Malaria

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2 nd stage

Medical microbiology II (Parasitology)

University of Basra

Lecture topics

- Introduction
- Epidemiology
- Life cycle
- Morphological features
- Pathogenesis
- Clinical feature
- Diagnosis
- Treatment
- Prevention
- Control

Introduction

- The word “malaria” come from the Italian mala aria, meaning “bad air”. When the term was coined, it was commonly believed that malaria was caused by breathing in bad air

- Protozoal infection caused by genus *Plasmodium* that are transmitted to human by the bite of infected **female anopheles mosquitoes**

- Malaria is the 2nd leading cause of death from infectious diseases in African, after HIV/AIDS.

Introduction

- **Etiology**

Four Plasmodium species are :

1- *P. falciparum* (Malignant tertian malaria)
2- *P. vivax* (Benign tertian malaria)
3- *P. ovale* (Ovale tertian malaria)
4- *P. malariae* (Quartan malaria)

- There are two phases in the life cycle

1- Sexual cycle , which occurs primarily in mosquitoes

2- Asexual cycle , which occurs in humans (the intermediate hosts)

➔ Two phases :

- Exoerythrocytic (involves infection of live)
- Erythrocytic (involves infection of RBCs)

Epidemiology

- **3.2 billion people (half the world's population) live in areas at risk of malaria transmission in 106 countries and territories**
- **In 2016, malaria caused an estimated 216 million clinical episodes, and 445,000 deaths. An estimated 91% of deaths in 2016 were in the WHO African Region.**

World Health Organization

Epidemiology

Condition that reduce the incidence of malaria

Sickle cell anemia

- Individual with sickle cell trait are partially protected against malaria
- Their RBCs have too little ATPase activity and cannot produce sufficient energy to support the growth of the parasite.

Glucose- 6-phosphate dehydrogenase (G6PD)

- Patient with G6PD deficiency may also be protected against malarial infection to a lesser degree

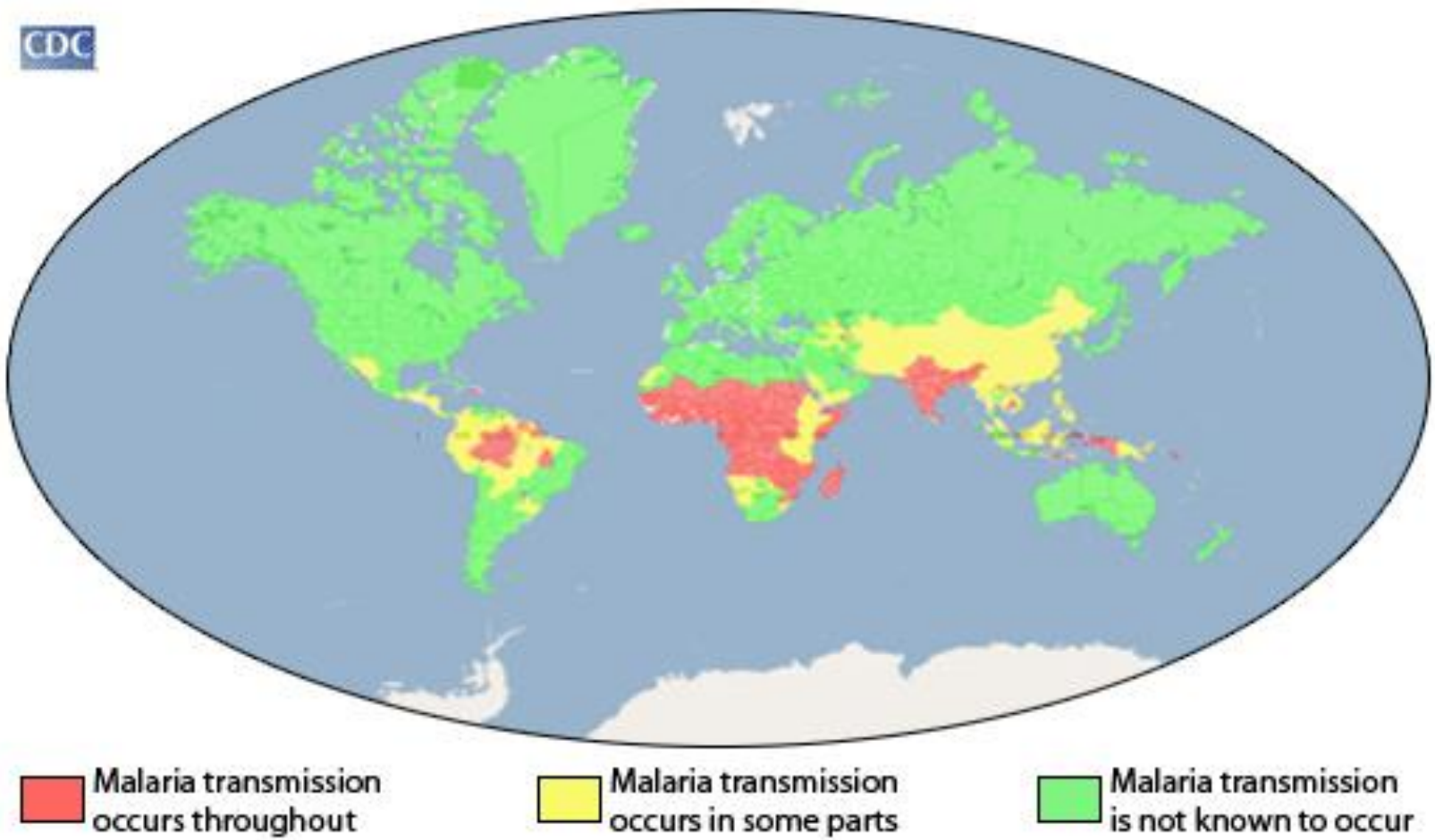
Duffy antigen

- Absence of the Duffy blood group determinants (Fy^a and Fy^b) results in a relative insusceptibility of these individuals to *P. vivax* infection.

Question

Why people with sickle cell trait have less susceptibility to malarial infection?

