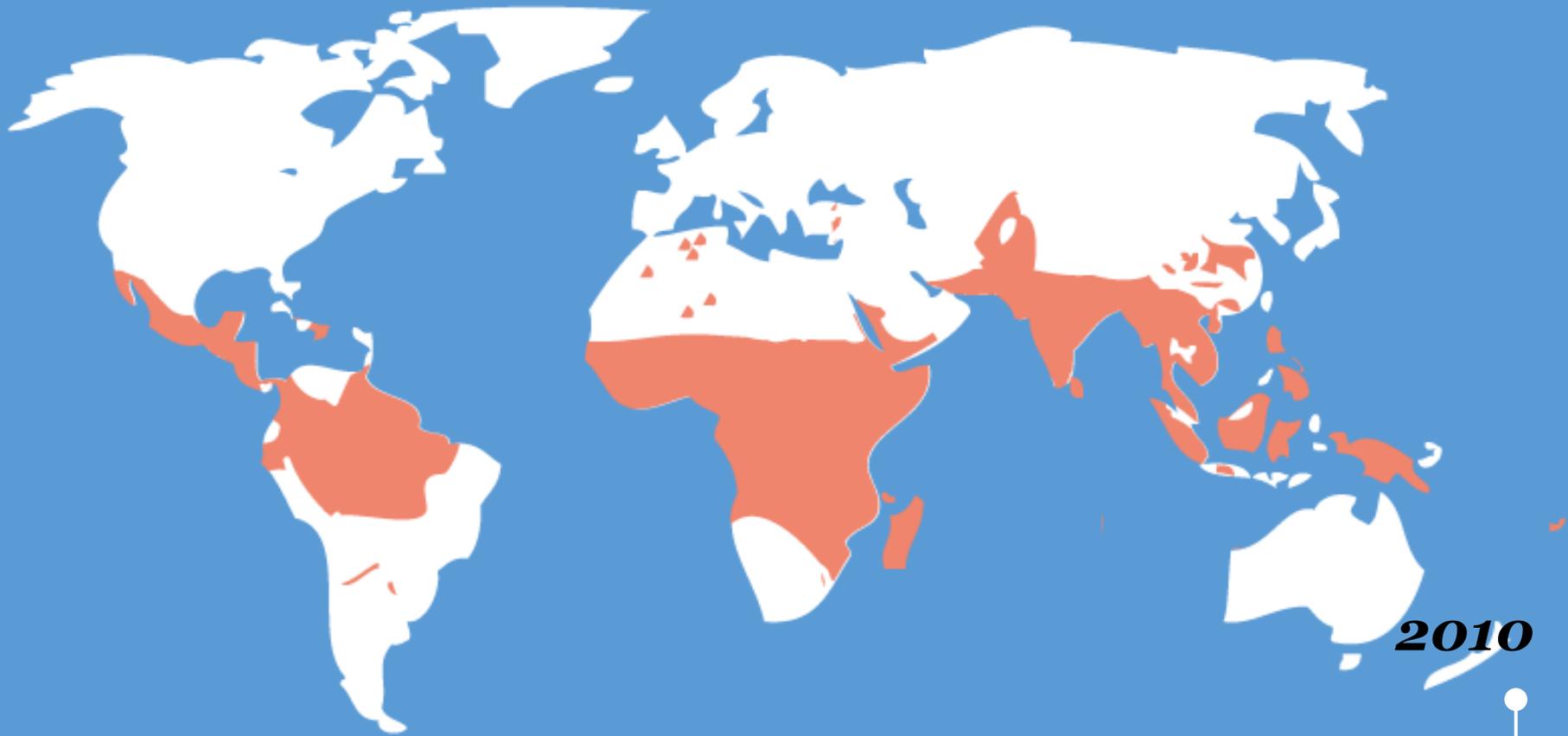


Malaria endemic areas



2002

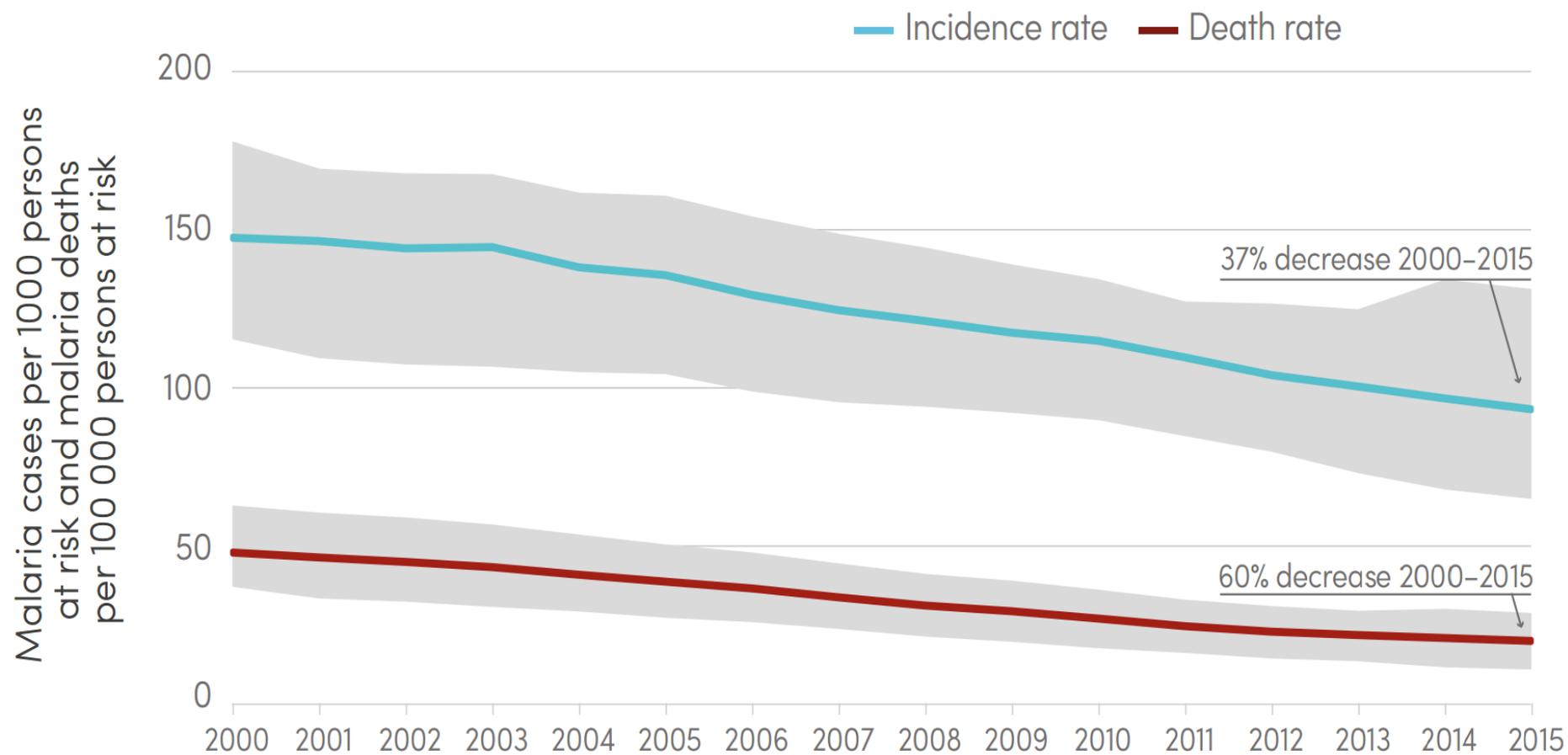
 *Malaria endemic areas*



2010



Malaria endemic areas





VISION	A WORLD FREE OF MALARIA			
	Goals	Milestones		Targets
		2020	2025	
1. Reduce malaria mortality rates globally compared with 2015	> 40%	> 75%	> 90%	
2. Reduce malaria case incidence globally compared with 2015	> 40%	> 75%	> 90%	
3. Eliminate malaria from countries in which malaria was transmitted in 2015	At least 10 countries	At least 20 countries	At least 35 countries	
4. Prevent re-establishment of malaria in all countries that are malaria free	Re-establishment prevented	Re-establishment prevented	Re-establishment prevented	

20 April 2016: The WHO European Region is malaria free

Ahead of World Malaria Day 2016, WHO announces that the European Region hit its 2015 target to wipe out malaria, thus contributing to the global goal to "End malaria for good".

**3 years with NO local
malaria transmission =
*ELIMINATION***



Elimination worldwide =
ERADICATION



Eradication =
LIBERATION

**from malaria for
all of humanity**



- **عود یا Relapse** به معنای پیدایش مجدد انگل در خون پس از محو شدن کامل آن ها در عفونت اولیه است. این حالت در ویواکس و اوآله به علت وجود هیپنوزوئیت دیده می شود. در این دو پلاسمودیوم تعدادی از اسپوروزوئیت ها به شکل سلول های کوچک در سلول های پارانشیم کبد برای مدت طولانی به طور خفته و غیر فعال باقی می ماند و هرچند وقت یک بار تعدادی از آن ها فعال می شوند

- **بازگشت یا recrudeescence** به معنای ظهور مجدد نشانه ها در فرد بیماری است که انگل های موجود در خون او قبلاً در حدی بوده است که منجر به بروز نشانه های بالینی نمی شده است. در فالسیپاروم و مالاریه ظهور مجدد می تواند یک ماه تا دو سال بعد اتفاق بیفتد

- **Malaria is a potential medical emergency and should be treated accordingly. Delays in diagnosis and treatment are leading causes of death in many countries**

How to diagnose malaria

- [Microscopy](#)
- The only correct way to diagnose a case of malaria is by examining the patient's stained blood film with a microscope.
- This is a highly skilled job.
- [Rapid diagnostic tests](#)
- especially in remote areas with limited access to good quality microscopy services.
- [Nucleic acid amplification-based diagnostics](#)

RESEARCH

Open Access



Comparison of three diagnostic methods (microscopy, RDT, and PCR) for the detection of malaria parasites in representative samples from Equatorial Guinea

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Abstract

Background: Malaria in Equatorial Guinea remains a major public health problem. The country is a holo-endemic area with a year-round transmission pattern. In 2016, the prevalence of malaria was 12.09% and malaria caused 15% of deaths among children under 5 years. In the Continental Region, 95.2% of malaria infections were *Plasmodium falciparum*, 0.5% *Plasmodium vivax*, and eight cases mixed infection in 2011. The main strategy for malaria control is quick

- More than a century later, microscopic detection and identification of *Plasmodium species* in ***Giemsa-stained thick blood films*** (for screening the presenting malaria parasite), and thin blood films (for species' confirmation) remains the **gold standard** for laboratory diagnosis.
- The wide acceptance of this technique by laboratories all around the world can be attributed to its **simplicity, low cost**, its ability to identify the **presence of parasites**, the infecting **species**, and assess parasite density—all parameters useful for the management of malaria.
- the staining and interpretation processes are **labor intensive, time consuming**, and require considerable **expertise** and trained healthcare workers, particularly for identifying species accurately at low parasitemia or in mixed malarial infections.

- **a thick blood film:** After drying, the spot is stained with diluted Giemsa (1 : 20, vol/vol) for 20 min, and washed by placing the film in buffered water for 3 min. The slide is allowed to air-dry in a vertical position
- As they are unfixed, the red cells lyse when a water-based stain is applied.
- **A thin blood film:** The film is then allowed to air-dry and is fixed with absolute methanol. After drying, the sample is stained with diluted Giemsa (1 : 20, vol/vol) for 20 min and washed by briefly dipping the slide in and out of a jar of buffered water (excessive washing will decolorize the film). The slide is then allowed to air-dry in a vertical position and examined under a light microscope

Blood films

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

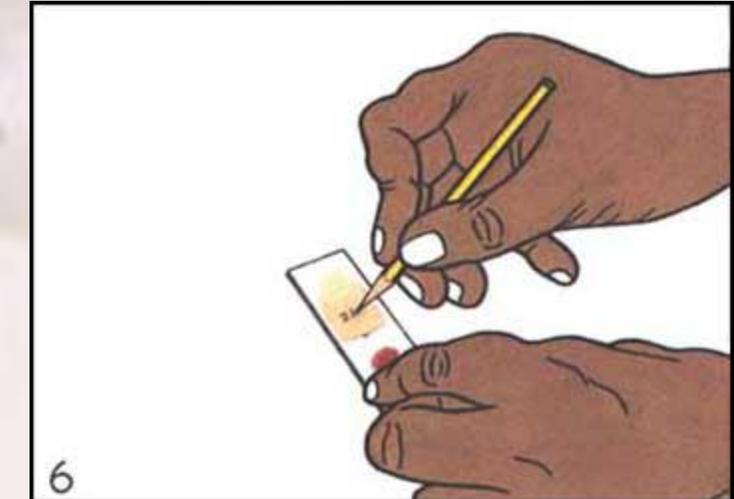
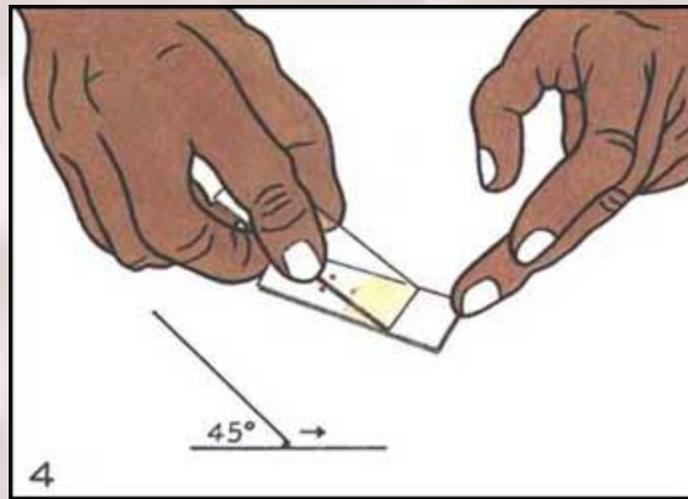
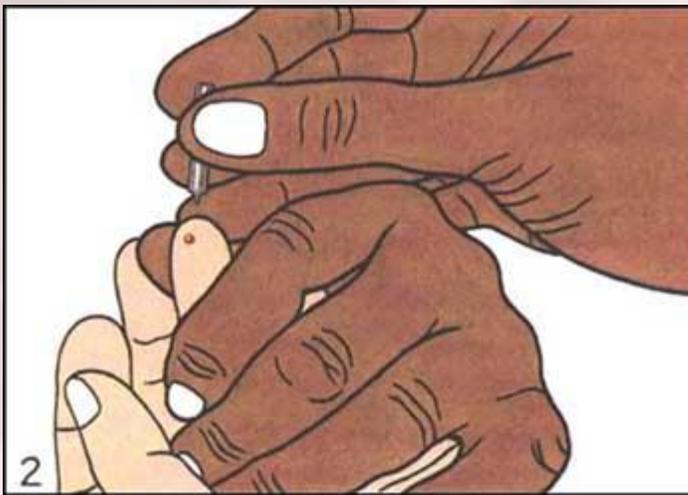
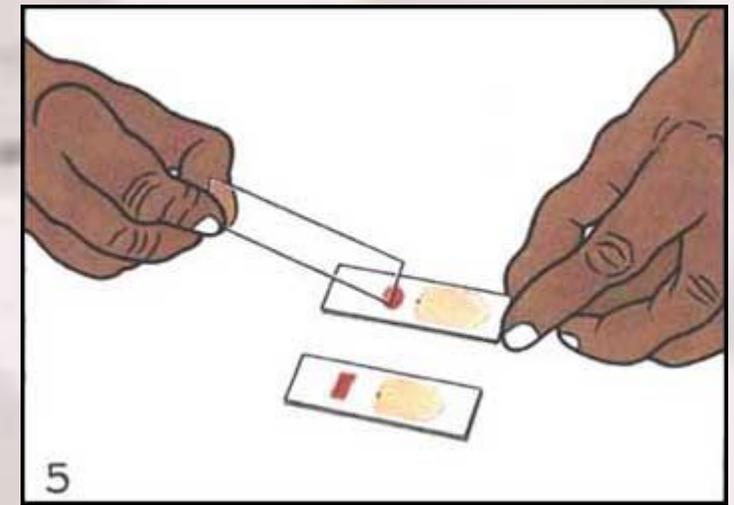
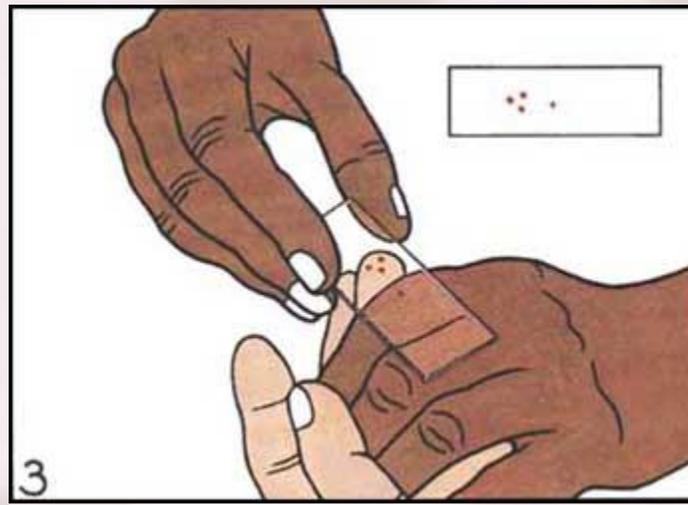
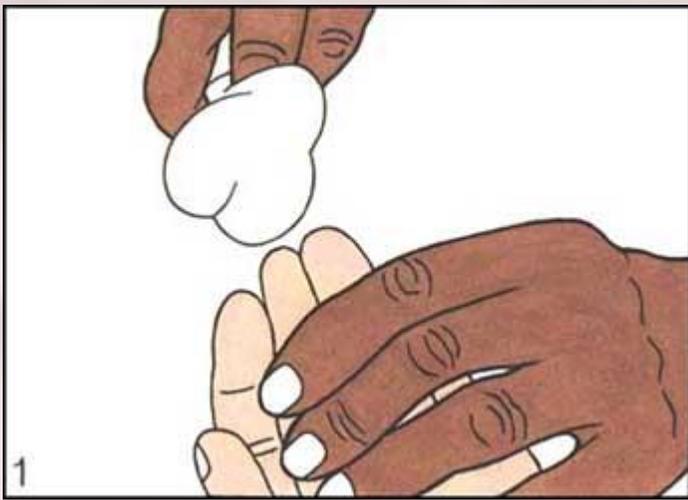
Thin film

