MALARIA

MALARIAL PARASITES

PHYLUM: APICOMPLEXA

Generic character:

- The parasite belonging to this group of protozoa do not possess any special organ of locomotion (cilia/flagella).
- They reproduce *asexually* by *schizogony* followed by sexual union (*syngamy*).

Protozoa infecting the human is divided into two groups:

1. **Intestinal parasite**: Here infection is transmitted by contamination and after sexual union, development of oocysts occur in the faeces in the soil.
2. **Blood inhabiting parasite**: Here infection is transmitted by inoculation and sexual union occurs within the insect host.

PLASMODIUM

Systematic placement:

- **PHYLUM**: APICOMPLEXA
- **SUBORDER**: HAEMOSPORINA
- **GENUS**: PLASMODIUM

GENUS PLASMODIUM

Generic character:

- There are two cycles: *asexual cycle* and *sexual cycle*.
- *The asexual cycle/ schizogony occurs inside RBC of the vertebrate host.*
- *The sexual cycle/ sporogony occurs in an invertebrate host.*
• The product of schizogony is called a merozoite.
• The product of sporogony is called a sporozoite.

Malarial parasites of man: (According to the age of discovery)

1. *Plasmodium malariae*
2. *Plasmodium vivax*
3. *Plasmodium falcioarum*
4. *Plasmodium ovale*

Malarial parasites of primates capable of infecting man:

<table>
<thead>
<tr>
<th>Type of malaria</th>
<th>Species responsible</th>
</tr>
</thead>
<tbody>
<tr>
<td>BENIGN TERTIAN TYPE</td>
<td><em>P. cynomolgi</em></td>
</tr>
<tr>
<td>OVALE TERTIAN TYPE</td>
<td><em>P. simium</em></td>
</tr>
<tr>
<td>QUARTAN TYPE</td>
<td><em>P. inui</em></td>
</tr>
<tr>
<td></td>
<td><em>P. brasilianum</em></td>
</tr>
<tr>
<td></td>
<td><em>P. shortii</em></td>
</tr>
<tr>
<td>QUOTIDIAN TYPE</td>
<td><em>P. knowlesi</em></td>
</tr>
</tbody>
</table>

**MALARIAL PARASITES OF MAN**

General life cycle of all 4 species of plasmodium infecting man:

1. **In Man:**

   Human represents the intermediate host of malarial parasites. In human, the parasite resides inside the hepatocytes and RBC and divides by asexual method (schizogony).

2. **In female anopheles mosquito:**

   Mosquito are the definitive host of malarial parasites. After sexual forms (male and female) develop within human host, they are transferred to the mosquito by bite. Within the mosquito, sexual reproduction takes place and sporozoites are formed.
Malaria infection is transmitted through the following routes:
   a. It may be acquired congenitally from mother to baby across the placenta,
   b. From platelet or blood transfusions and
   c. From the use of shared needles;
   d. It is most frequently initiated with the bite of an infected, female *Anopheles* mosquito, which injects the sporozoite stage of the parasite with its bite.

**PRE-ERYTHROCYTIC SCHIZOGONY**

- After entering into the human body, sporozoites do not directly enter into a RBC, but undergoes a developmental phase inside the host tissue.
- This developmental phase is called *pre-erythrocytic schizogony*, which occurs inside the parenchyma cells of liver.
Duration of malarial parasites in the hepatic stage of development:

<table>
<thead>
<tr>
<th>Name of parasite</th>
<th>Duration of pre-erythrocytic schizony</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. vivax</td>
<td>8 days</td>
</tr>
<tr>
<td>P. falciparum</td>
<td>6 days</td>
</tr>
<tr>
<td>P. ovale</td>
<td>9 days</td>
</tr>
</tbody>
</table>

At the end of this hepatic stage of development, a single sporozoite can develop into a schizont that contains thousands of daughter parasites that fill the hepatocyte. Infected hepatocytes burst and release numerous merozoites into the bloodstream.