Epidemiology

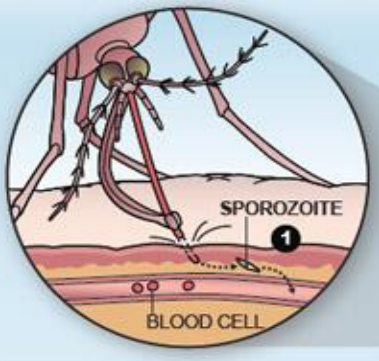


Life cycle

The malaria parasite life cycle involves two hosts. During a blood meal, a malaria-infected female *Anopheles* mosquito inoculates sporozoites into the human host . Sporozoites infect liver cells and mature into schizonts , which rupture and release merozoites . (Of note, in *P. vivax* and *P. ovale* a dormant stage [hypnozoites] can persist in the liver and cause relapses by invading the bloodstream weeks, or even years later.) After this initial replication in the liver (exo-erythrocytic schizogony), the parasites undergo asexual multiplication in the erythrocytes (erythrocytic schizogony). Merozoites infect red blood cells . The ring stage trophozoites mature into schizonts, which rupture releasing merozoites . Some parasites differentiate into sexual erythrocytic stages (gametocytes) . Blood stage parasites are responsible for the clinical manifestations of the disease.

The gametocytes, male (microgametocytes) and female (macrogametocytes), are ingested by an Anopheles mosquito during a blood meal . The parasites' multiplication in the mosquito is known as the sporogonic cycle . While in the mosquito's stomach, the microgametes penetrate the macrogametes generating zygotes . The zygotes in turn become motile and elongated (ookinetes) which invade the midgut wall of the mosquito where they develop into oocysts . The oocysts grow, rupture, and release sporozoites , which make their way to the mosquito's salivary glands. Inoculation of the sporozoites into a new human host perpetuates the malaria life cycle .

The Life Cycle of Malaria

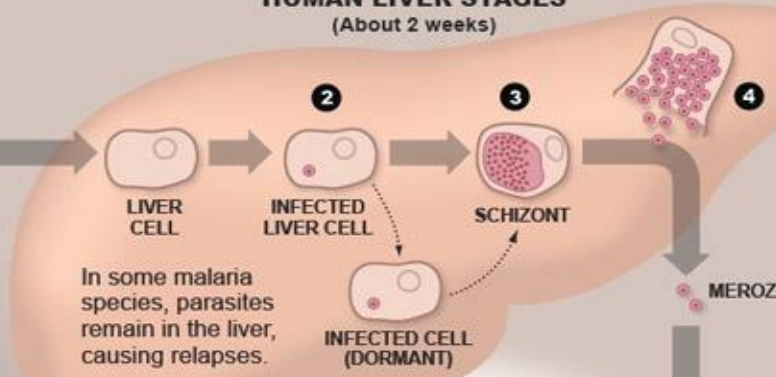


1. To start the cycle, an infected female *Anopheles* mosquito injects sporozoites into the skin while feeding.
2. Sporozoites enter the blood stream and are carried to the liver, where they infect liver cells.
3. Within liver cells, the parasites develop into schizonts.
4. The schizonts rupture, releasing thousands of individual merozoites into the bloodstream.

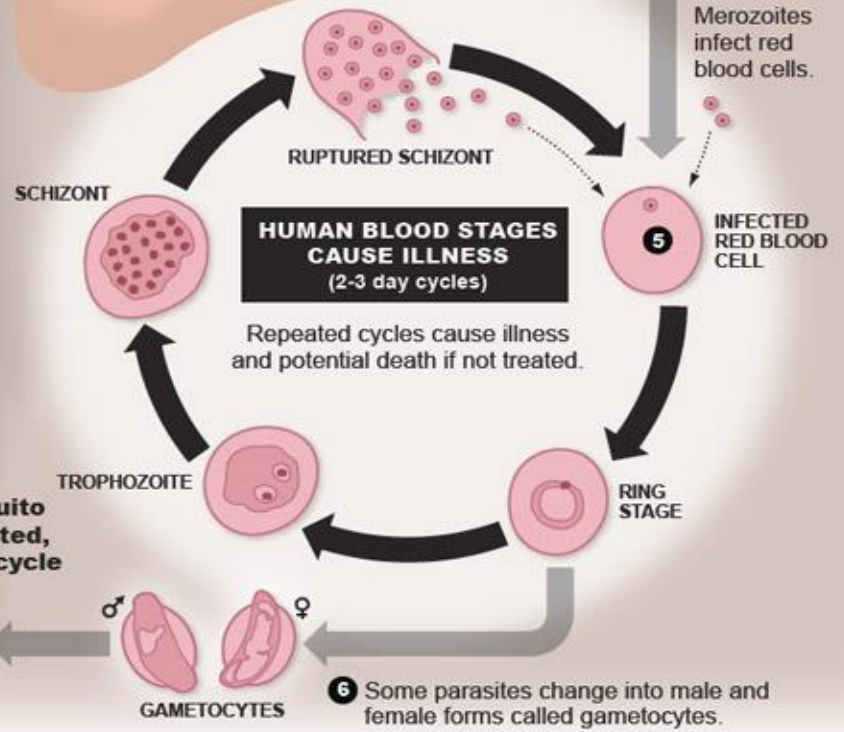
An infected mosquito starts the cycle

SPOROZOITES

HUMAN LIVER STAGES
(About 2 weeks)