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

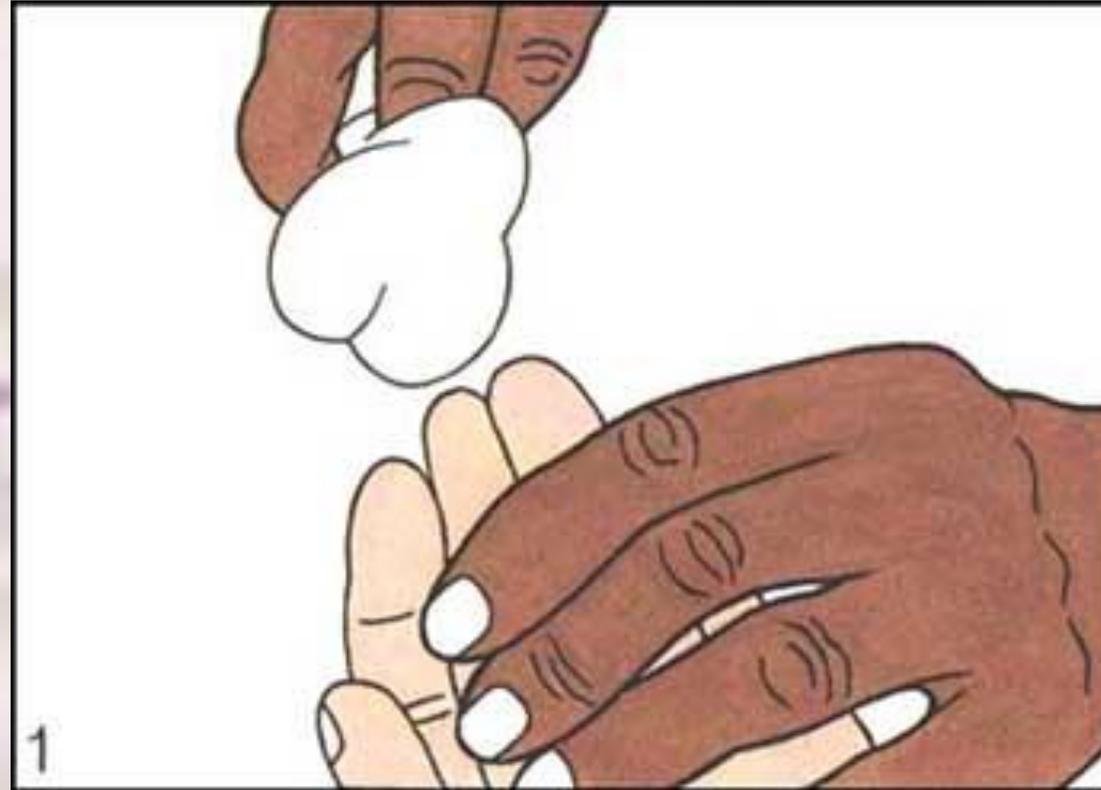
Thick film

The thick film is made up of **large numbers of dehaemoglobinized red blood cells**.

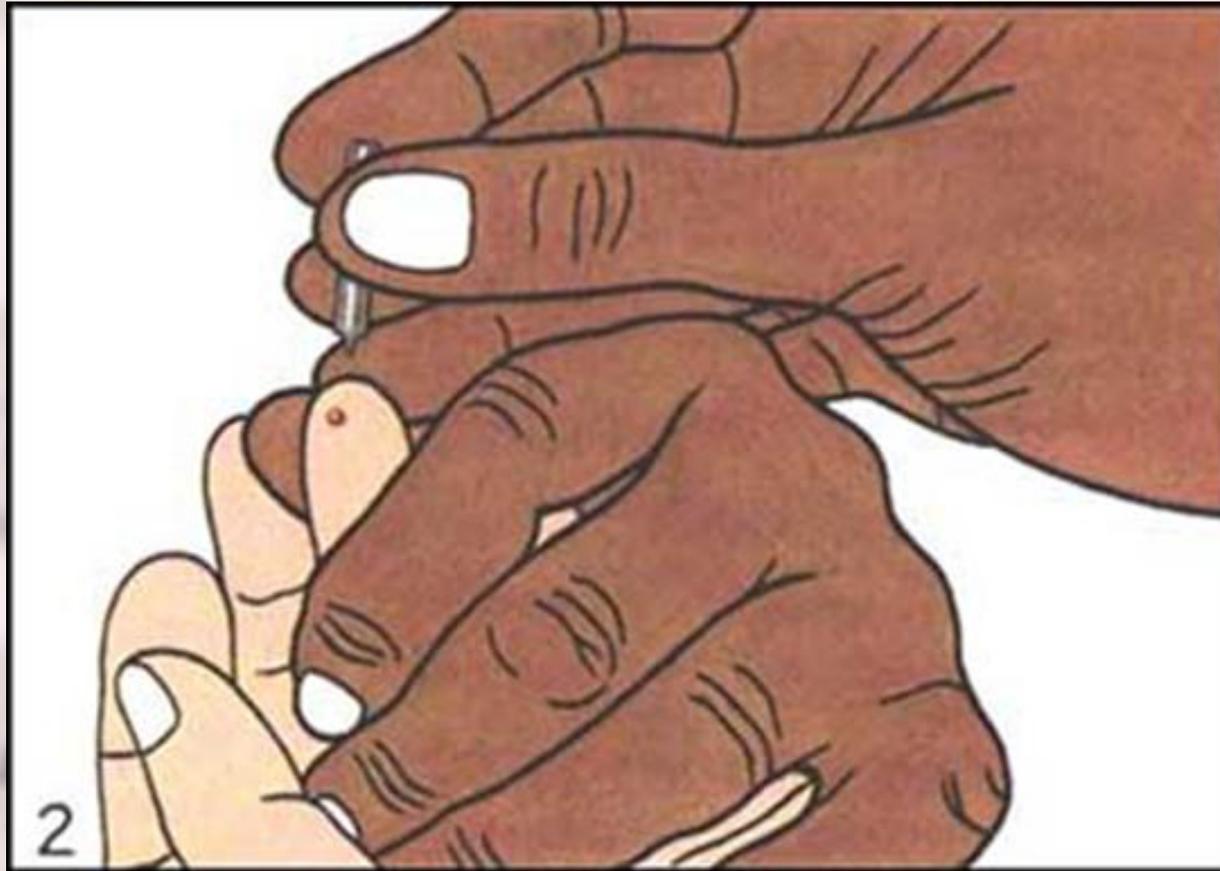
Any parasites present are concentrated in a smaller area than in the thin film and so are **more quickly** seen under the microscope.



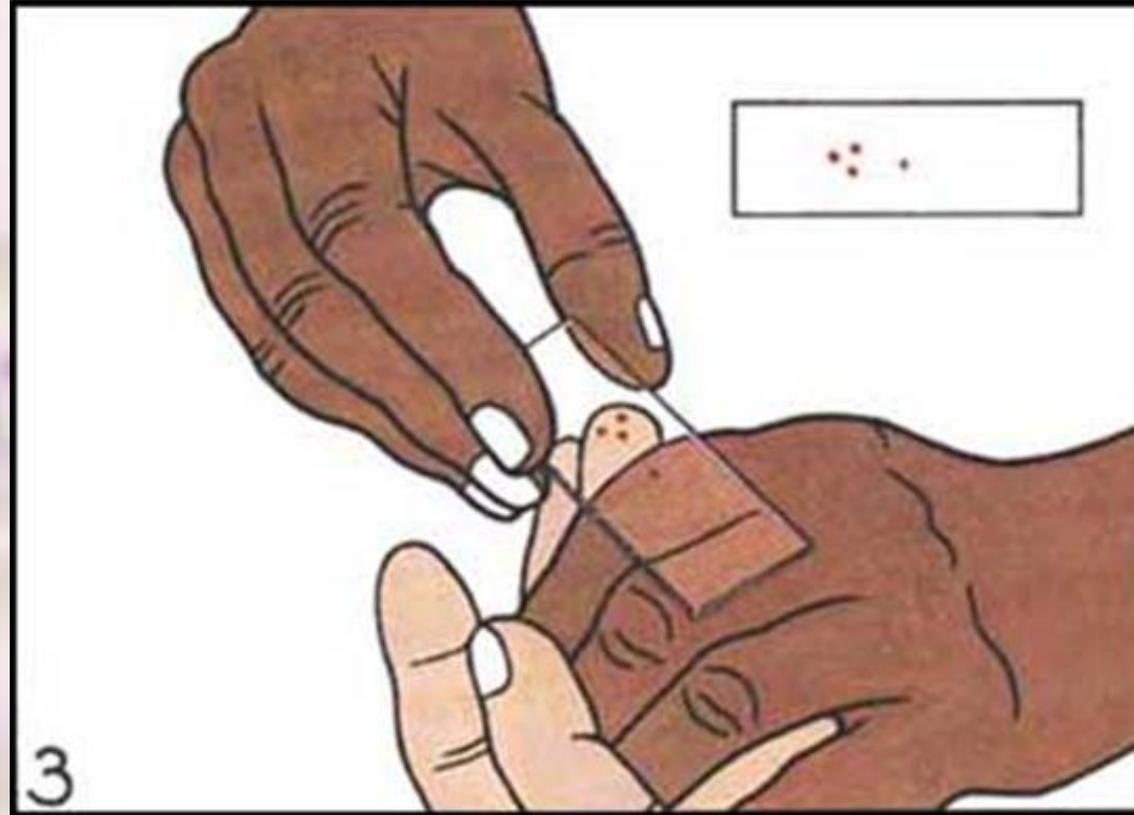
Preparation of thick and thin blood films on the same slide



1. Holding the patient's left hand, palm upwards, select the third finger from the thumb. (The big toe can be used with infants. The thumb should never be used for adults or children). Clean the finger with a piece of cotton wool lightly soaked in alcohol, using firm strokes to remove dirt and grease from the ball of the finger. Dry the finger with a clean cotton cloth, using firm strokes to stimulate blood circulation.



2. Puncture the ball of the finger with a sterile lancet, using a quick rolling action. Apply gentle pressure to the finger to express the first drop of blood and wipe it away with a dry piece of cotton wool. Make sure that no strands of cotton remain on the finger to be later mixed with the blood.

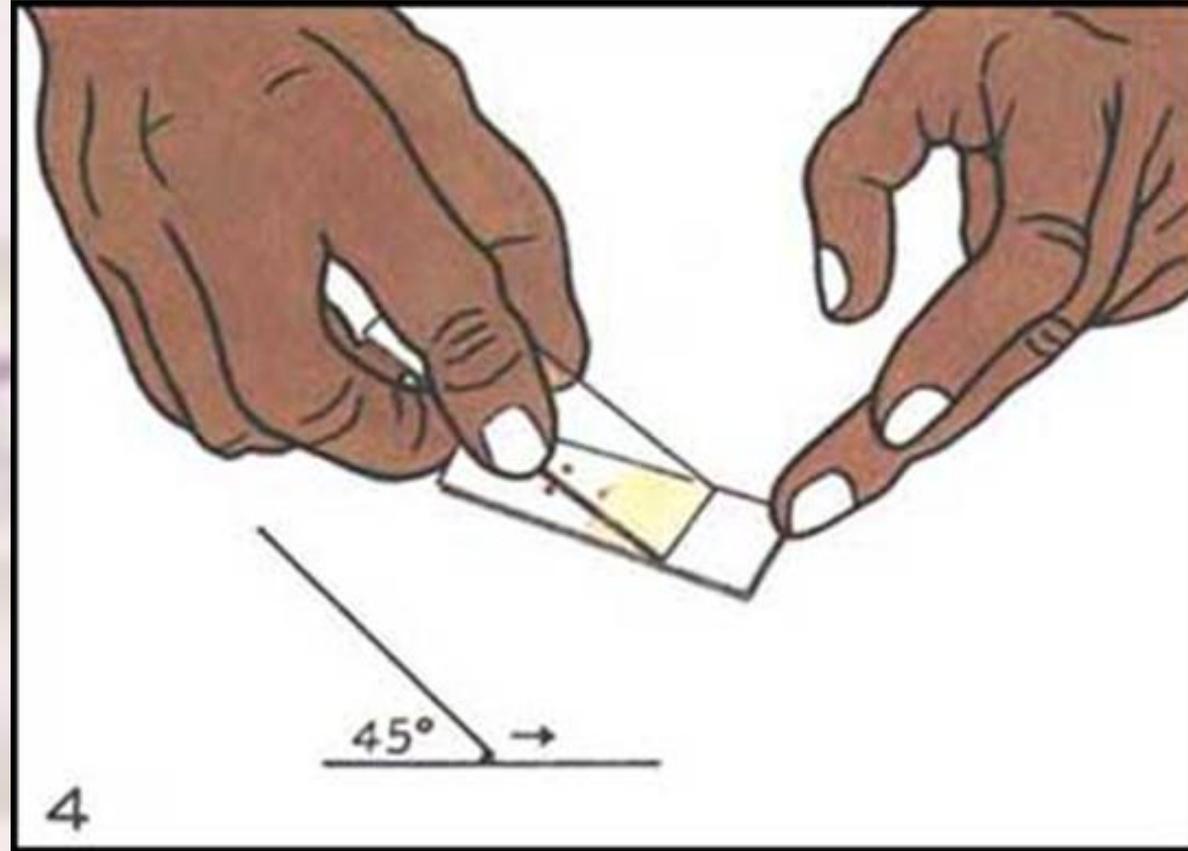


3. Working quickly and handling clean slides only by the edges, collect the blood as follows.

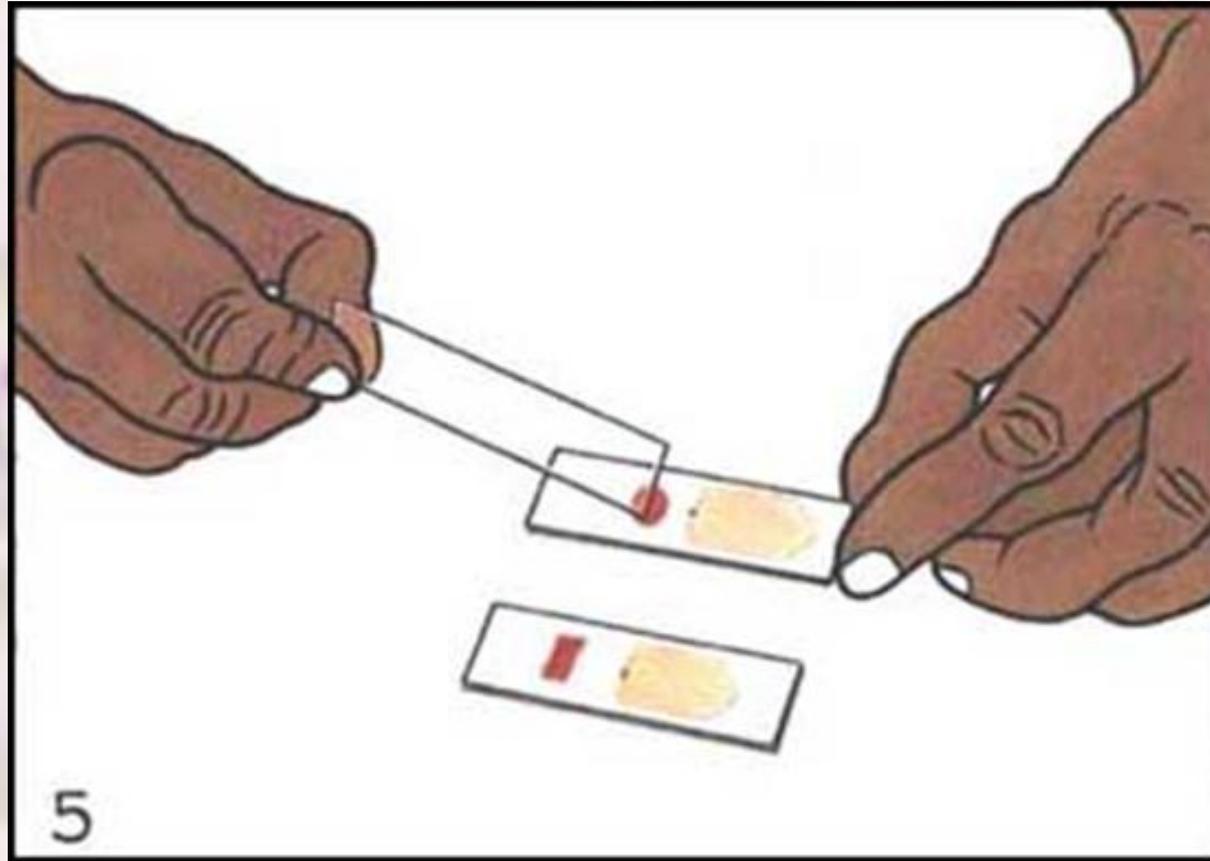
Apply gentle pressure to the finger and collect a single small drop of blood on the middle of the slide. This is for the thin film.

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

Wipe the remaining blood away from the finger with a piece of cotton wool.