After the developmental phase, merozoites are liberated into the circulation. They are then called cryptozoites.

The smaller merozoites (micromerozoites) enter the circulation and the larger ones (macromerozoites) re-enter the hepatocytes.

During this phase, no parasites are found in the circulation and inoculation of such blood does not cause any infection, so, is sterile. No clinical manifestation/pathological damage to liver occurs in this phase.

ERYTHROCYTIC SCHIZOGONY

During this phase, the exo-erythrocytic merozoites from the liver, invade RBCs.

Merozoites of *P. falciparum* can infect RBCs of all ages, whereas those of *P. vivax* and *P. ovale* infect reticulocytes and those of *P. malariae* invade only older RBCs.

<table>
<thead>
<tr>
<th>Malarial parasites</th>
<th>Infected stage of RBC</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. falciparum</em></td>
<td>All stages of RBC</td>
</tr>
<tr>
<td><em>P. vivax / P. ovale</em></td>
<td>Reticulocytes</td>
</tr>
<tr>
<td><em>P. malariae</em></td>
<td>Older RBCs</td>
</tr>
</tbody>
</table>

Shortly after merozoites are released from hepatocytes, they invade RBCs and over a period of 2 or 3 days, develop asexually.

The stages of asexual development include the ring (early trophozoite), trophozoite and schizont stages.

The ring stage derives its name from its signet ring-like appearance, with a blue-stained nucleus and a pink-stained ring of cytoplasm.
• The **trophozoite** is the feeding stage of the parasite and contains a single nucleus with **pigment granules**, called **hemozoin** (*a product of hemoglobin digestion*), located within the cytoplasm of the parasite.

• The **schizont stage** is initiated by the **division of the trophozoite nucleus**. *Each individual nucleus then becomes surrounded by parasite cytoplasm to form a merozoite.* **At maturation, the schizont bursts and releases merozoites into the blood circulation.**

• **These asexual forms may be demonstrated in the circulation by observing thick smear of peripheral blood 3-4 days after completion of pre-erythrocytic schizogony.**

• The parasitic multiplication during the erythrocytic phase is responsible for a clinical attack of malaria.

**GAMETOGONY**

• **Most of the released merozoites re-invade a new erythrocyte, thereby repeating their asexual life cycle (blood stage cycle).**
In some instances, however, invasion of an erythrocyte by a merozoite initiates sexual development instead of asexual development. Thus, merozoites may develop into male gametocytes (microgametocytes) or female gametocytes (macrogametocytes) in the RBCs of capillaries of spleen and bone marrow. These gametocytes mature completely in 4 days. Only mature gametocytes are demonstrable in the peripheral blood. The individual carrying gametocytes are called carriers.

P. falciparum gametocytes (banana shaped)

These gametocytes can develop further only when they are taken up by an appropriate species of Anopheles mosquito during a blood meal.

**LATENT STAGE (HEPATIC)**

After the establishment of clinical infection, the pre-erythrocytic phase completely disappears in *P. falciparum* infection, whereas in *P. vivax* and *P. ovale* infection, it is the dormant phase and is called the “resting phase.” This resting phase of parasite is also known as hypnozoites and they are capable of producing merozoits. This form is now held responsible for relapses of *P. vivax* and *P. ovale* malaria.

**MOSQUITO CYCLE OF PLASMODIUM**

It is the sexual cycle of plasmodium. A female anopheles mosquito, during its blood meal from a human host, ingests both sexual and asexual forms of plasmodium.
• The asexual forms die immediately, but the sexual forms, being capable of developing in mosquito, survive.

• It has been estimated that the blood of human host should contain >12 gametocytes/cu.mm and the blood meal of a mosquito should contain females greater in number than males for infecting a mosquito.