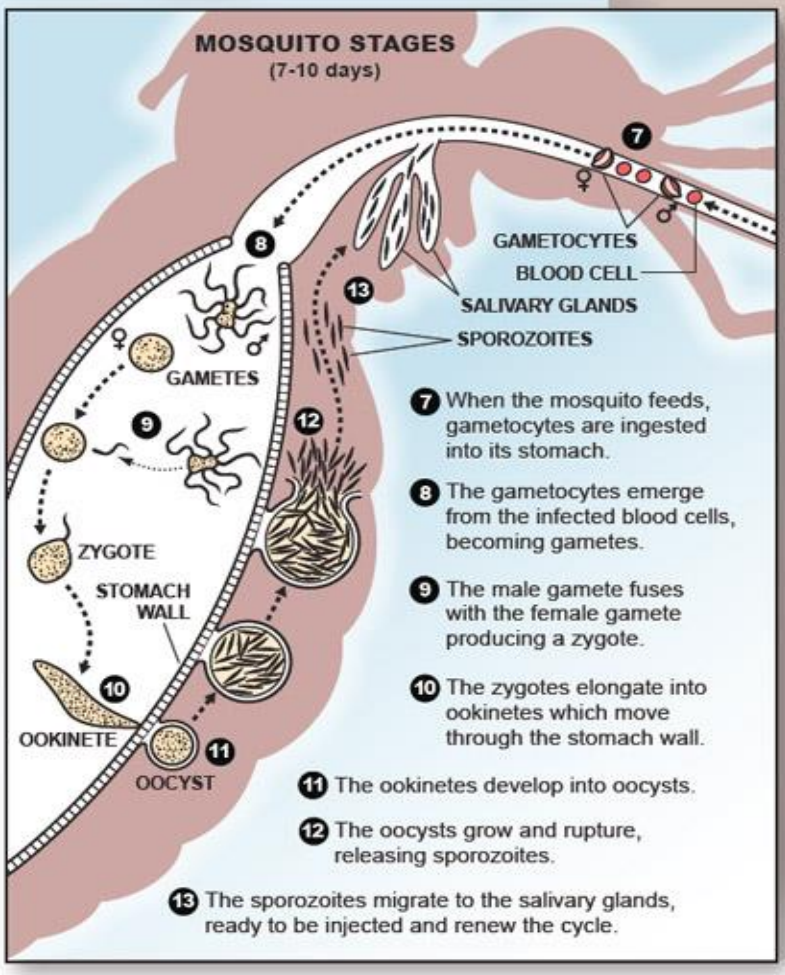
5 Merozoites infect red blood cells.



6 Some parasites change into male and female forms called gametocytes.

Another mosquito becomes infected, continuing the cycle

MOSQUITO STAGES
(7-10 days)



7 When the mosquito feeds, gametocytes are ingested into its stomach.

8 The gametocytes emerge from the infected blood cells, becoming gametes.

9 The male gamete fuses with the female gamete producing a zygote.

10 The zygotes elongate into ookinetes which move through the stomach wall.

11 The ookinetes develop into oocysts.

12 The oocysts grow and rupture, releasing sporozoites.

13 The sporozoites migrate to the salivary glands, ready to be injected and renew the cycle.

The Carter Center / Graphic by Al Granberg

Life cycle

To view video
press here

Animated life cycle of *Plasmodium spp.* in the insect vector and human

Question

Why does fertilization of gametocytes occur only in mosquito(not in human) ?

Pathogenesis

Pathogenesis of Malaria in Tissues and Blood

Beatrice Autino[1§*], Yolanda Corbett[2*], Francesco Castelli[1,3] and Donatella Taramelli[2]

- The pathogenicity of malaria is related to the erythrocytic infection
- As the number of parasites increases ➔ the number of erythrocytes is decreased :-
- Inside RBCs : Trophozoites feed on hemoglobin(partial metabolism) ➔ forming the malarial pigment ➔ darkening (spleen , brain)

- Rupture of the parasitized cells
- Lysis of non-parasitized cells

➔ Normocytic and normochromic anemia

Free in blood stream : -

- The debris of the ruptured cells
- Parasite (merozoite)
- Parasite by produce

➔ Pyrogens stimulate chemoreceptors of the temperature –regulating mechanism of the host to conserve heat

➔ The characteristic chills and fever of a malarial attack

Pathogenesis

- *Plasmodium falciparum* is the species responsible for fatal malaria due to :
  - It invades erythrocytes of all ages
  - Tendency for more than one parasite to develop in a single erythrocyte
  - Invade erythrocyte accumulated and adhere to the lining blood vessels ➔ blockage in vital areas (brain, lungs, and kidneys)➔ Ischemia
- Spleen: enlarged (congestion, hemorrhage)
- Liver: enlarged (hypertrophy, congestion)
- Bone marrow: (congestion, hemorrhage)

Clinical feature

Malaria

Kathryn N. Suh, Kevin C. Kain, Jay S. Keystone

- **Primary attack (Febrile paroxysm)**

It includes several paroxysms of symptoms of gradually decreasing intensity over a period of 3 weeks:-

P. vivax, p. ovale and P. malariae

- Sudden uncontrollable shivering followed by
- High grade fever > 40 °C accompanied by headache, muscular pains, nausea and vomiting (N/V) , abdominal pain and increased pulse and respiration rates
- Drenching sweat the patient is exhausted but feels marked relief.
- Pernicious symptoms (coma, convulsions, cardiac failure) rarely occur .

P. falciparum

- Initial chill is less pronounced and the fever more prolonged
- Pernicious manifestations are common

Clinical feature Malaria

Nicholas J White, Sasithon Pukrittayakamee, Tran Tinh Hien, M Abul Faiz, Olugbenga A Mokuolu, Arjen M Dondorp

- **Relapse**

- ✓ Several attacks occur after long period of remission (> one year)
- ✓ *P. vivax and P. ovale* ➔ due to persistence of exoerythrocystic development (dormant parasite)

- **Recrudescence**

- ✓ Renewal of clinical manifestation after long period of remission without re-exposure
- ✓ *P. Falciparum and P. malariae* ➔ due to persistence of parasites in the blood at levels too low to be detected or to produce symptoms

Clinical feature

Malaria

Kathryn N. Suh, Kevin C. Kain, Jay S. Keystone

- **Complications in malaria**

✓ *Mainly due to P. Falciparum* infection *:*

1- Cerebral malaria

- Congestion and anoxia of brain ➔ Hyperpyrexia , coma , convulsions, Paralysis and death

2- Black water fever :-

- Massive destruction of uninfected RBCs by autoantibodies activated by antigen (newly parasitized or quinine treated RBC)
- Sever chills with rigor, high fever, jaundice, vomiting , rapidly progressive anemia and dark red or black urine (color of cola)

3- Gastrointestinal disorders

4- Vascular collapse and shock (Algid malaria)

5- Malarial hyperpyrexia : Temperature > 42 C

✓ Complication of *P. malariae* include nephrosis ➔ Nephortic syndrome in children

Question

Why is *P. falciparum* more dangerous than other species of malaria ?