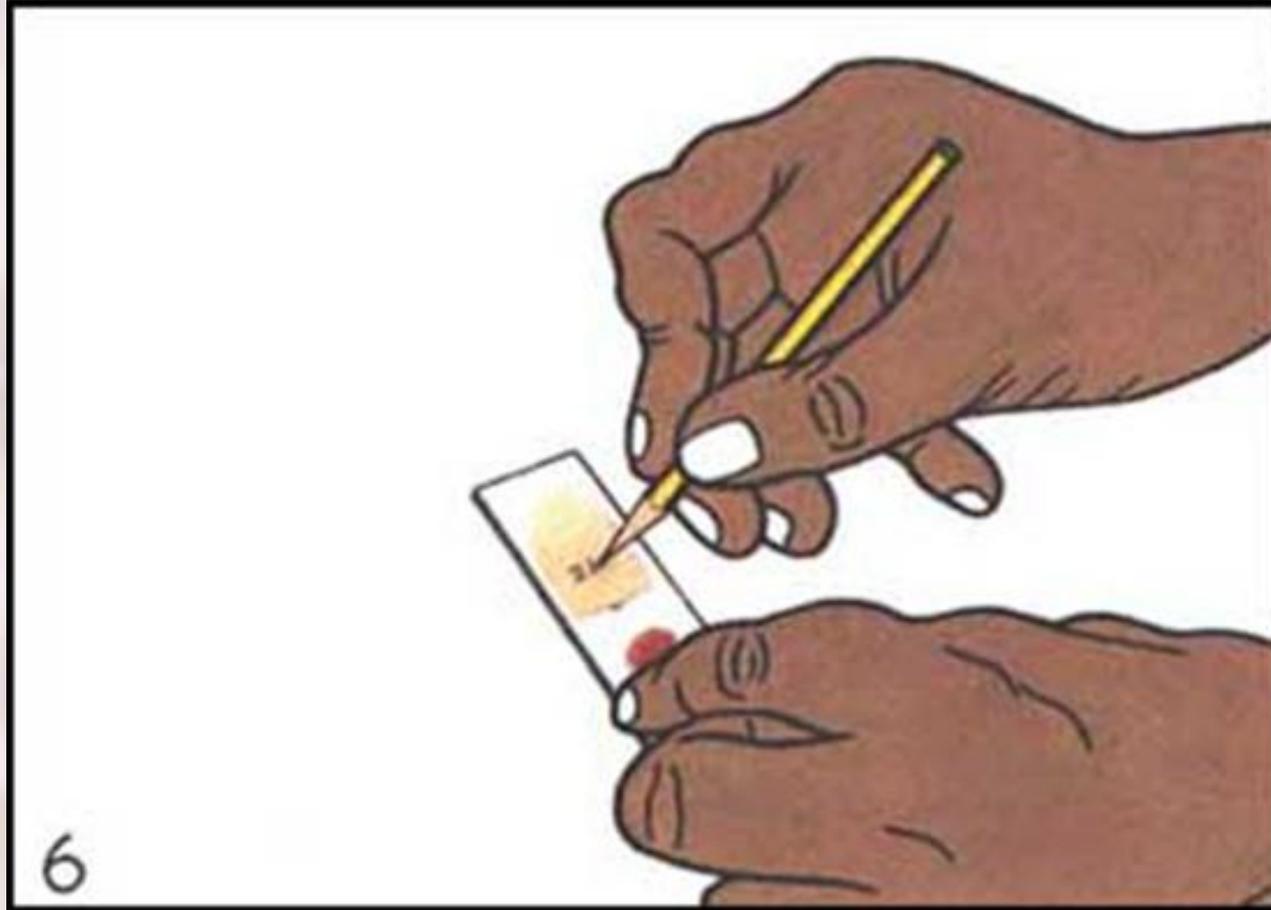


4. *Thin film.* Using a second clean slide as a “spreader” and, with the slide with the blood drops resting on a flat, firm surface, touch the small drop with the spreader and allow the blood to run along its edge. Firmly push the spreader along the slide, keeping the spreader at an angle of 45° . Make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



5. *Thick film.* Always handle slides by the edges or by a corner to make the thick film as follows. Using the corner of the spreader, quickly join the drops of blood and spread them to make an even, thick film. The blood should not be excessively stirred but can be spread in circular or rectangular form with 3 to 6 movements. The circular thick film should be about 1 cm ($\frac{1}{3}$ inch) in diameter.

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

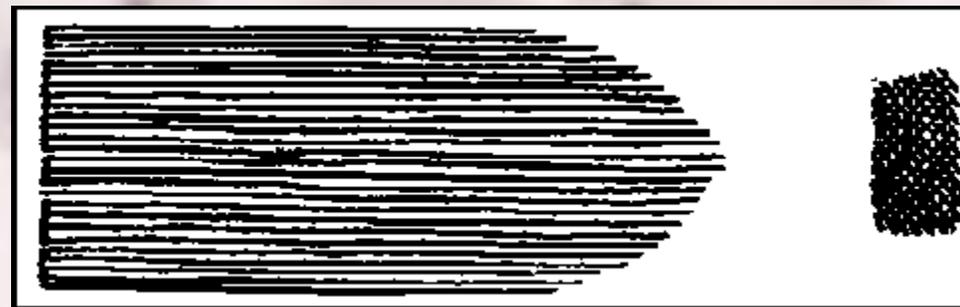


6. Label the dry thin film with a soft lead pencil by writing across the thicker portion of the film the patient's name or number and the date. Do not use a ballpoint pen for labelling the slide. Allow the thick film to dry with the slide in a flat, level position, protected from flies, dust and extreme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

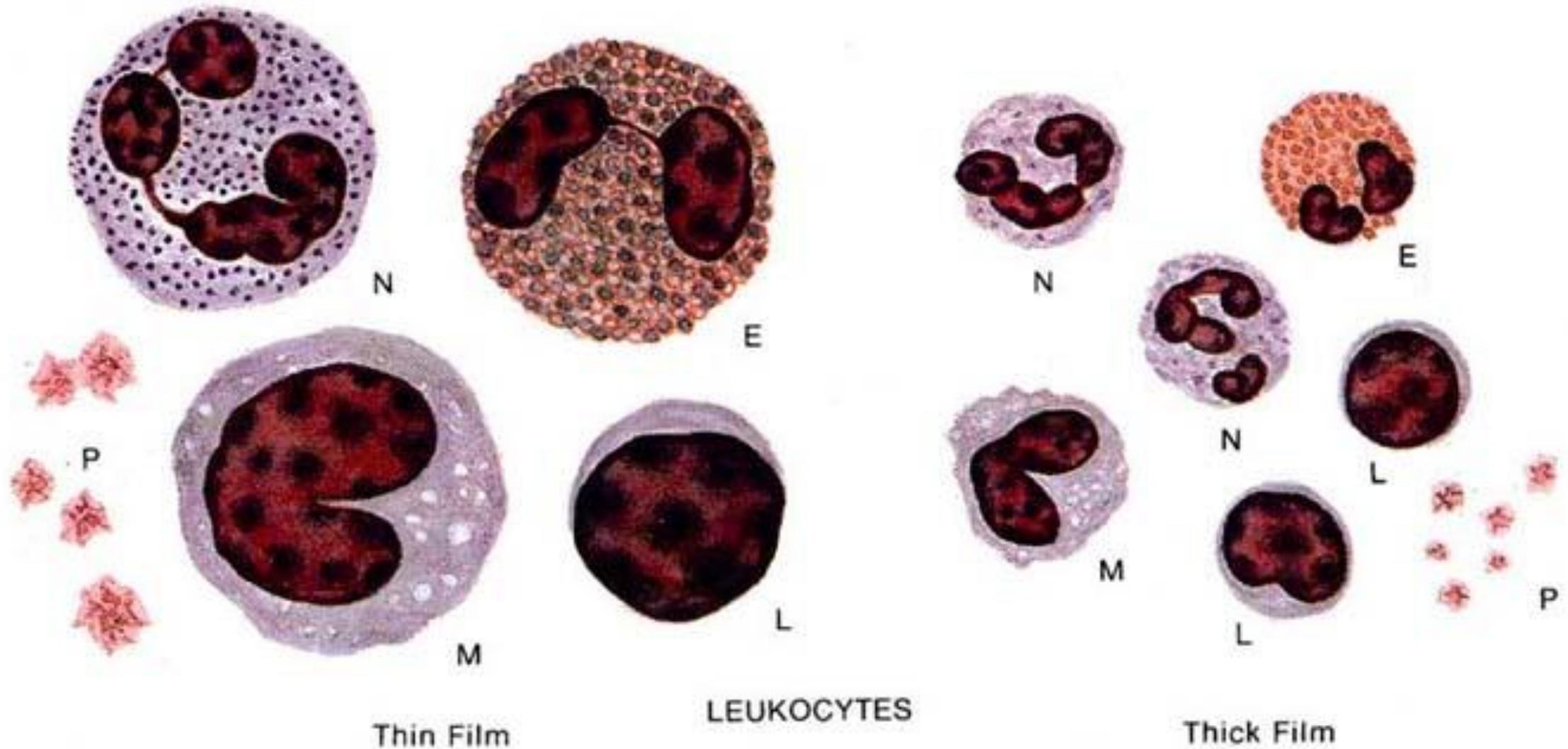


example of well made and correctly labelled thick and thin films

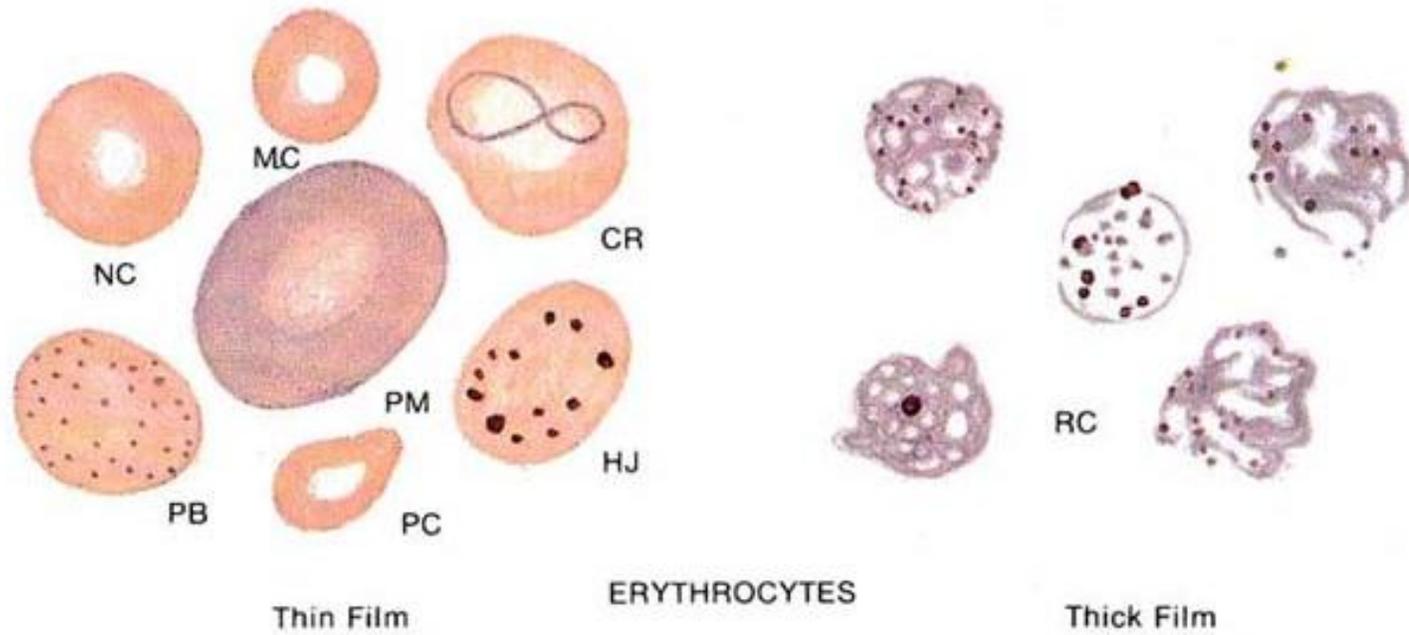
Common faults in making blood films



the white blood cells appear to be smaller, with the cytoplasm more compact around the nuclei.



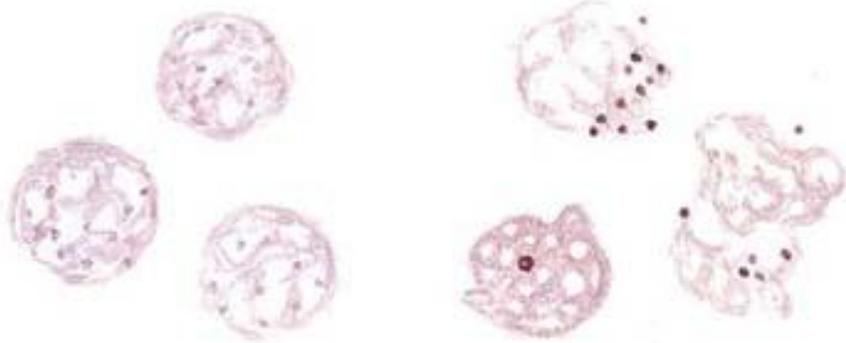
N = Neutrophil, E = Eosinophil, M = Monocyte, L = Lymphocyte, P = Platelets



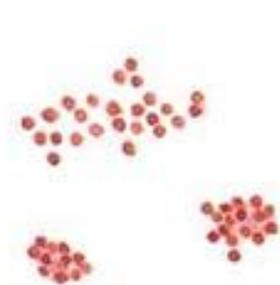
NC = Normocyte, MC = Microcyte, PM = Polychromatic macrocyte, PC = Poikilocyte, PB = Punctate basophilia, CR = Cabot's ring, HJ = Howell-Jolly bodies, RC = Reticular 'clouds' and chromatoid bodies in severe anaemia



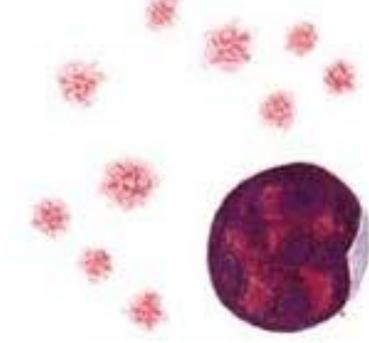
MALARIA STAINING AND pH



'Clouds' and chromatoid debris derived from immature erythrocytes in severe anaemia

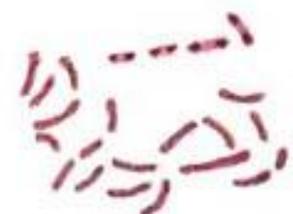
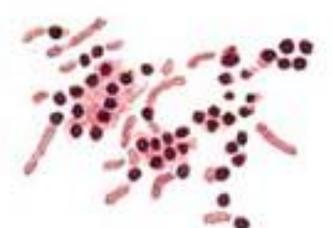


Isolated groups of eosinophilic granules

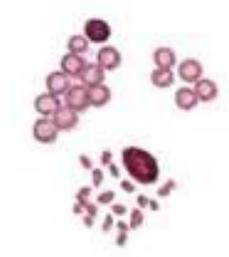
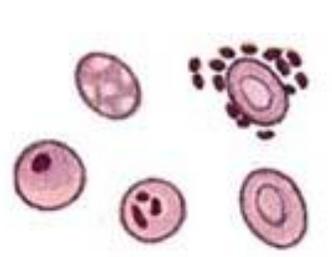


Blood platelets. Lymphocyte for comparison of size

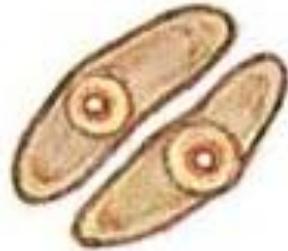
BLOOD ELEMENTS



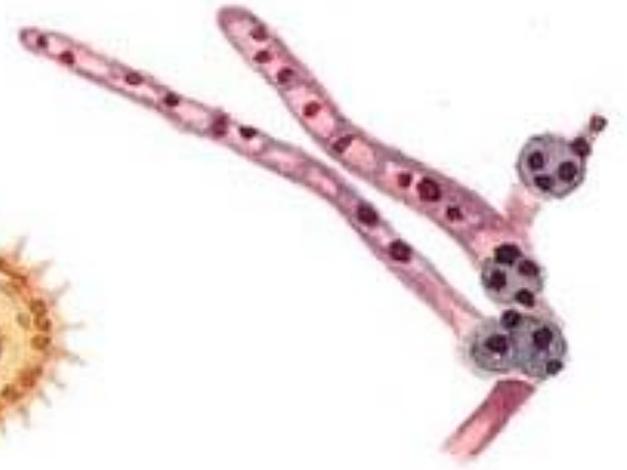
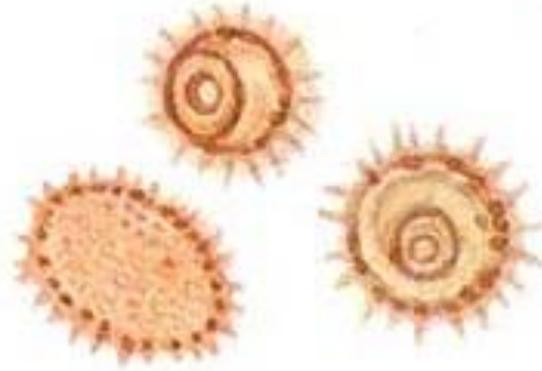
BACTERIA