The stage of development is described here:

Development of microgamete

- This phase of development occurs inside the midgut of a mosquito. From 1 microgametocyte, 4-8 thread like filamentous microgametes are formed.*

Formation of zygote (After 20 min- 2 hours of a blood meal of mosquito)

- The microgametes are attracted towards macrogametes by chemotaxis and one of the male gametes attaches to the periphery of a female gamete and penetrates that. Then fusion of male and female pronuclei occurs and zygote is formed.

Formation of ookinete (In the next 24 hours)

- The zygote lengthens and develops into an ookinete. It then penetrates the gut wall and is engulfed by gut mucosal cell. Then it rests on the external border of the cell and basement membrane.

Formation of oocyst

- When the ookinete lies on the external border of gut mucosa and basement membrane, it develops into an oocyst. Oocyst has a single vesicular nucleus and pigment granules. As it matures, it increases in diameter.

Formation of sporozoites

- By the meiotic and mitotic divisions of oocyst, a large numbers of haploid sporozoites develop.

Release of sporozoites

- About 10th day after infection of a mosquito, the oocyst matures fully and ruptures, releasing sporozoites releasing into the body cavity (haemocele)

On the salivary glands

- The released sporozoites are specially directed towards the salivary glands and are concentrated in the ducts. In this stage, a single bite of mosquito can transmit the infection to human.
*In the above chart:
If the process of development of microgametes from microgametocytes is observed in a moist preparation of blood, there will be a special feature: that the microgametocytes will not show any flagellation and only one microgamete will be formed from a microgametocyte. This incident is called **ex-flagellation**.

**STAINING METHOD**

The structures of malarial parasites are best demonstrated by using any of the modifications of Leishman and Giemsa stain.

- Leishman’s stain is prepared by dissolving the dry powder in acetone free pure methyl alcohol in .15% strength.
- Giemsa’s stain is prepared by above procedure but it is made as a readymade watery solution. Here, **fixation with alcohol is necessary**.

**CULTIVATION**

By using **Bass and John’s technique**, only erythrocytic schizogony of one generation can be observed in artificial culture media.

**IMMUNOLOGY OF MALARIA**

**Plasmodial antigens:** They are soluble antigens derived from **asexual erythrocytic phases** and which can be detected from the sera of the infected patient by **Ouchterlony double diffusion precipitation technique**. It can be divided into 3 types:

1. **Labile (L)**- It is degraded by heating at 56°C for 30 minutes. It has been sub classified into La and Lb antigens.
2. **Resistant (R)**- It is stable at 56°C for 30 minutes.
3. **Stable (S)**- It is not degraded after heating at 100°C for 5 minutes.

**Malarial antibodies:** They are of 2 types:

1. **Protective antibodies**- IgG.
2. **Precipitating antibodies**- IgG and IgM.

These antibodies can be secreted in milk and cross placenta and appear in the sera of an infant born from an **immune mother**. This gives a protective immunity to the infant for 6 months.
**Type of immunity:**
- Development of cell mediated and humoral immunity following malarial infection occur with the help of RE cells present in spleen and liver. This can be manifested by disappearance of clinical symptoms despite parasitemia.
- However, this *immunity is active only against free merozoites and mature schizonts*, but *not against gametocytes and hypnozoits*.