Diagnosis

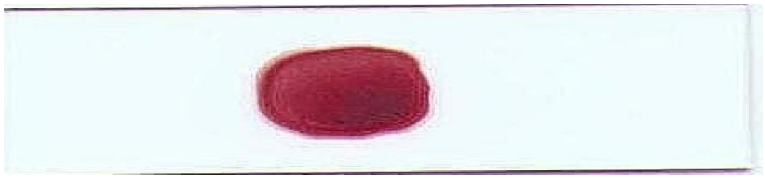
Malaria

Kathryn N. Suh, Kevin C. Kain, Jay S. Keystone

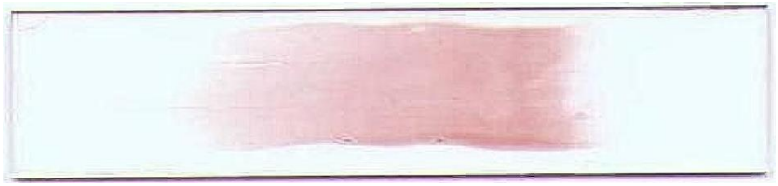
- **Clinical feature are suggested but not diagnosis**
- **Laboratory Diagnosis**
  - ***Microscopic demonstration still the Gold standard in Diagnosis:***

- ✓ Thick and thin film using Giemsa's stain : Thick blood smears are more sensitive in detecting malaria parasites because the blood is more concentrated allowing for a greater volume of blood to be examined ; however , thick smears are more difficult to read

- ✓ Timing of blood smear: Acute stage➔ early or at peak of paroxysmal attack
  Chronic stage ➔ at any time



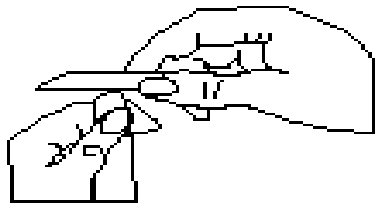
Thick smear used to screen for presence of parasite



Thin smear is used for species identifcation

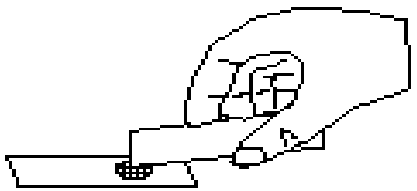
Diagnosis

FIGURE A-2. Preparation of a thin and thick blood film on the same slide



1

Touch the blood drop with a clean slide.

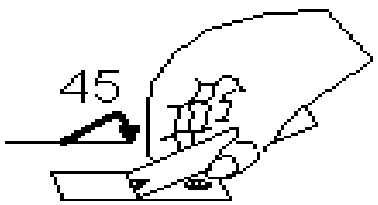


2

Using the corner of another slide, spread the blood drop into the shape of a circle or square of ~1 cm^2.

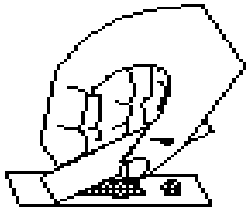
3

Gently squeeze the patient's finger again, and touch the edge of a clean slide to the newly formed blood drop.



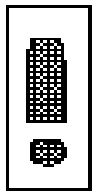
4

Take this slide and hold the edge that has the blood drop at an ~45 angle against the surface of the first slide. Wait until the blood completely spreads along the edge of the second slide.



5

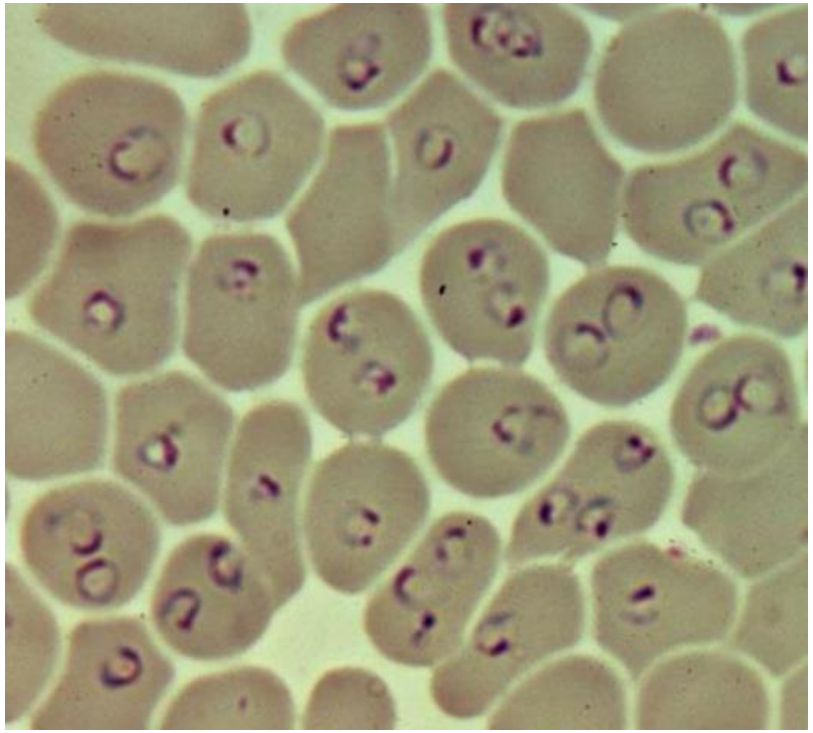
While holding the second slide at the same angle, rapidly and smoothly push the slide forward.