SPORES



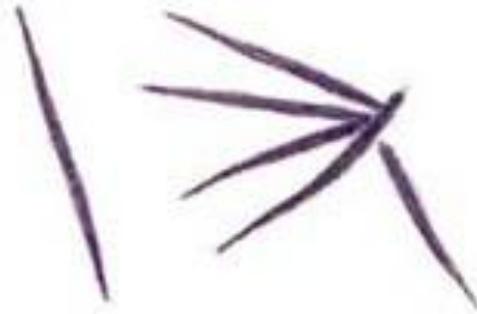
VEGETABLE CELLS



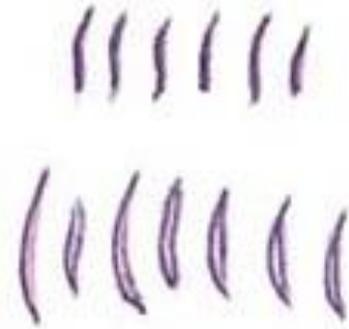
Hyphae and spores
FUNGUS



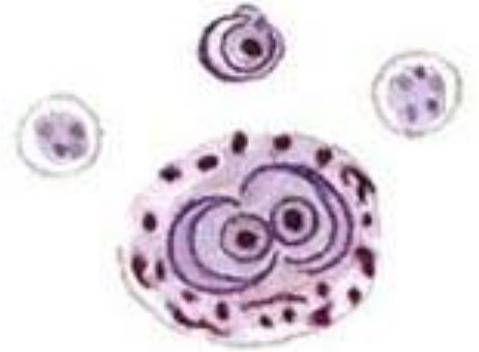
Dust particles



Giemsa stain
crystals



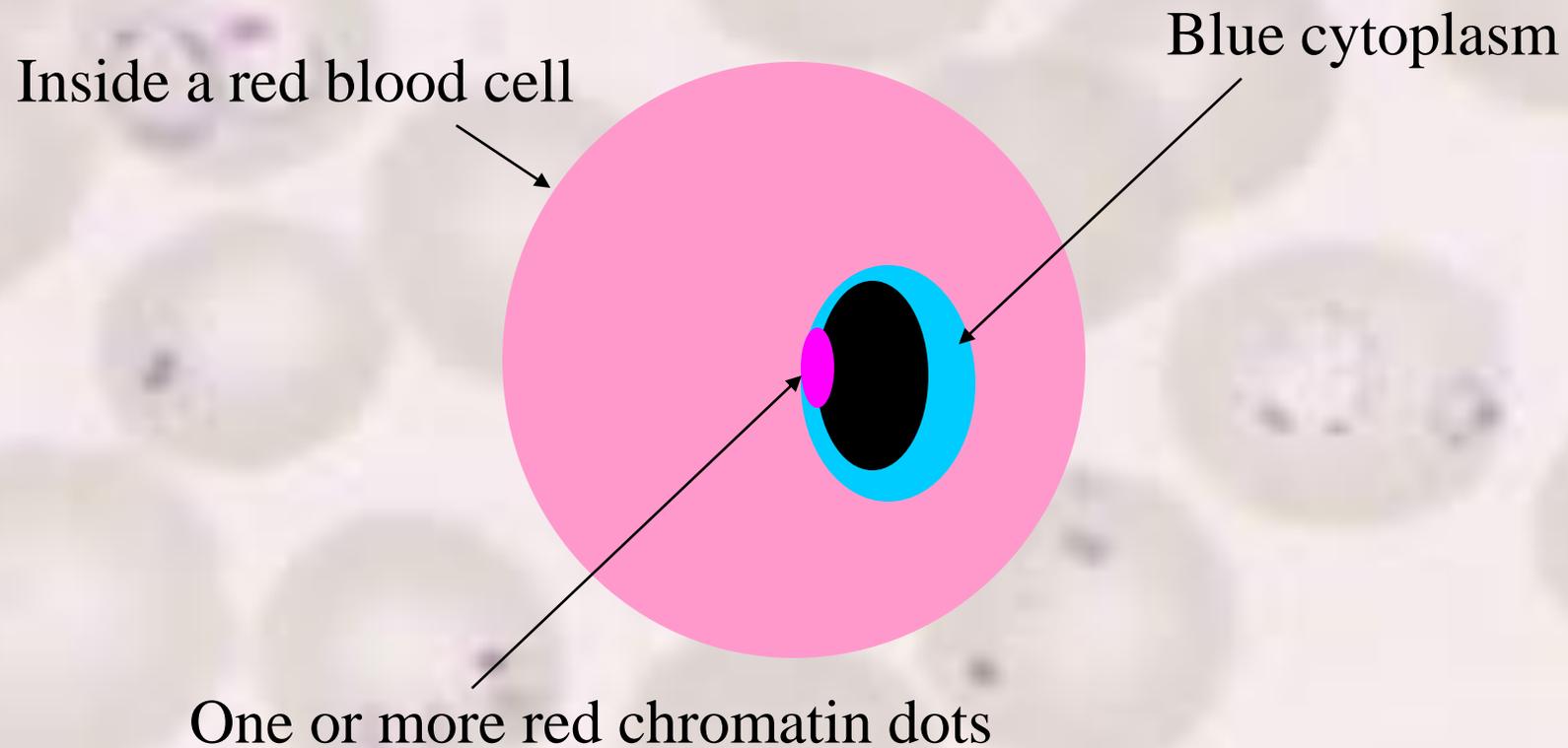
Herring-bone
scratches in
glass slide



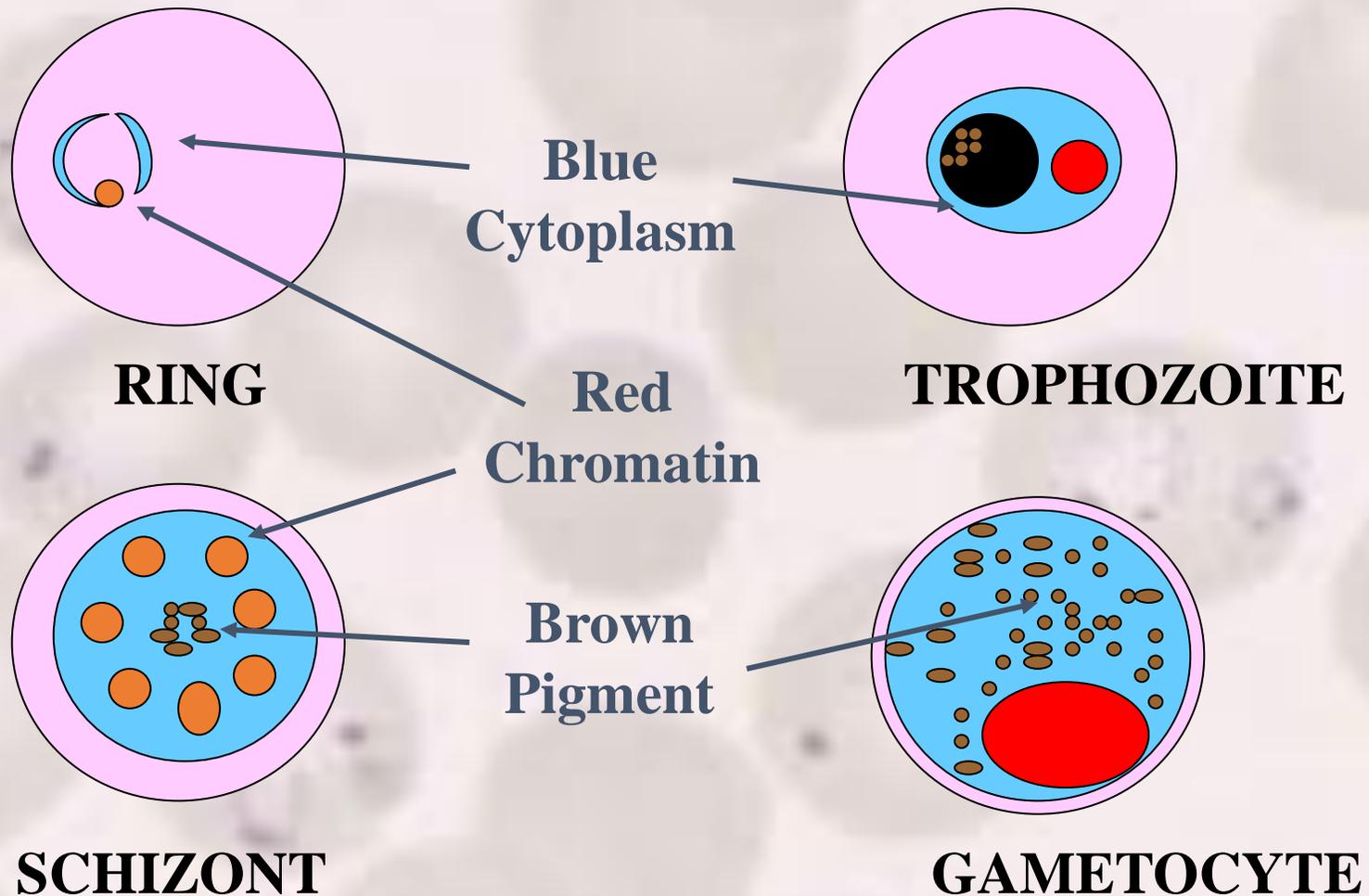
Crystalline 'pits'
in devitrified slide

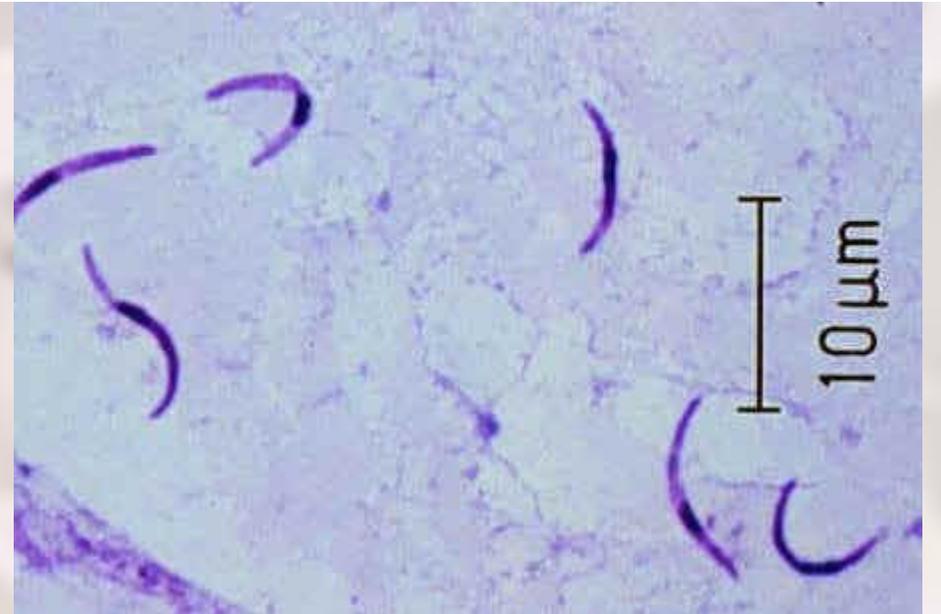
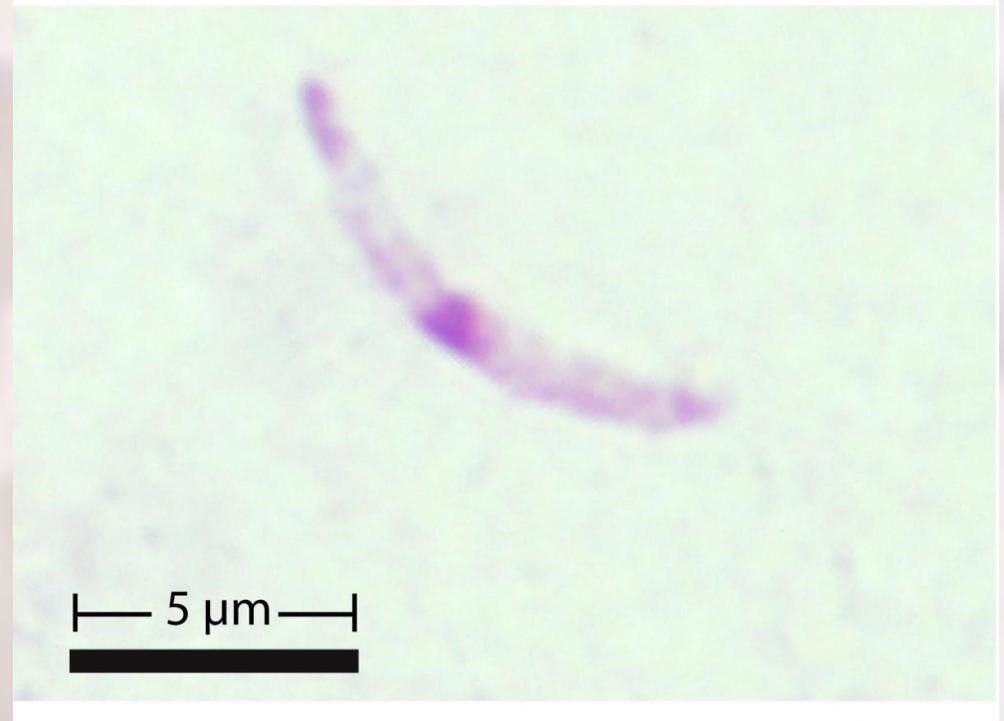
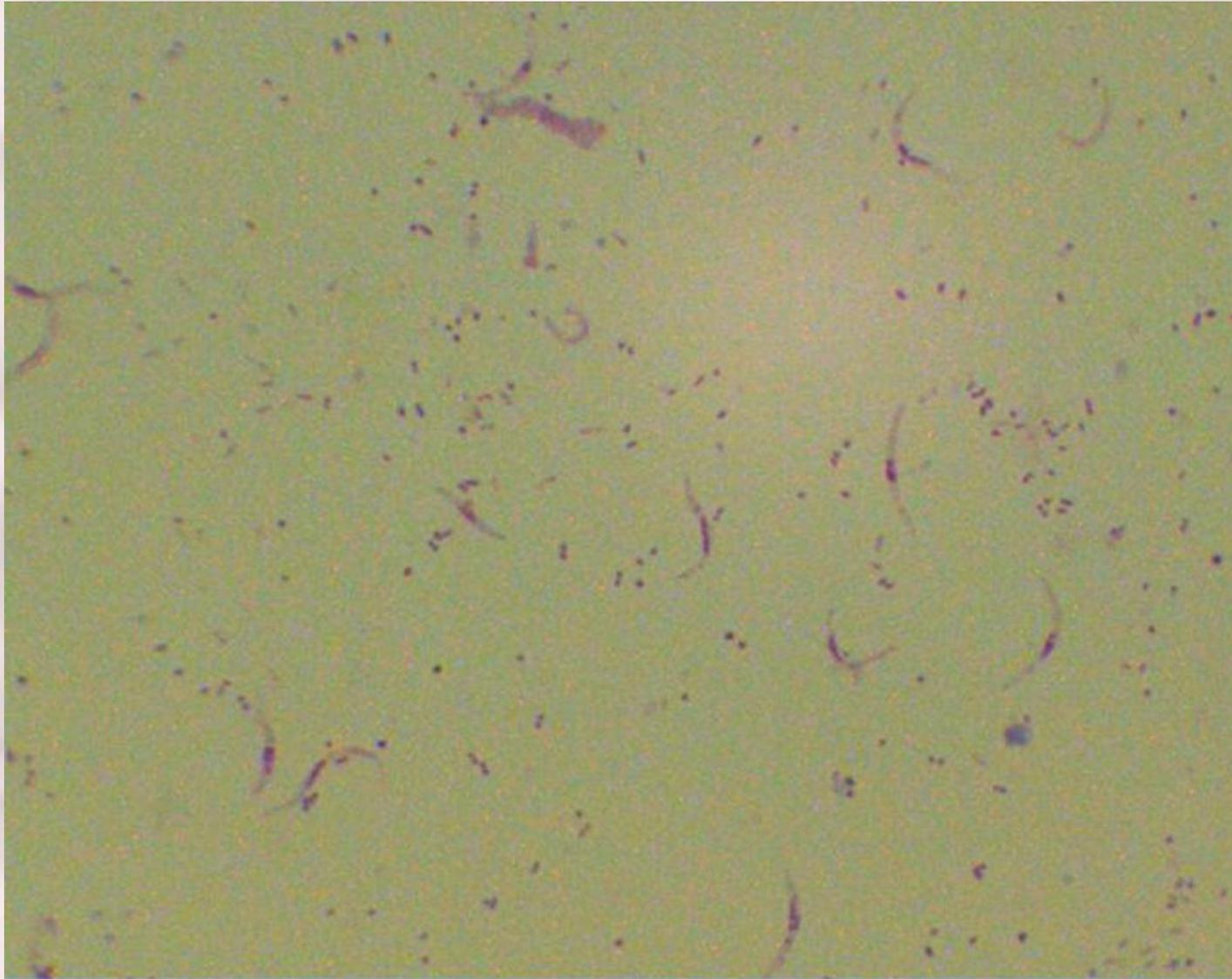
VARIOUS SOURCES