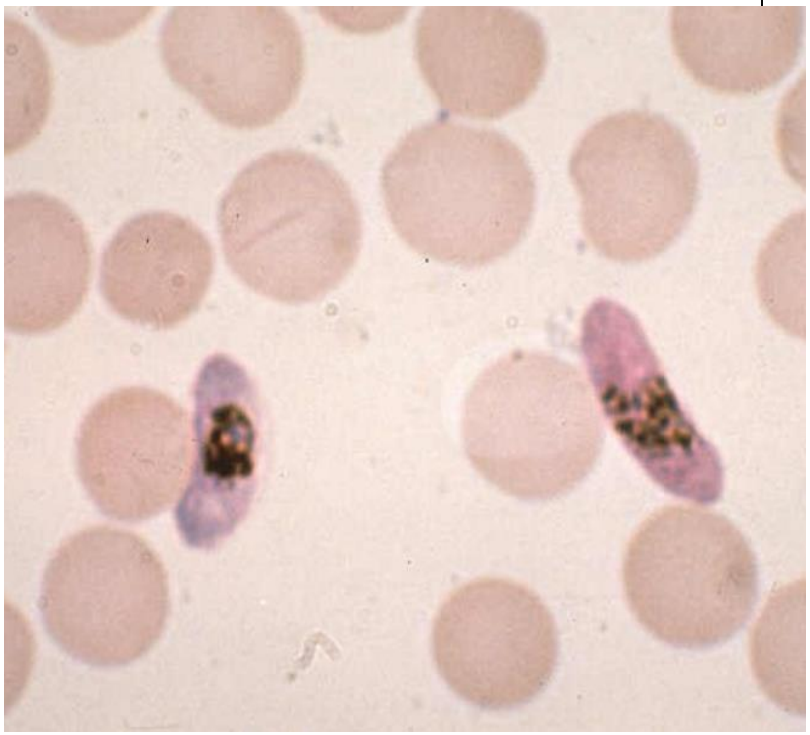
6

Write the identification number on the slide. Wait until the thick film is completely dry before staining it.

Diagnosis



P. falciparum infection of RBCs showing Ring form



P. falciparum infection of RBCs showing gametocytes

Diagnosis

- ***Quantitative Buffy Coat (QBC)***

is a laboratory test to detect infection with malaria or other blood parasites. The blood is taken in a QBC capillary tube which is coated with acridine orange (a fluorescent dye) and centrifuged; the fluorescing parasites can then be observed under ultraviolet light at the interface between red blood cells and buffy coat. This test is more sensitive than the conventional thick smear, however it is unreliable for the differential diagnosis of species of parasite

Procedure:

1-Draw samples of blood (55 µl) in to the QBC tube by capillary action.

2-Rotate the tubes for 10 seconds to dissolve the contained residues in the blood.

3-Insert a close fitting cylindrical insert or plastic float inside a acridine orange-coated capillary tube.

4-Centrifuge the tubes at 12,000 g for 5 minutes.

After gentrification blood components and malaria parasites separate based on density, and concentrate in distinct layers

5-Insert the centrifuged QBC Malaria test into the Previewer. Position the tube so the closure end extends over the depressed area of the holder.

Diagnosis

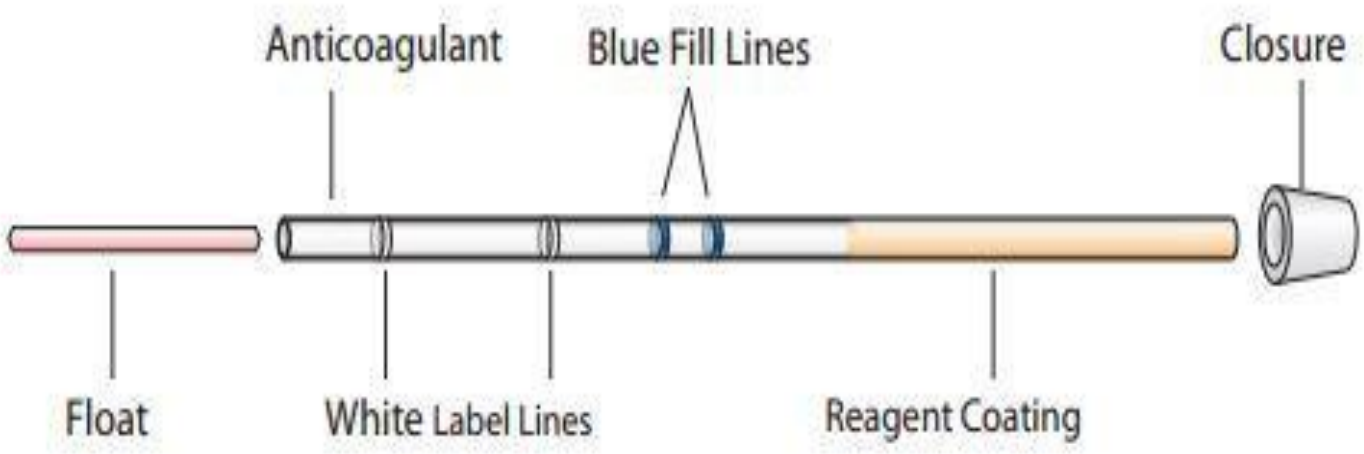
- ***Quantitative Buffy Coat (QBC)*** (Cont.)

Procedure:

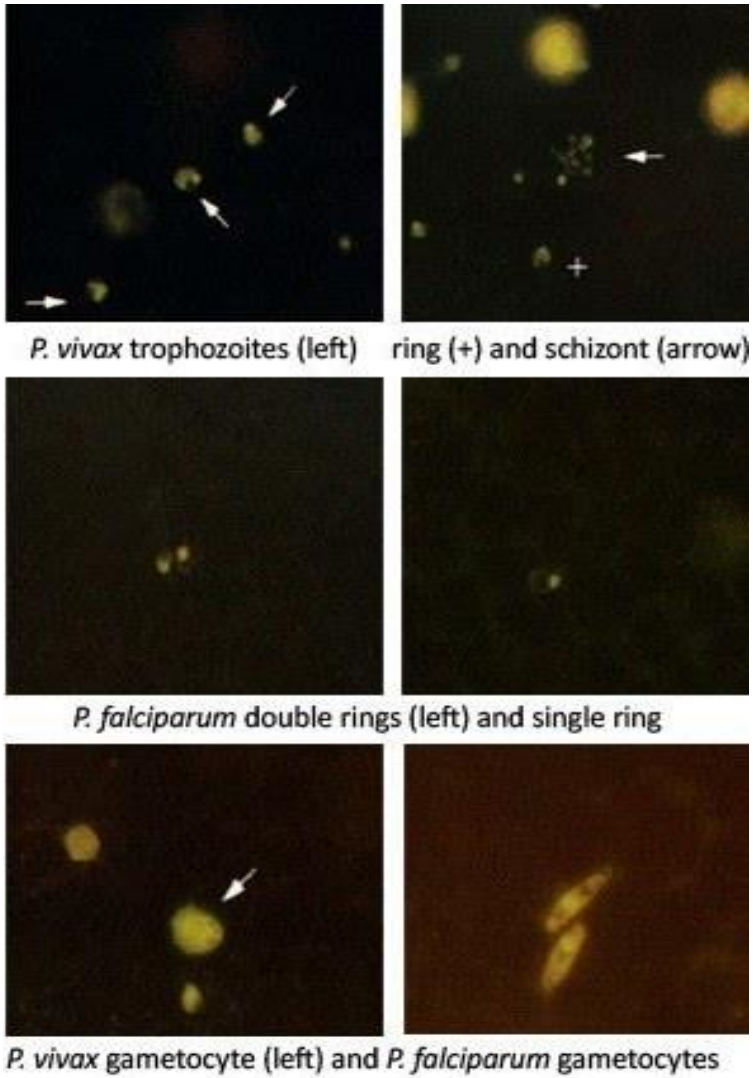
6-The area surrounding the float just beneath the buffy coat was examined under oil immersion. Individual cells within this layer were easily seen by microscopy; the malaria parasites staining green (DNA) and orange (RNA) under blue-violet light.

7-The entire circumference of the tube was examined systematically while moving away from the buffy coat through the erythrocyte layer.

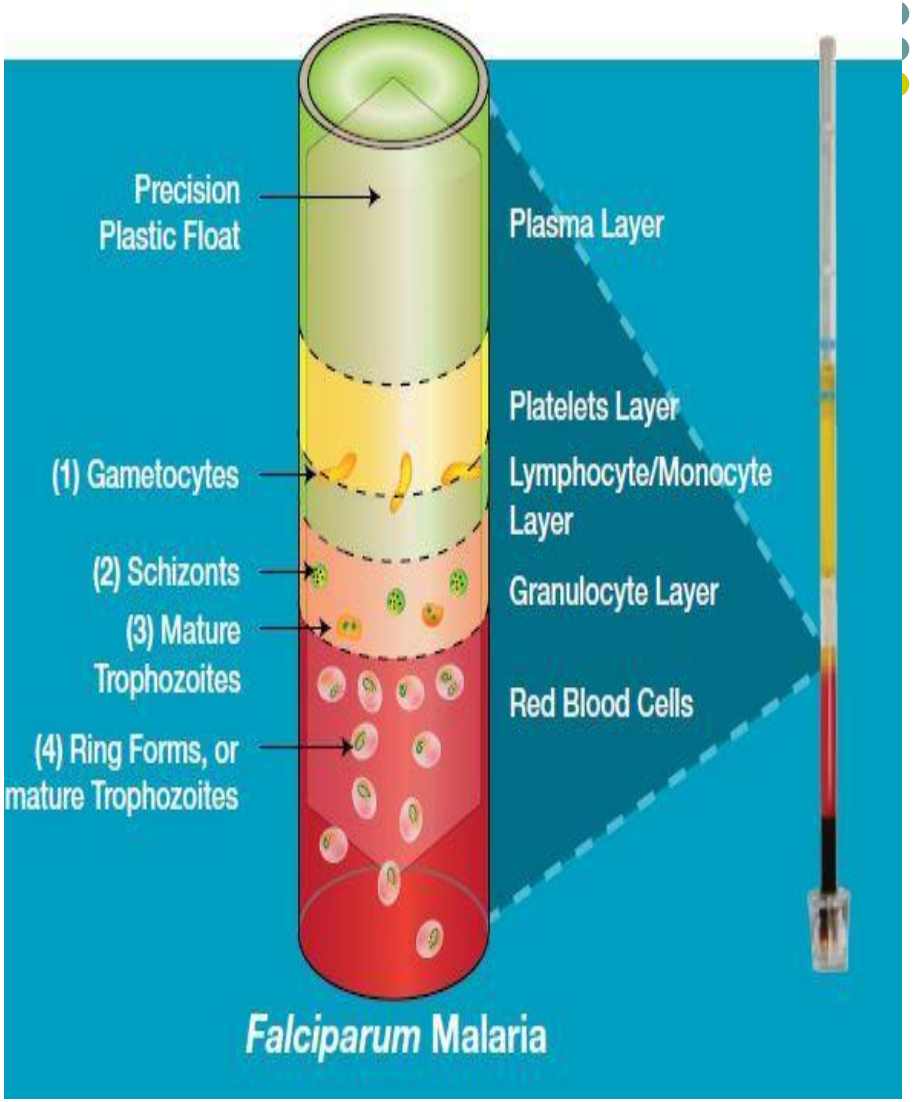
8-Each tube was examined until parasites were detected or for a maximum of 5 minutes.



Diagnosis



Appearance of malarial parasite in QBC system under microscopy



QBC capillary tube

Diagnosis

- ***Antigen detection method***

Various test kits are available to detect antigens derived from malaria parasites. Such immunologic (immunochromatographic) tests most often use a dipstick or cassette format , and provide results in 2-15 minutes. These " Rapid Diagnostic Tests"