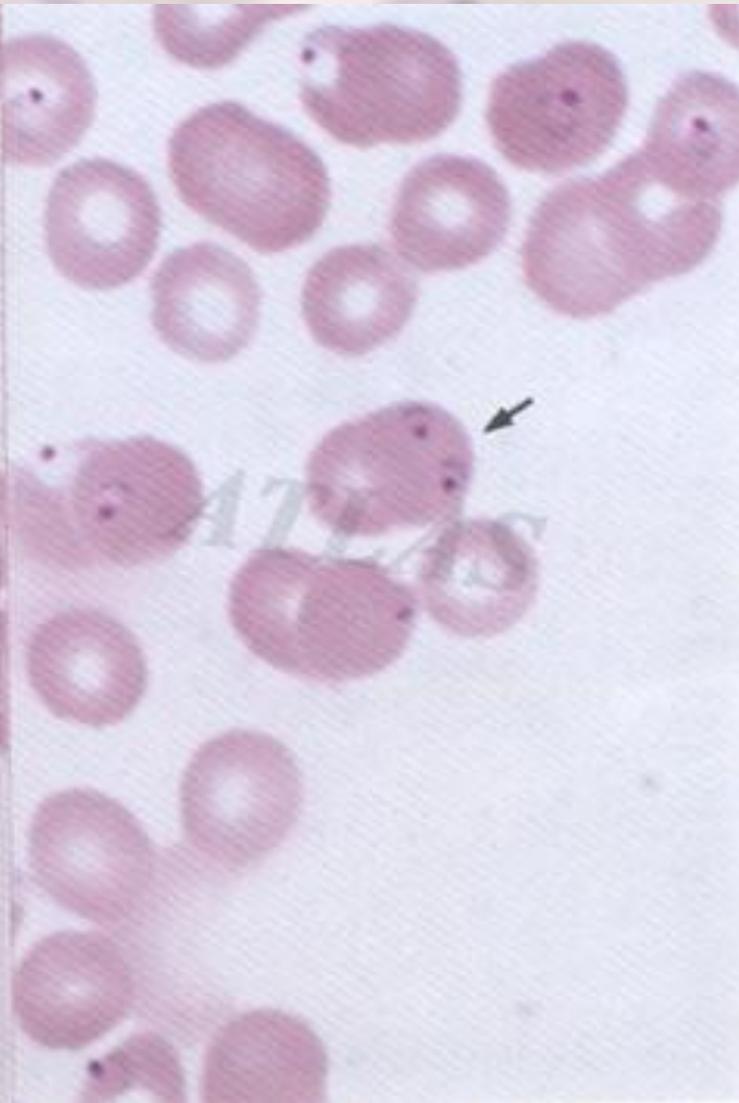
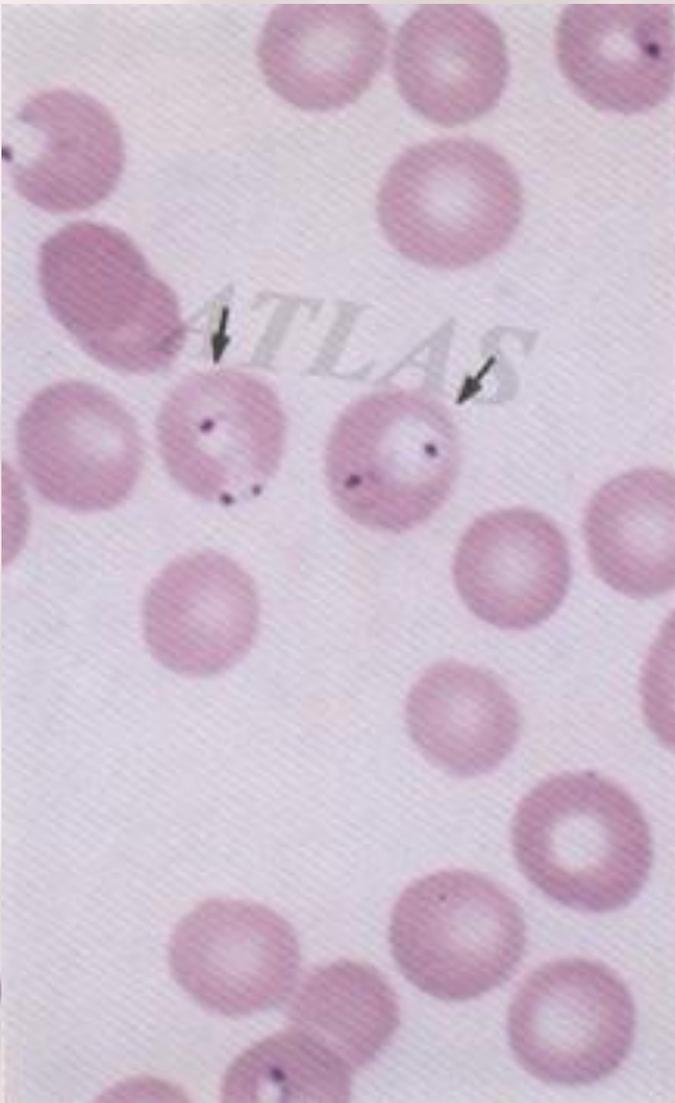
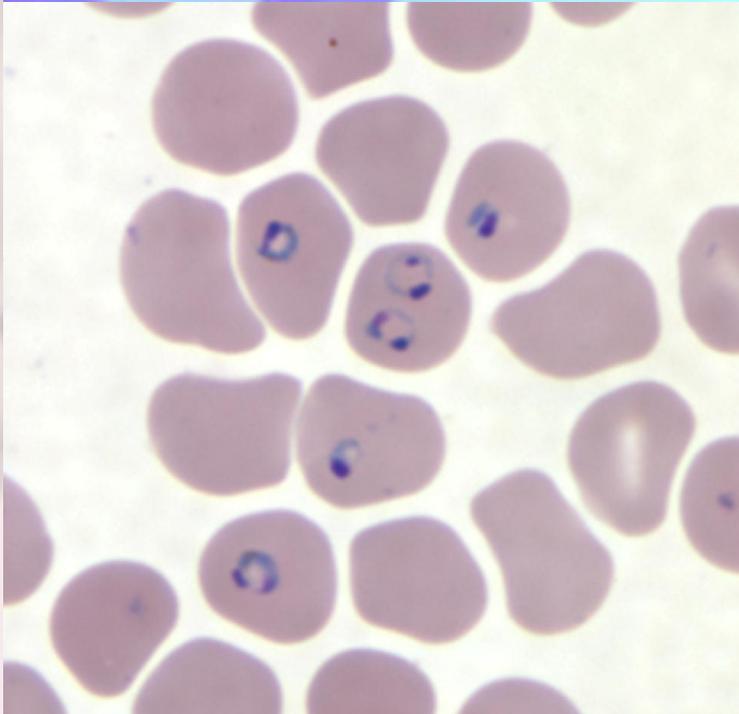
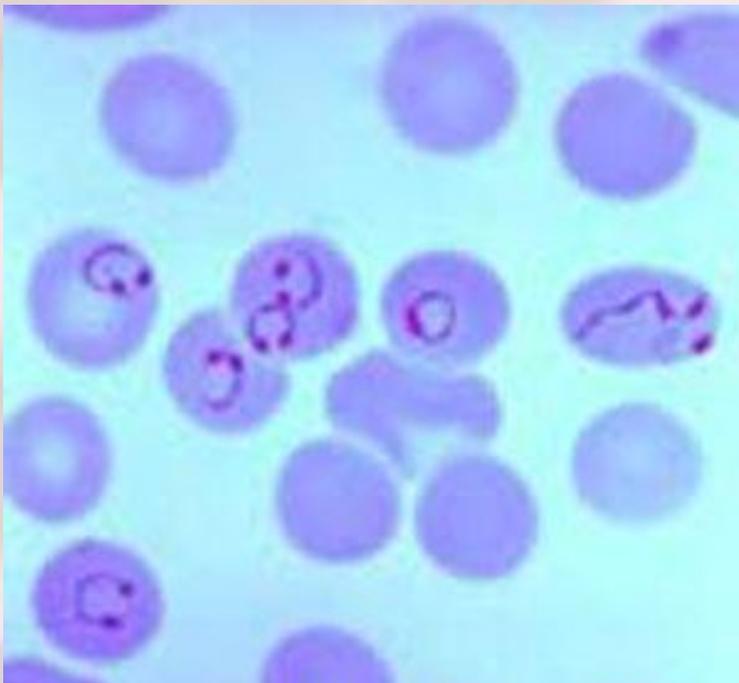
Recognizing Malaria Parasites



Recognizing Erythrocytic Stages: Schematic Morphology



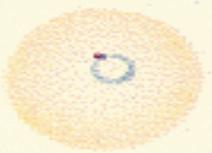




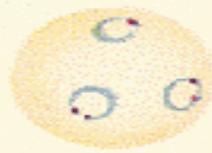
P. falciparum



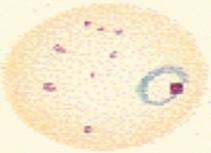
marginal form



ring form



double dotted rings



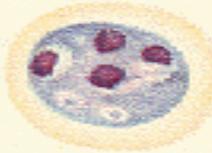
ring form



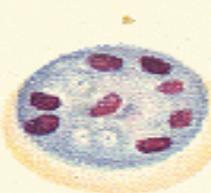
young trophozoite



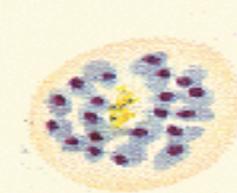
trophozoite



early schizont



schizont



mature schizont



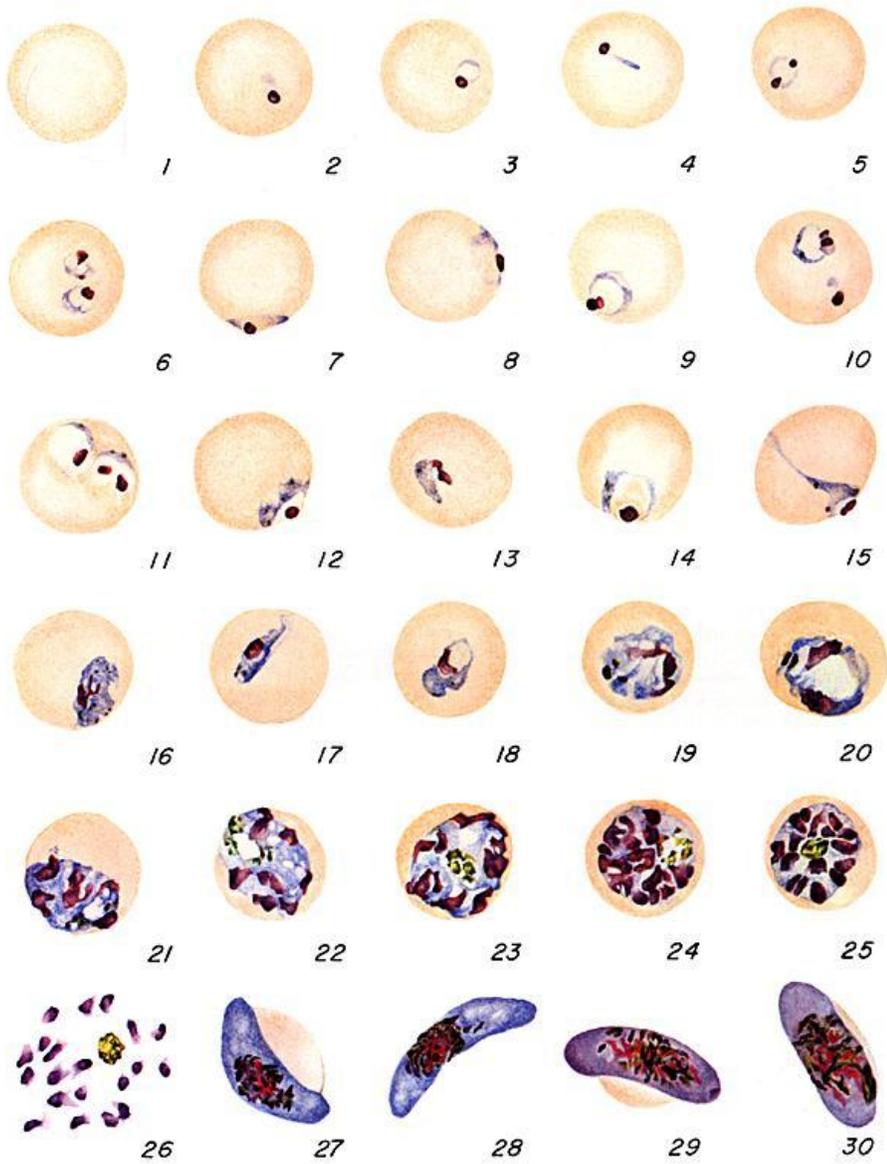
female gametocyte



male gametocyte

پلاسمودیوم فالسیپاروم

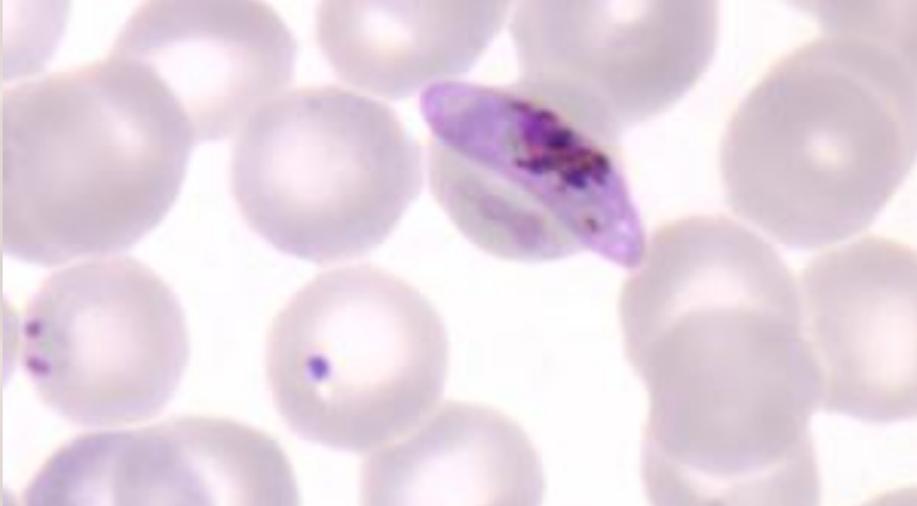
- وجود رنگدانه های مورر به رنگ قرمز
- شیزونت رسیده حاوی 8-24 مروزوئیت
- گامتوسیت های کشیده و هلالی
- حمله به گلوبول قرمز در هر سنی