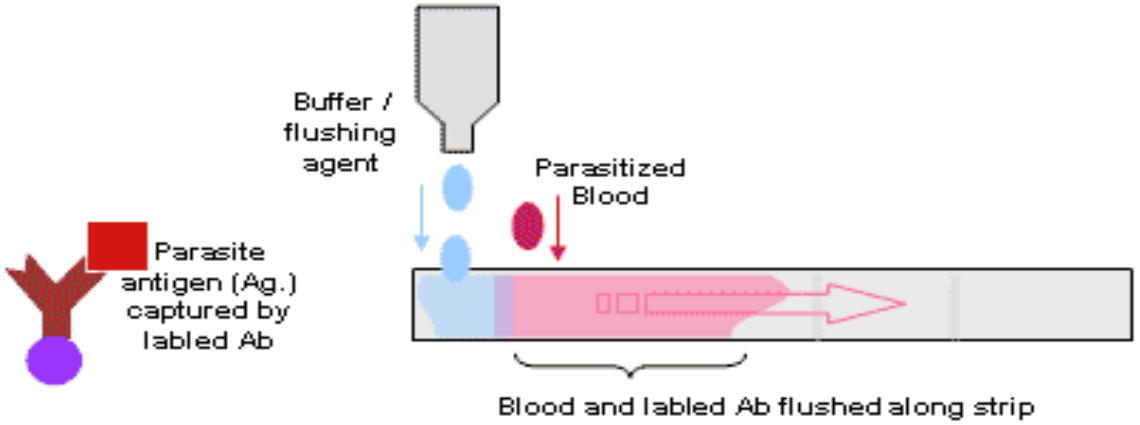
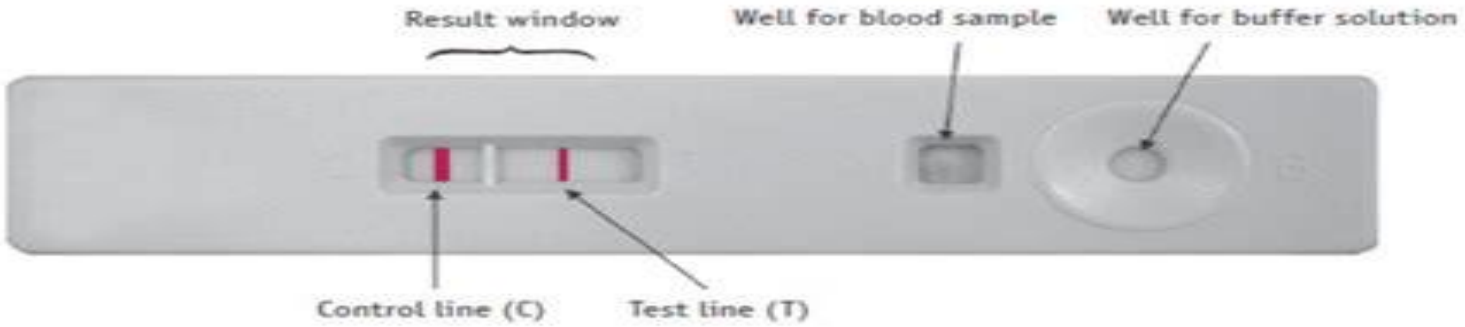


Figure 1. Typical RDT for Malaria Diagnosis



Source: Good practices for selecting and procuring rapid diagnostic tests for malaria. Geneva: WHO; 2011.

Diagnosis

- ***Serology***

Serology detects antibiodies against malaria parasites, using either indirect immunofluorescence (IFA) or enzymes-linked immunosorbent assay (ELISA).

Serology does not detect current infection but rather measures past experience.

- ***Molecular***

Parasite nucleic acids are detected using polymerase chain reaction (PCR). This technique is more accurate than microscopy.

Review Article: Malaria Diagnostics in Clinical Trials

Sean C. Murphy,* Joseph P. Shott, Sunil Parikh, Paige Etter, William R. Prescott, and V. Ann Stewart

Treatment

Malaria

The pharmacology of antimalarials

Kathryn N. Suh, Kevin C. Kain, Jay S. Keystone

Class definition example	Objectives	Drugs
Blood Schizonticidal drugs	Act on (erythrocytic) stage of the parasite thereby terminating clinical illness	Quinine,Artemisinins,Amodiaquine, Chloroquine, Lumefantrine, Tetracycline [a] , Atovaquone, Sulphadoxine, Clindamycin [a] , Proguanil [a]
Tissue schizonticidal drugs	Act on primary tissue forms of plasmodia which initiate the erythrocytic stage. They block further development of the infection	Primaquine, Pyrimethamine, Proguanil, Tetracycline
Gametocytocidal drugs	Destroy sexual forms of the parasite thereby preventing transmission of infection to mosquitoes	Primaquine, Artemisinins, Quinine [b]

[a] slow acting , cannot be used alone to avert clinical symptoms

[b] Weakly gametocytocidal

Dr. Ayad almakki

Treatment

Malaria

Kathryn N. Suh, Kevin C. Kain, Jay S. Keystone

The pharmacology of antimalarials (Cont.)

Class definition example	Objectives	Drugs
Hypnozoitocidal drugs	These act on persistent liver stages of P. ovale and P. vivax which cause recurrent illness	Primaquine, Tafenoquine
Sporozontocidal drugs	These act by affecting further development of gametocytes into oocytes within the mosquito thus abating transmission	Primaquine, Proguanil, Chlorguanil

Treatment

- ✓ Chloroquine

Mechanism of action

1- The parasite digests the host cell's hemoglobin to obtain essential amino acids

2- The process releases large amounts of heme, which is toxic to the parasite

3- To protect itself the parasite ordinarily polymerizes the heme to nontoxic hemozoin, which is sequestered in the parasite's food vaculo

4- Cholroquine prevents the polymerization to hemozoin

5- The accumulation of heme results in lysis of both the parasite and the red blood cell.