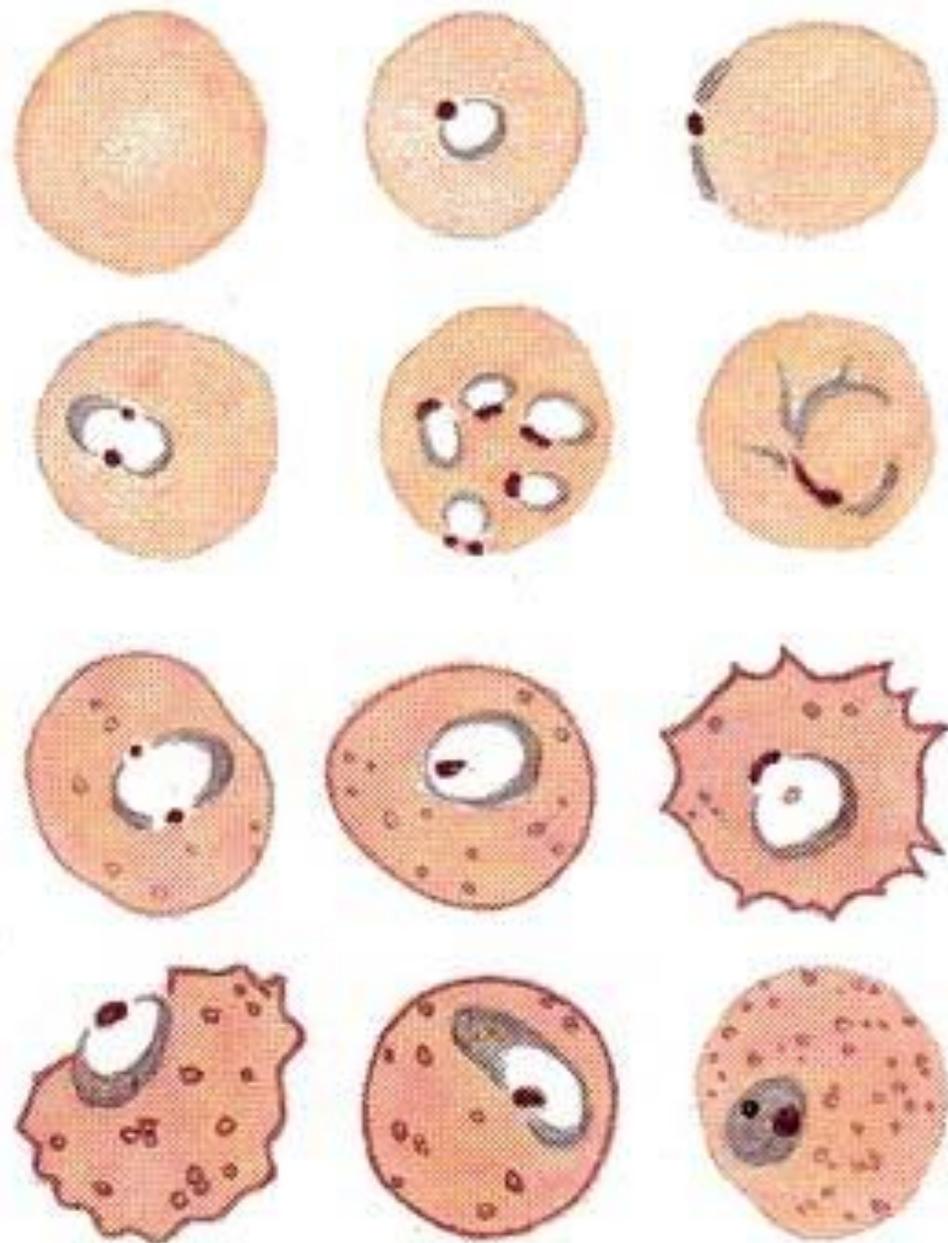


0 10μ

PLASMODIUM FALCIPARUM

S. H. Nicholson

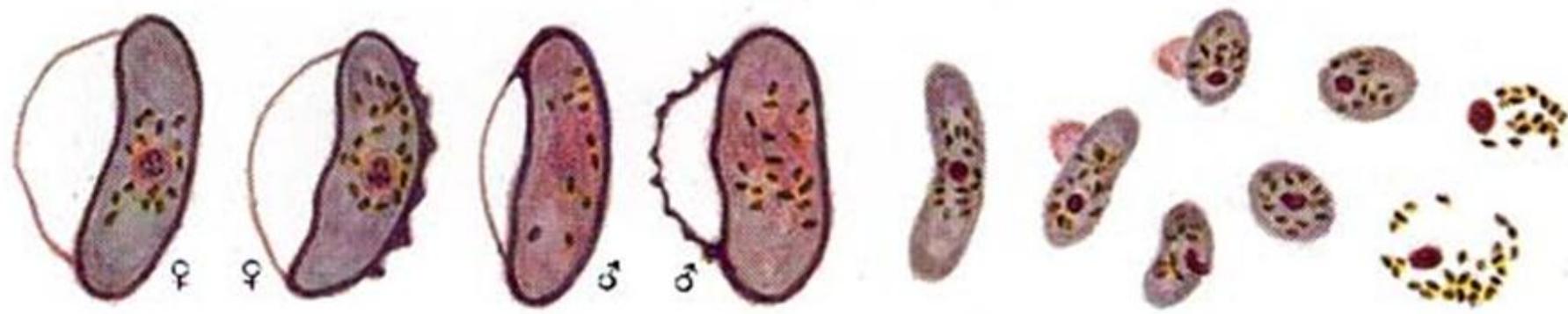




TROPHOZOITES



SCHIZONTS



GAMETOCYTES

Thin film

Thick film

پلاسمودیوم ویواکس

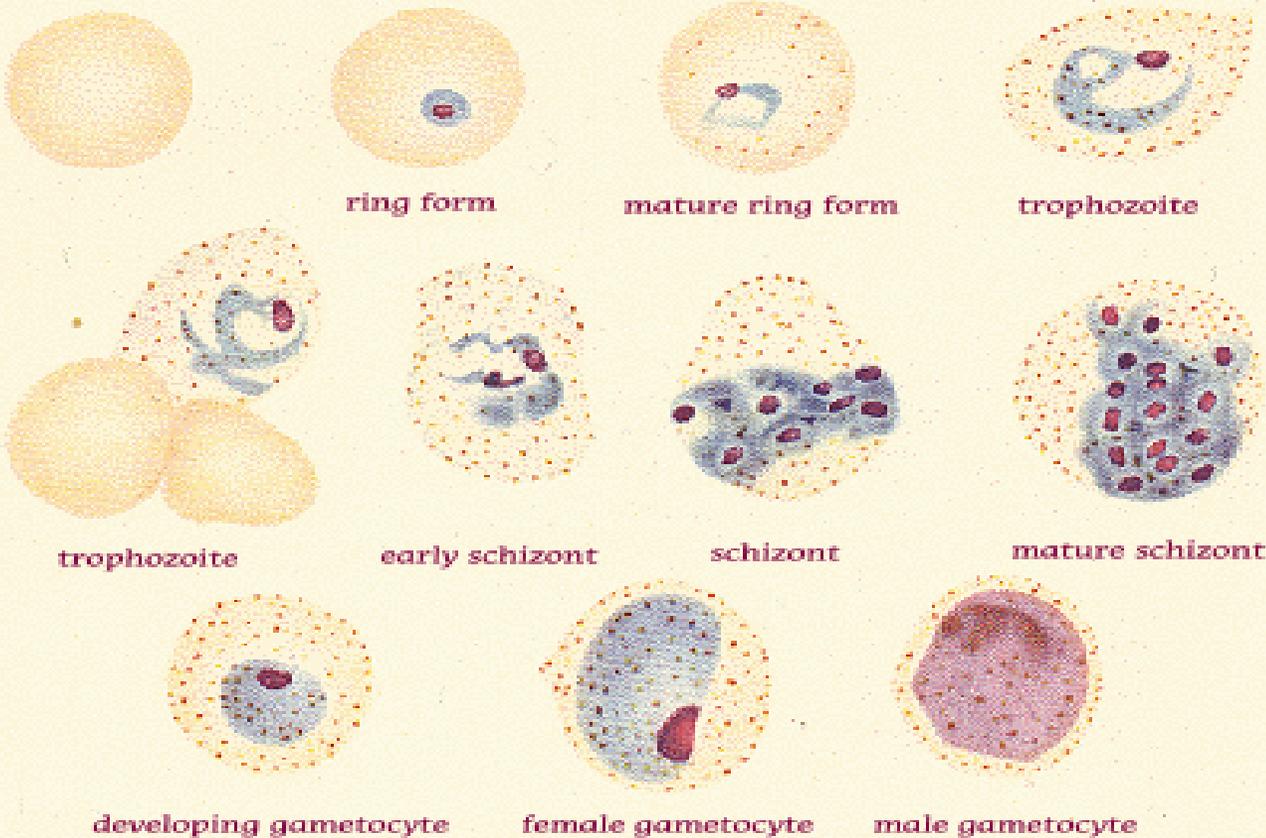
- رینگ و تروفوزوئیت جوان به شکل آمیب

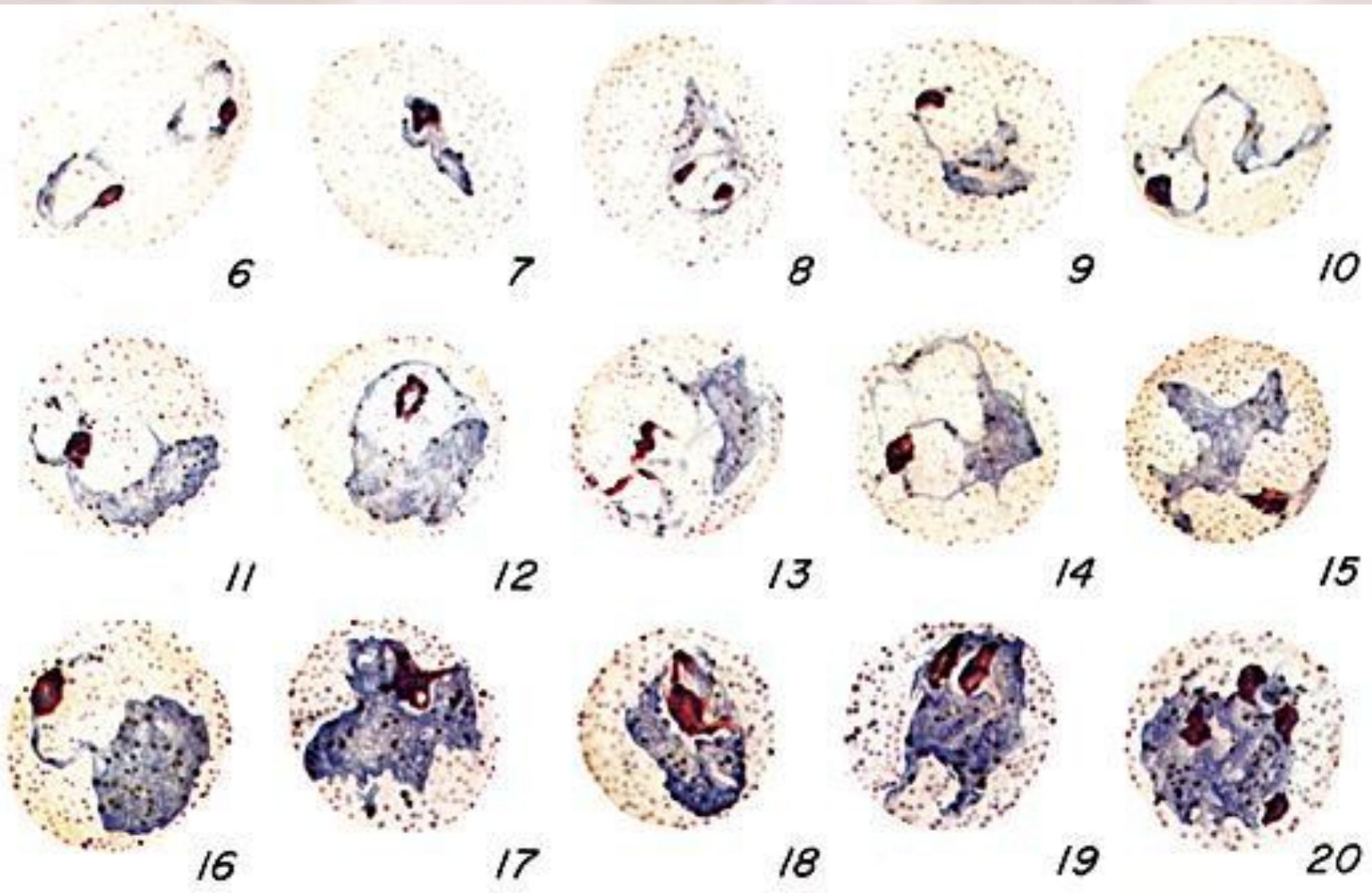
- شیزونت حاوی 12-18 مروزوئیت

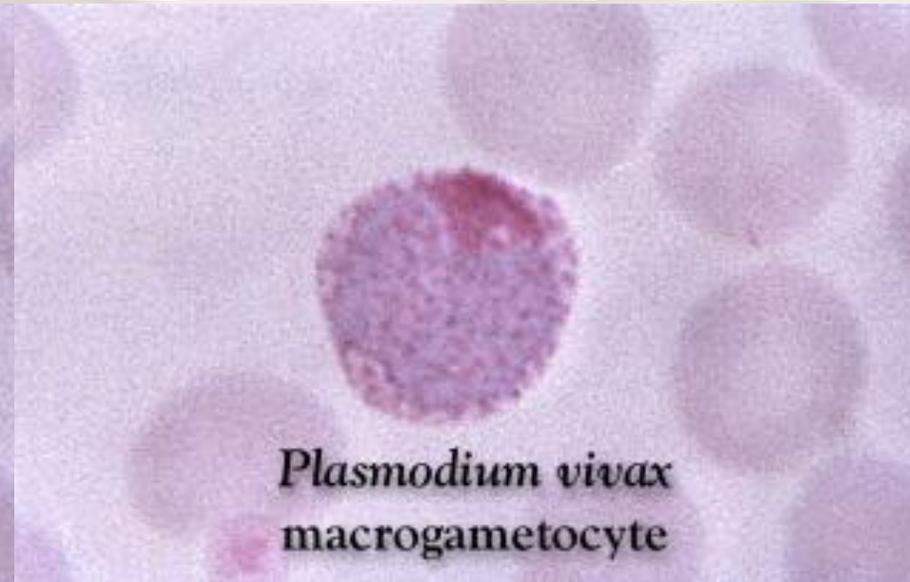
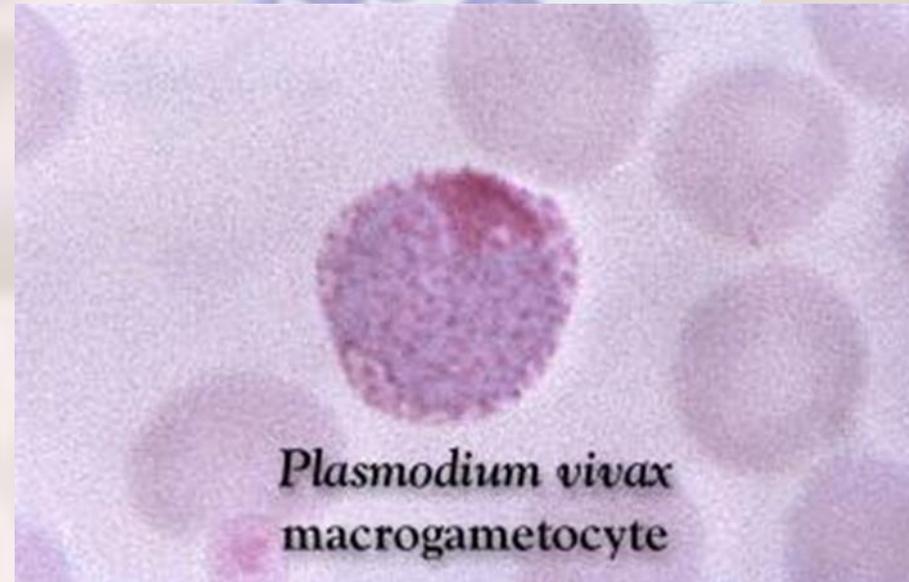
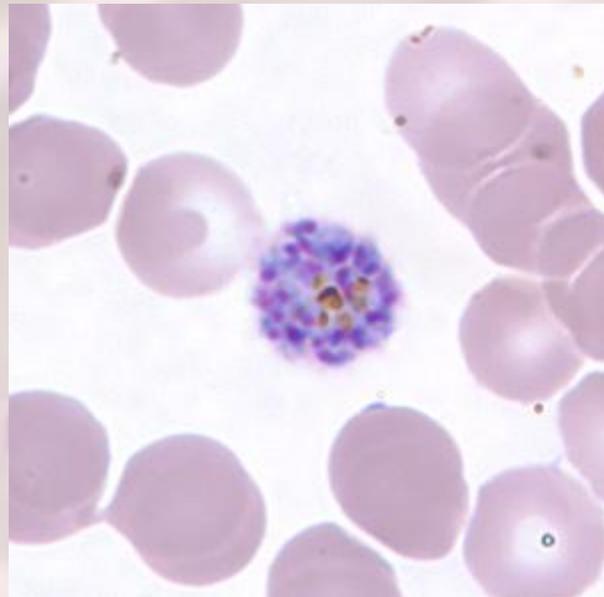
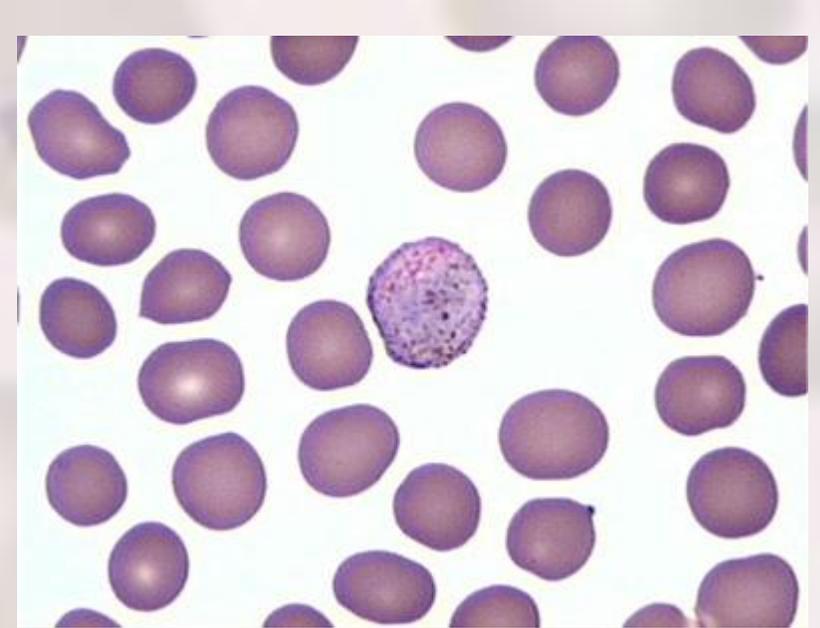
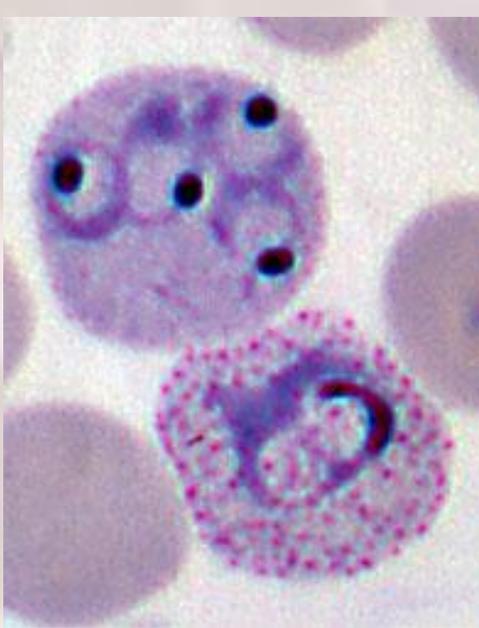
- گلبول قرمز آلوده بزرگ تر از حد طبیعی رنگ پریده و حاوی دانه های شافنر

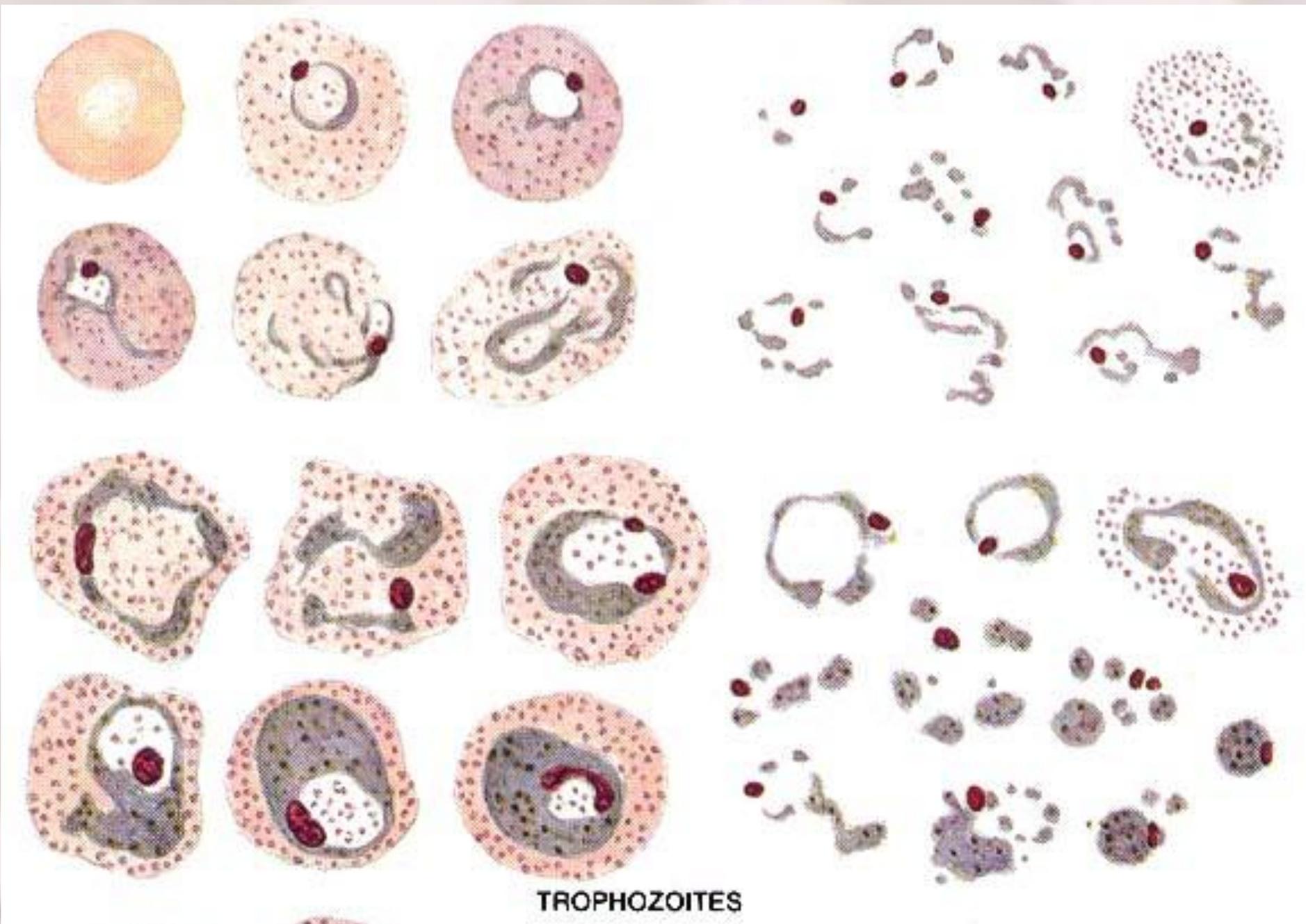
- حمله عمدتاً به رتیکولوسیت ها

P. vivax

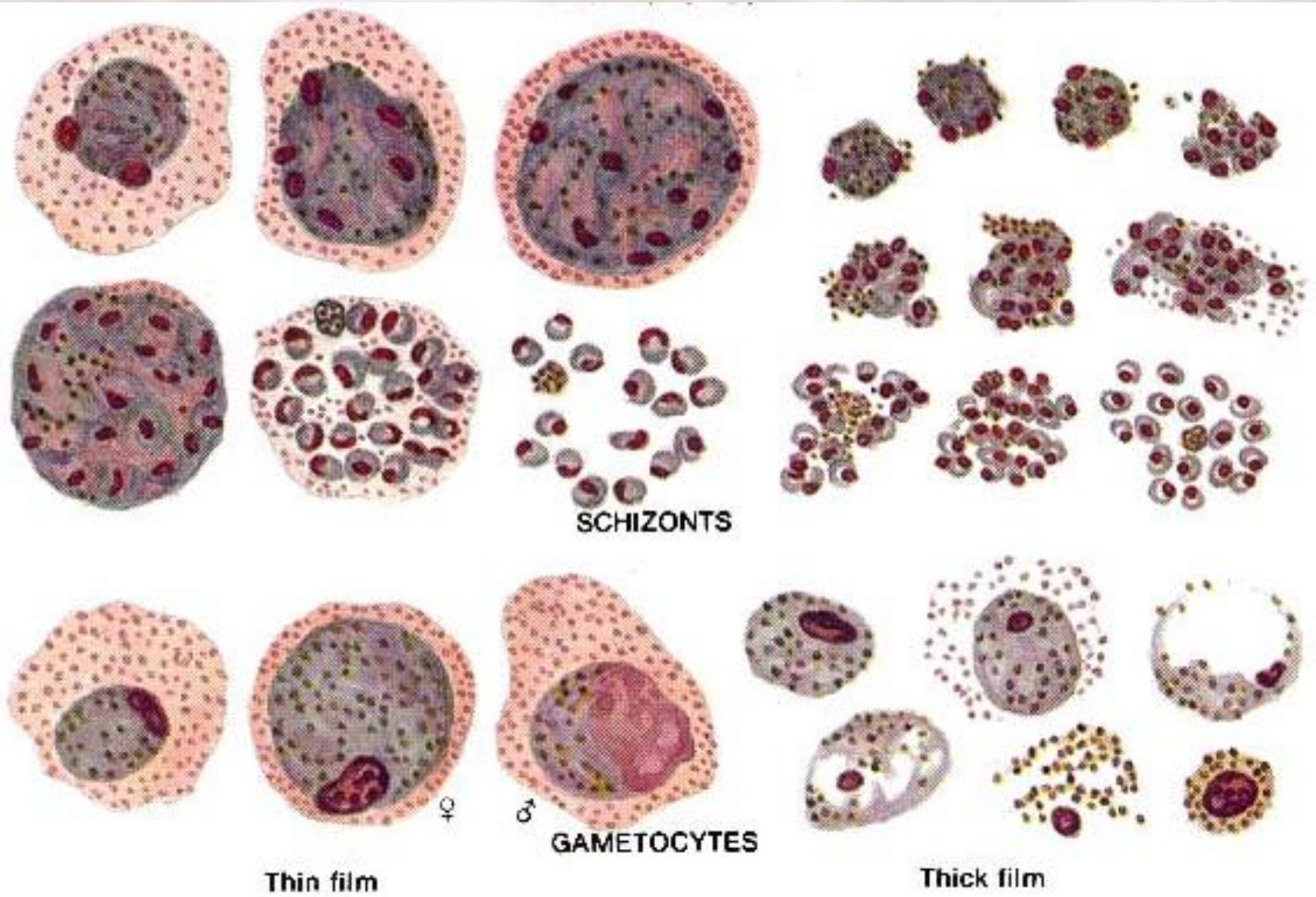








TROPHOZOITES



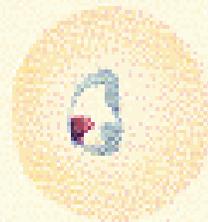
Thin film

GAMETOCYTES

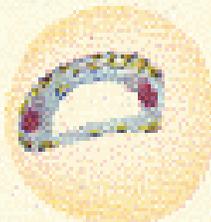
Thick film

پلاسمودیوم مالاریه

P. malariae



ring form



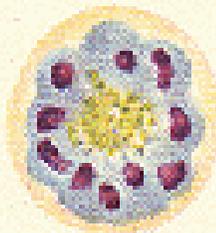
early band form



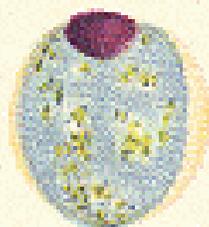
band form



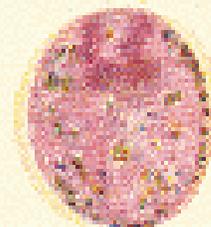
early schizont



mature schizont



female gametocyte

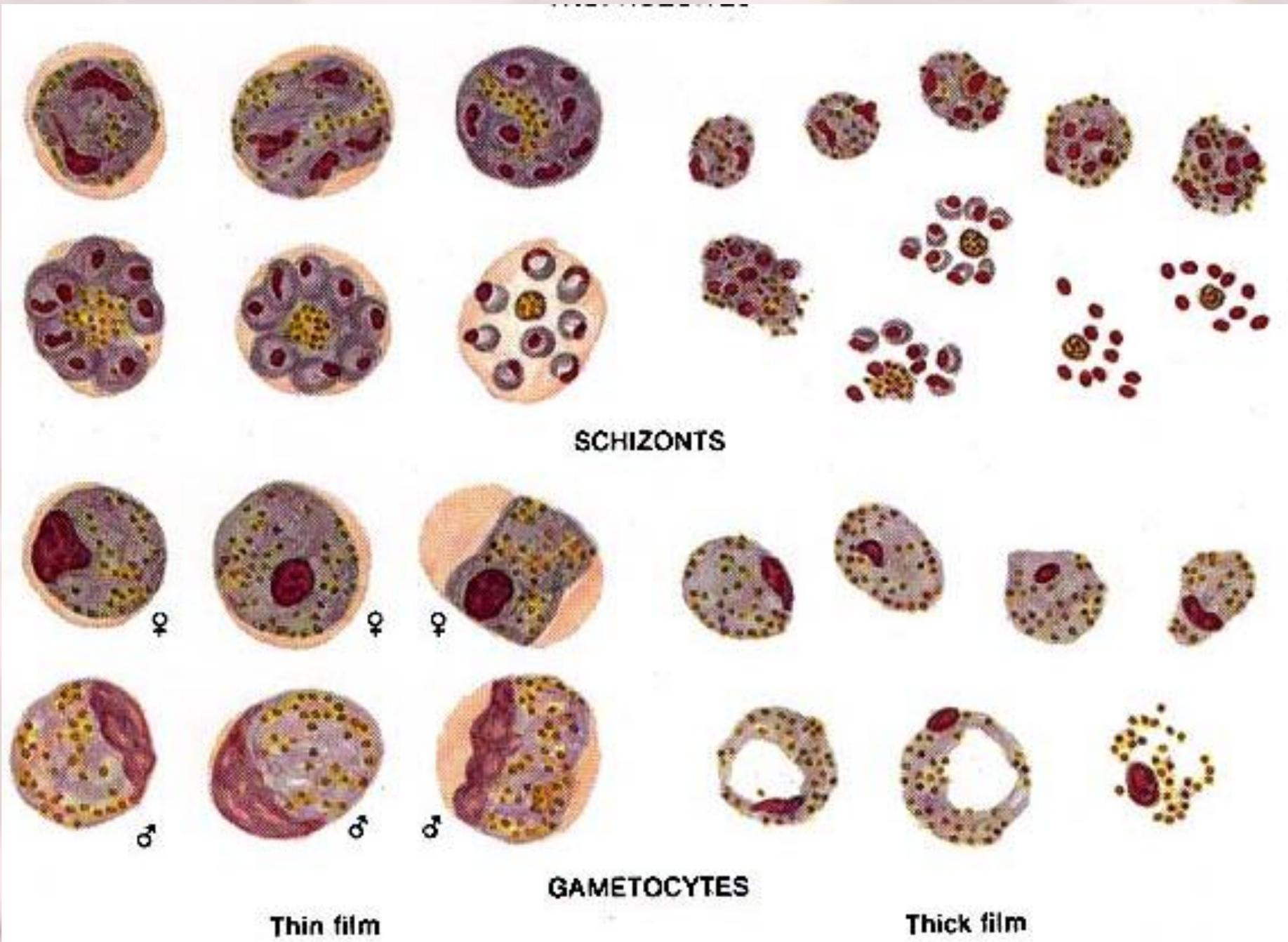


male gametocyte

- تروفوزوئیت های نواری شکل که گاه تمام عرض گلوبول را فرا می گیرد
- شیزونت رسیده گل مینایی دارای 6-12 مروزوئیت
- اندازه گلوبول قرمز آلوده طبیعی و گاه کوچکتر از حد معمول به علت تمایل انگل به سلول های پیر



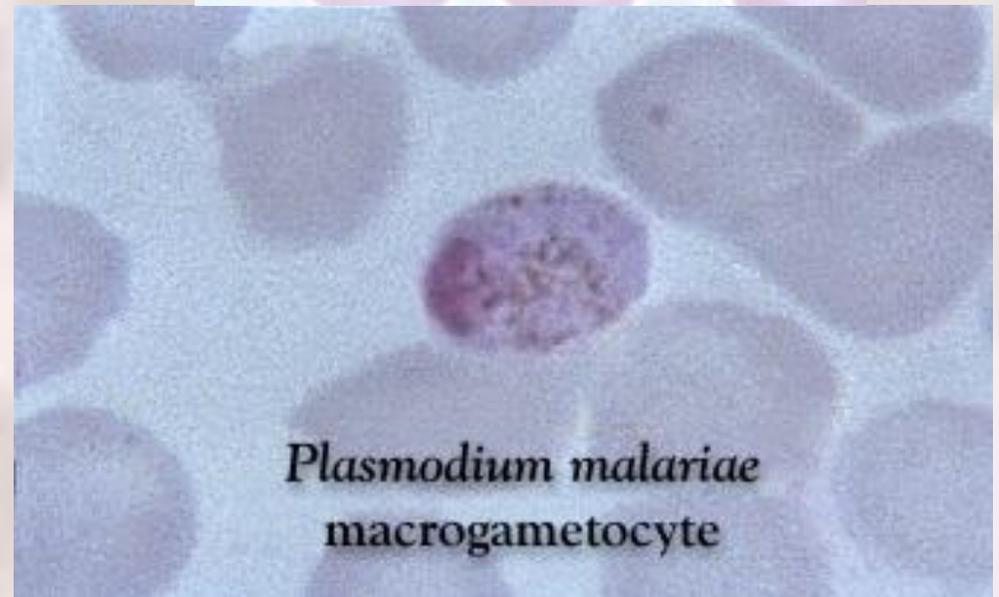
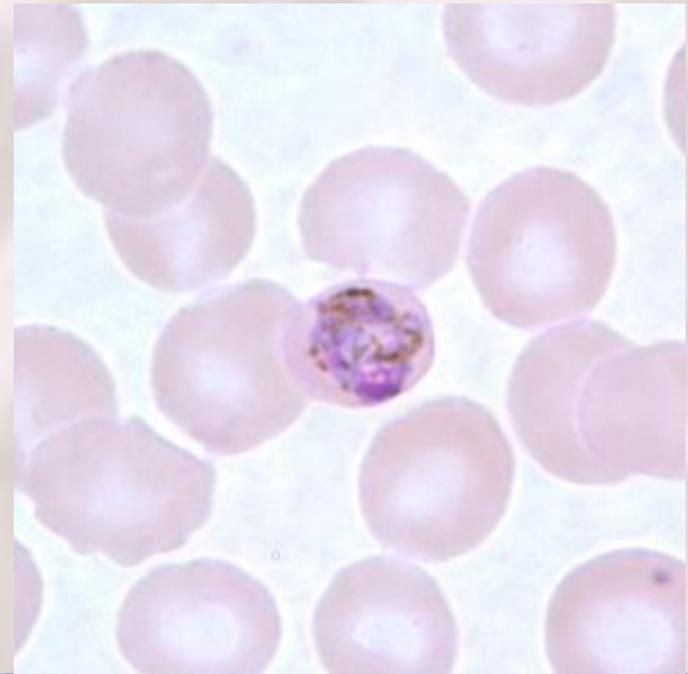
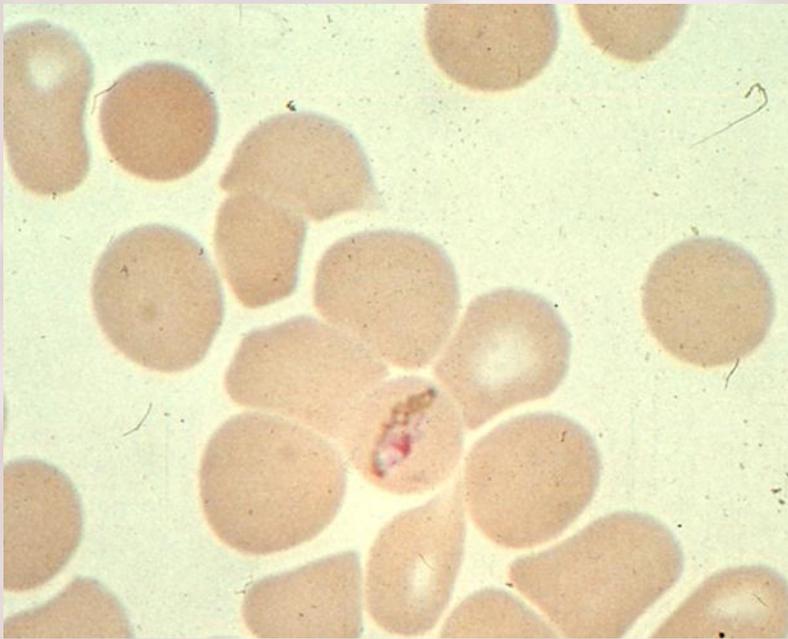
TROPHOZOITES