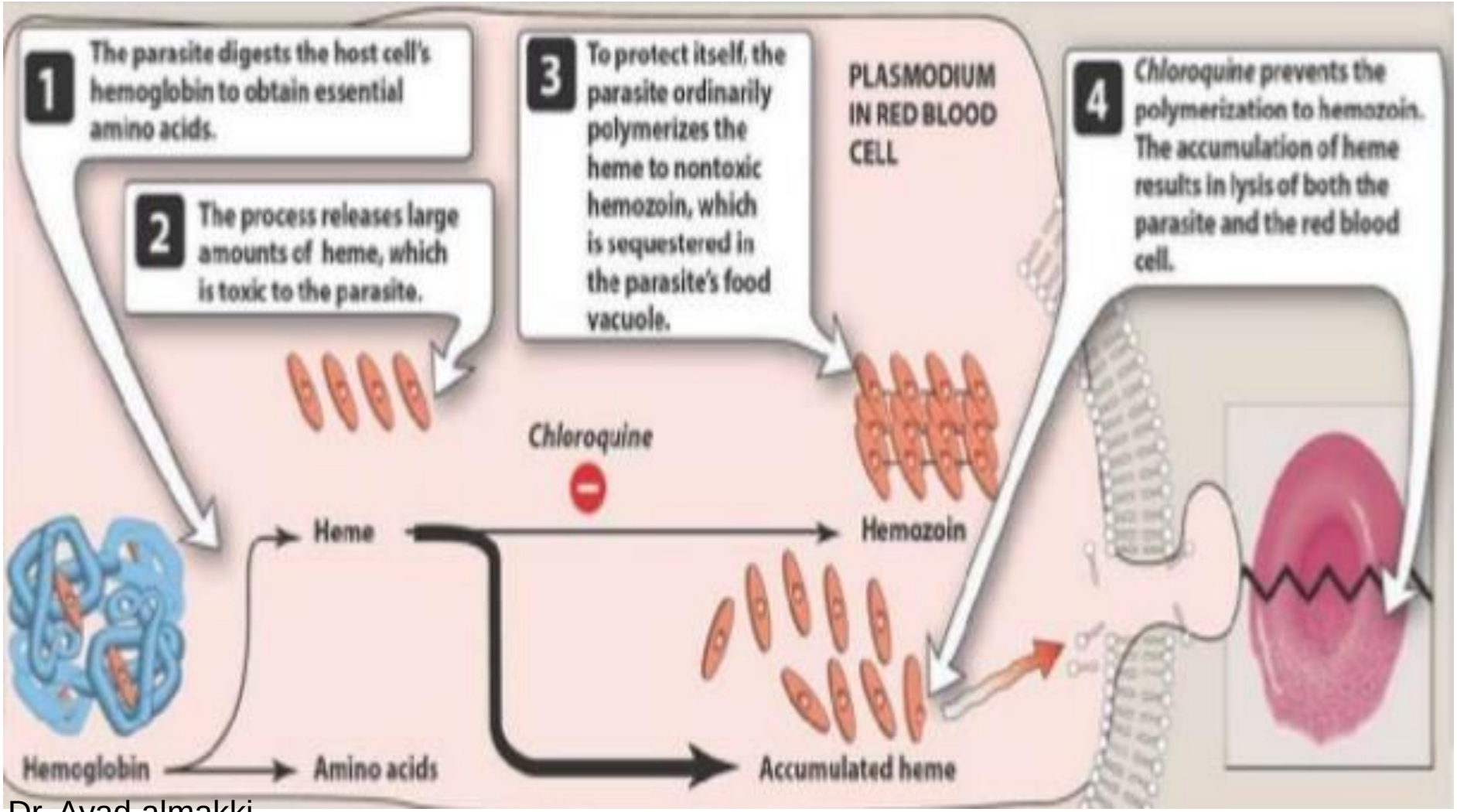
CHLOROQUINE: MECHANISM OF DRUG ACTION AND RESISTANCE IN *PLASMODIUM FALCIPARUM*

ANDREW F. G. SLATER

Treatment

- ✓ Chloroquine

Mechanism of action



Treatment

The Molecular Mechanism of Action of Artemisinin—The Debate Continues

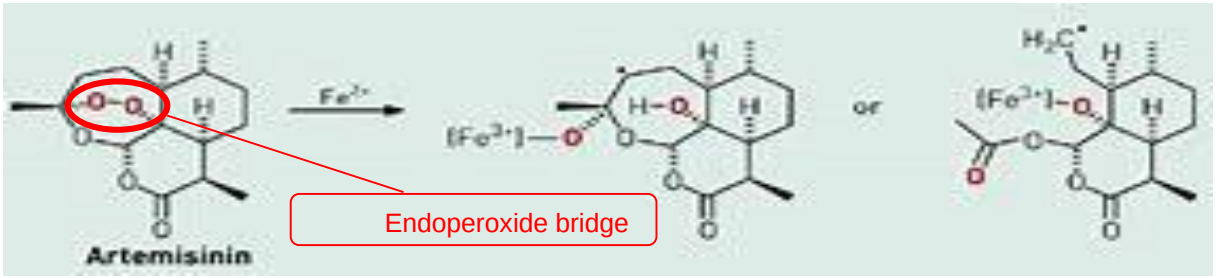
Paul M. O'Neill [1,*], Victoria E. Barton [1] and Stephen A. Ward [2]

✓ Artemisinin derivative

3 more semi-synthetic derivatives that are actually more active than artemisinin itself, it includes :-

1- Artesunate 2- Artemether 3- Arteether

Mechanism of action



In the acid vacuole of parasite, cleavage of endoperoxide bridge of artemisinin compounds by heme iron

⇩

Free radicals generated

⇩

Damage parasite membrane by covalently binding to membrane proteins

⇩

Death of parasite

Treatment

✓ Quinine

- A first-line treatment for malaria, and it should be used only when artemisinins are not available

Mechanism of action

- Same like chloroquine

Pharmacokinetics

- Absorption :- Rapidly absorbed both orally & parenterally
- Metabolism :- liver
- Excretion :- urine

Side effect

Dose dependent toxicity

- Like Cinchonism is a pathological condition caused by an overdose of quinine :-
  - Mild form : Tinnitus , slight reversible impairment of hearing
  - Severe form : vertigo, vomiting, abdominal pain
- Hypotension : Occur if drug is given too rapidly
- Hypoglycemia : I.V. quinine which is due to release of insulin
- Black water fever : a fetal condition in which acute haemolytic anaemia is associated with renal failure(dark urine)

Question

What is the drug used for treating relapse of malaria?

Prevention

Malaria

Kathryn N. Suh, Kevin C. Kain, Jay S. Keystone

- Chemoprophylaxis

- Should be given 1 week before traveling, and continued 4 weeks after leaving
- Depends on the area of travel(ie. Chloroquine resistance or not)

Antimalarial tablets	Adult prophylactic dose	Regimen
Chloroquine resistance high		
Mefloquine	250 mg weekly	Started 2-3 weeks before travel and continued until 4 weeks after
Or Doxycycline	100 mg daily	Started 1 week before and continued until 4 weeks after travel
Or Malarone	1 tablet daily	From 1-2 days before travel Until 1 week after return
Chloroquine resistance absent		
Chloroquine	300 mg base weekly	Started 1 week before and continued until 4 weeks after travel
and proguanil	100-200 mg daily	

Control

- Treatment of human infections with antimalarial drugs
- Elimination of Mosquito breeding places
- National improvements on health and hygiene
- Use of Mosquito nets, treated with Pyrithrin
- Clothing with sleeves, and long trousers
- Use of Mosquito repellents

Malaria

Nicholas J White, Sasithon Pukrittayakamee, Tran Tinh Hien, M Abul Faiz, Olugbenga A Mokuolu, Arjen M Dondorp


Thank you for
your attention