Thin film

GAMETOCYTES

Thick film

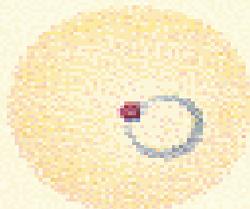


Plasmodium malariae
macrogametocyte

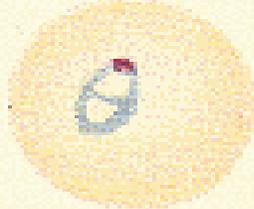
P. ovale



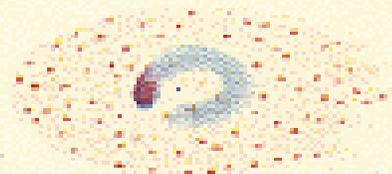
young ring



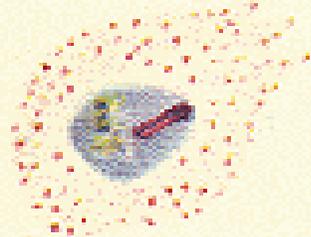
older ring



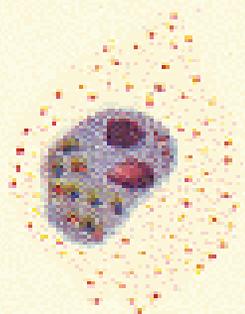
comet form



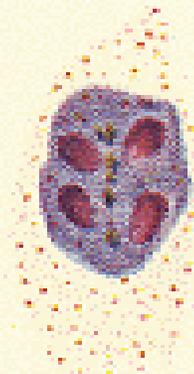
trophozoite



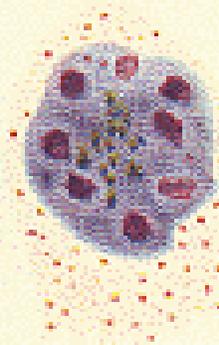
trophozoite



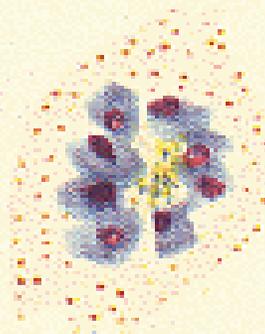
young schizont



schizont



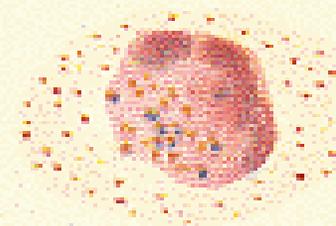
mature schizont

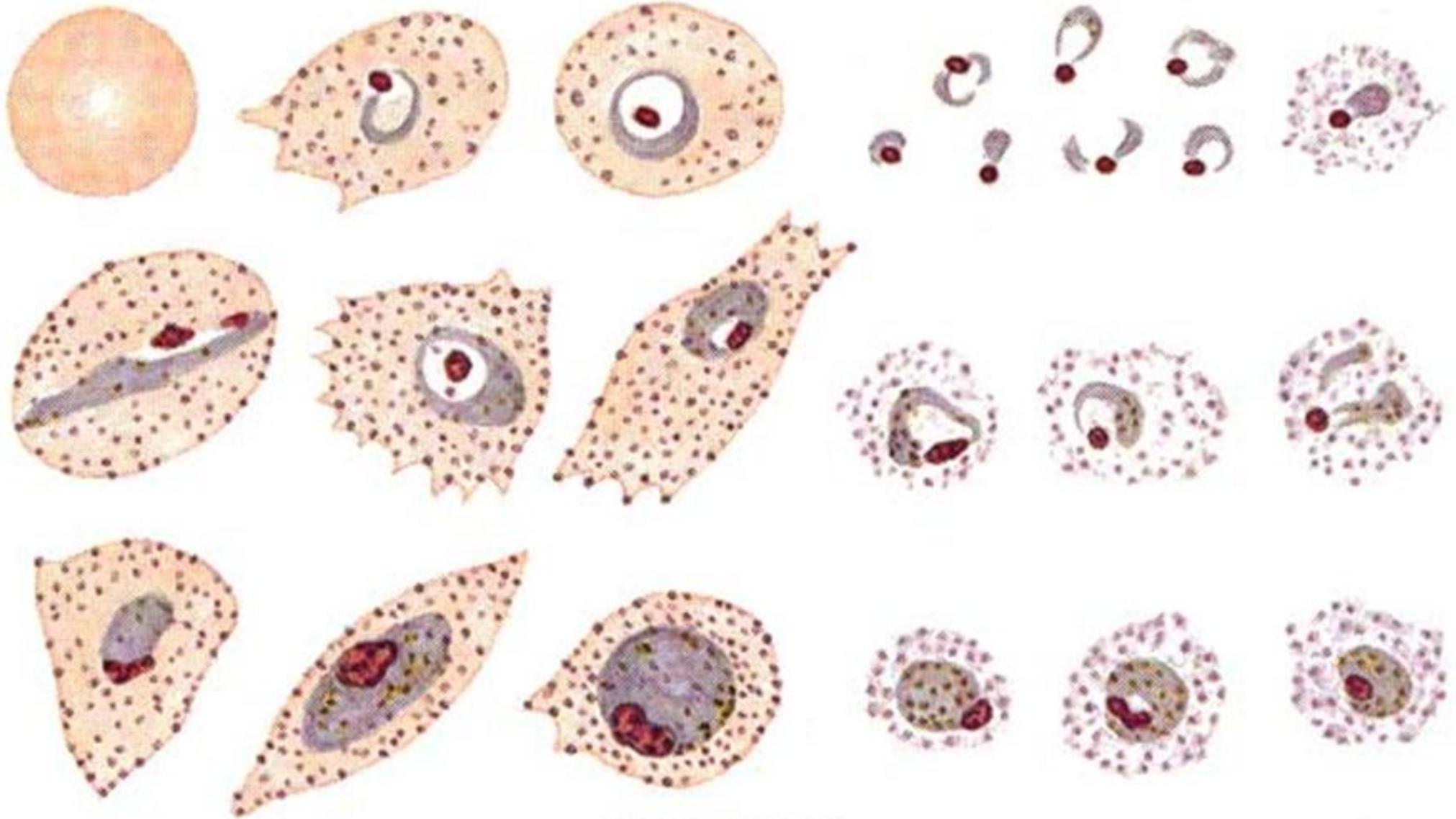


female gametocyte

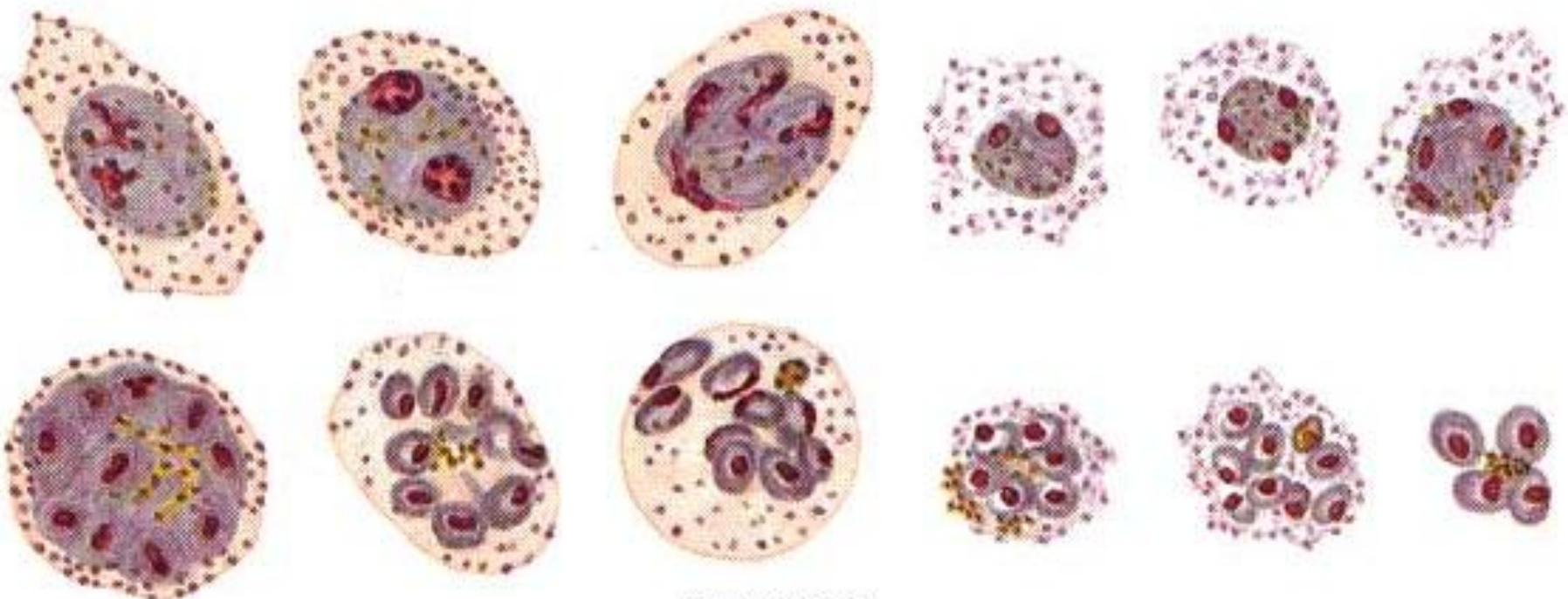


male gametocyte

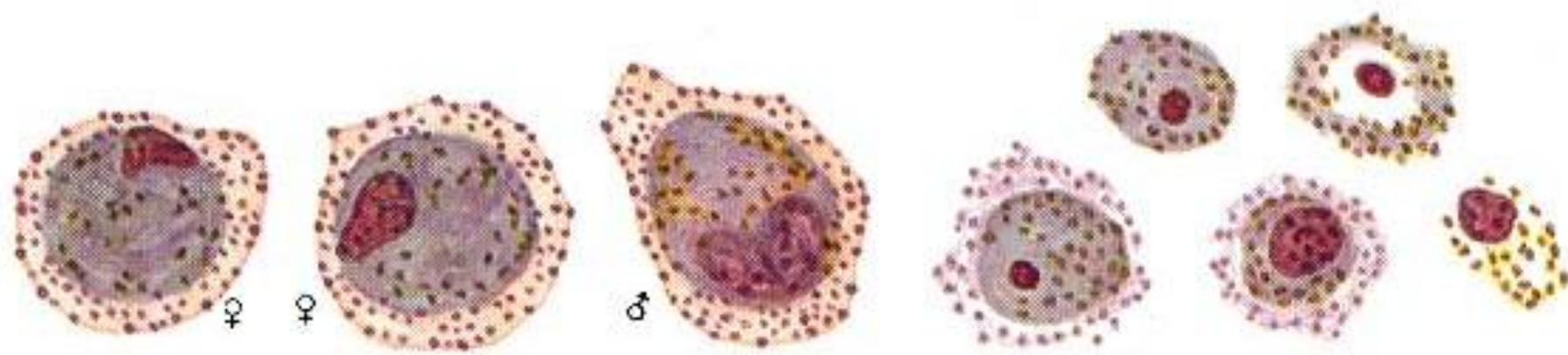




TROPHOZOITES



SCHIZONTS



GAMETOCYTES

Thin film

Thick film

Examining the thin film

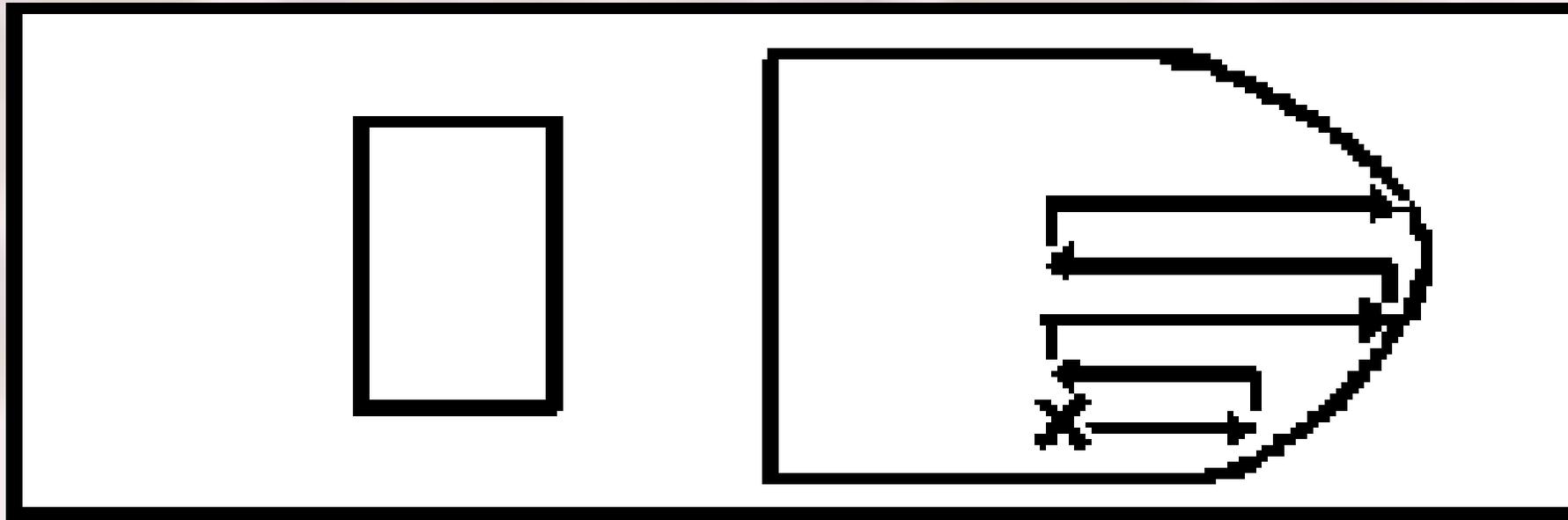
Since it takes almost 10 times as long to examine a thin film as to examine a thick film, routine examination of thin films is not recommended. Only a very few could be properly examined in a day's work.

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

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

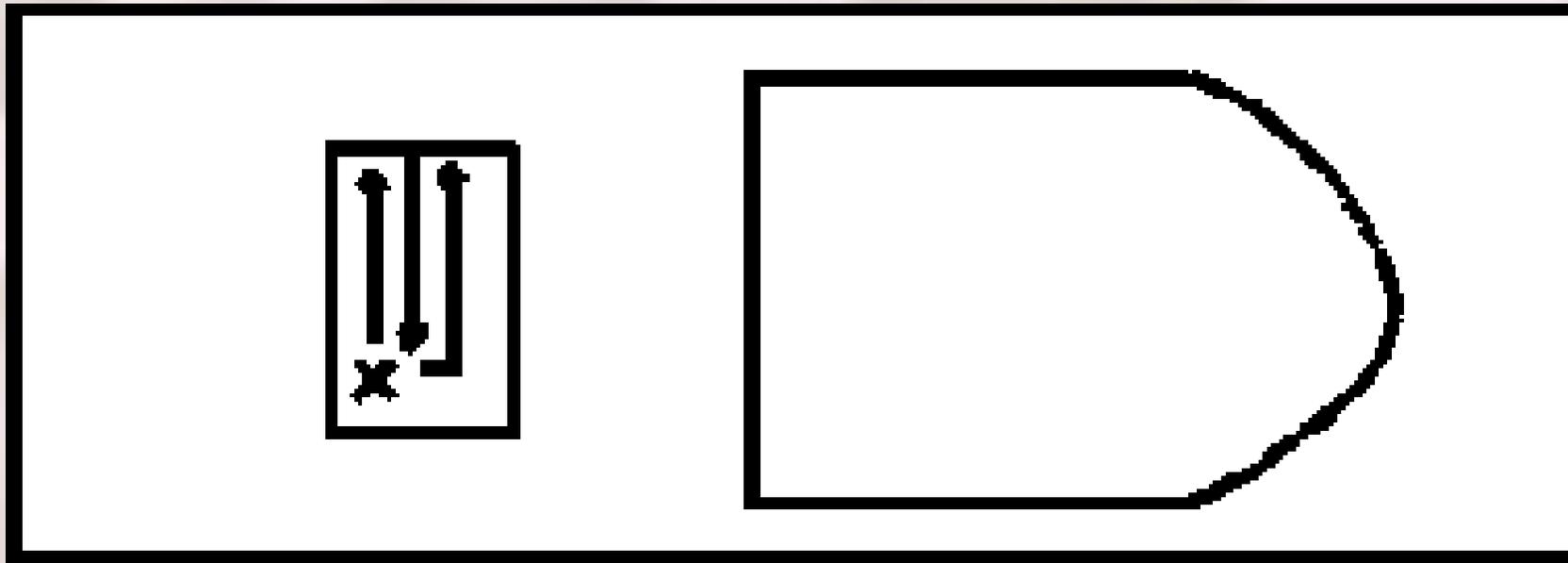
a systematic, standard way:

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



Examining the thick film

- I. Routinely, it is thick blood films that are examined.
- II. Routine examination of a thick film is based on examination of 100 good fields.
- III. This ensures that there is little possibility of a mixed infection (more than one species present in the blood film) being overlooked.
- IV. Place immersion oil on the thick film.



Establishing a parasite count

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

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

Method 1: parasites per microlitre of blood

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

Step 1

- (a) If, after 200 leukocytes have been counted, 10 or more parasites have been identified and counted, record the results on the record form in terms of the number of parasites per 200 leukocytes.
- (b) If, after 200 leukocytes have been counted, 9 or fewer parasites have been counted, continue counting until you reach 500 leukocytes on your tally counter; then record the number of parasites per 500 leukocytes.

Step 2

- In each case, the number of parasites relative to the leukocyte count can be converted to parasites per microlitre of blood by the simple mathematical formula:

$$\frac{\text{number of parasites} \times 8000}{\text{number of leukocytes}} = \text{parasites per microlitre}$$

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

Method 2: the plus system

- A simpler method of counting parasites in thick blood films is to use the plus system. This system is less satisfactory, however, and should be used only when it is not possible to carry out the more acceptable count of parasites per microlitre of blood. The system entails using a code of between one and four plus signs, as follows:

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

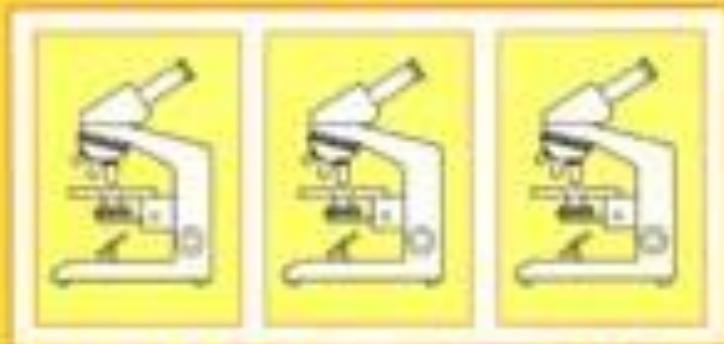
QBC technique

- staining parasite deoxyribonucleic acid (DNA) in micro-hematocrit tubes with fluorescent dyes, e.g. acridine orange, and its subsequent detection by epi-fluorescent microscopy.
- finger-prick blood is collected in a hematocrit tube containing acridine orange and anticoagulant. The tube is centrifuged at 12,000 g for 5 min and immediately examined using an epi-fluorescent microscope
- Parasite nuclei fluoresces bright green, while cytoplasm appears yellow-orange.
- Although the QBC technique is **simple, reliable, and user-friendly**, it **requires specialized instrumentation**, is **more costly** than conventional light microscopy, and is **poor at determining species and numbers of parasites**.

Rapid diagnostic tests (RDTs)

- containing specific anti-malaria antibodies; they do not require laboratory equipment. Most products target a *P. falciparum*-specific protein, e.g. histidine-rich protein II (HRP-II) or lactate dehydrogenase (LDH).
- verall, RDTs appears a highly valuable, rapid malaria-diagnostic tool for healthcare workers; however it must currently be used in conjunction with other methods to confirm the results, characterize infection, and monitor treatment.

Basic malaria microscopy



Part I. Learner's Guide



World Health Organization
Geneva