**FEATURES OF INFECTED RBCs IN INFECTION BY DIFFERENT SPECIES OF PLASMODIUM**

<table>
<thead>
<tr>
<th>Features</th>
<th>P.falciparum</th>
<th>P.vivax</th>
<th>P.malariae</th>
<th>P.ovale</th>
</tr>
</thead>
<tbody>
<tr>
<td>Characteristics of infected RBC</td>
<td>RBC not enlarged</td>
<td>Schuffner’s dots in cytoplasm of RBC; RBC enlarged</td>
<td>RBC not enlarged</td>
<td>Schuffner’s dots in cytoplasm of RBC; Compact trophozoite; RBC enlarged</td>
</tr>
<tr>
<td>Ring forms</td>
<td>Smaller rings; Multiple rings per cell; Double nuclei; Applique forms</td>
<td>Large rings</td>
<td>Medium sized rings</td>
<td>Large rings</td>
</tr>
<tr>
<td>Trophozoites</td>
<td>Seldom seen in peripheral blood</td>
<td>Parasite “active”; amoeboid shape with pseudopodia</td>
<td>“Band” forms</td>
<td>Compact</td>
</tr>
<tr>
<td>Mature schizonts</td>
<td>Seldom seen in peripheral blood</td>
<td>Schuffner’s dots in cytoplasm of RBC; RBC enlarged</td>
<td>“Rosette” forms</td>
<td>Schuffner’s dots; fewer merozoites per cell; RBC elongated</td>
</tr>
<tr>
<td>Gametocytes</td>
<td>“Crescent” shaped</td>
<td>Round; within enlarged RBC</td>
<td>Round; within non-enlarged RBC</td>
<td>Round; within enlarged RBC</td>
</tr>
</tbody>
</table>
PATHOLOGY OF MALARIA

On completion of erythrocytic schizogony, the following substances are liberated into the blood stream from a mature schizont-

1. Merozoites
2. Pigment granules (the main being haematin)
3. Cytoplasm of infected RBC

Role of pigments in pathology of malaria:

It should be mentioned at first that the term hemozoin is not recommended by WHO. But haematin is more appropriate.

- During erythrocytic schizogony and gametogony, the parasite takes oxygen directly from the oxy-haemoglobin of RBC and the amount of oxygen taken increases with the size of parasite.
- The protein material (globin) of RBC is broken down and resynthesized into parasitic protein.
- The waste product of globin is iron protoporphyrin/haematin, which is accumulated inside the infected RBC as pigment granules.
- These pigment granules, after being liberated in the plasma, are filtered out from the circulating blood by cells of RE system.
- Inside the RE cells, pigments may be found in any proportion.
- So the organs rich in RE cells (liver and spleen) become densely pigmented and assume a colour varying from grey to black. It is the cause of the characteristic pigmentation of organs in malaria.

Other non-specific pigments found in the sera of a patient of malaria:

These pigments are produced when oxy-haemoglobin released from ruptured parasitized RBCs are taken up by RE cells and metabolized. They are not specific for malaria:

1. Iron containing: Haemosiderin.
2. Iron free: Haematoidin.
Oxyhaemoglobin released from lysis of infected RBCS taken up by RE cells and converted into:

- **Haemosiderin (Iron containing)**
  - Converted into bilirubin
  - Excess bilirubin in the blood
    - Giving rise to hemolytic jaundice
    - Van den Bergh Reaction direct delayed positive.

- **Haematoidin (Iron free)**
  - Granales (Oxyhaemoglobin released from lysis of infected RBCS)
  - Haemofuscin (Oxyhaemoglobin released from lysis of infected RBCS)
  - Liver cells changes a major part of it into conjugated bilirubin
    - Eliminated as stercobilin in faeces and urobilin in urine
Pathological changes and other features of malaria:

- Malaria is often classified as uncomplicated or complicated/severe.
- Uncomplicated malaria can be caused by all four species and is characterized by periodic fever and chills, mild anemia and splenomegaly.
- Severe or complicated malaria is almost exclusively caused by *P. falciparum* infections (although occasionally by *P. vivax* and other species) and is associated with higher parasite burdens and vital organ dysfunction including:
  - CNS (coma, seizures etc.),
  - Pulmonary compromise (pulmonary edema, ARDS, respiratory distress etc.),
  - Acute renal failure,
  - Severe anemia and
  - Metabolic acidosis.
- Anemia arises in part from the destruction of erythrocytes when merozoites burst out of the infected RBC and RBC production is further compromised by bone marrow suppression or dyserythropoiesis.
- In falciparum malaria, anemia can be dramatic and life threatening.
- *The rise in temperature is correlated with the rupture of schizonts with release of pyrogens together with merozoites from the bursting infected RBCs.*
- Most malaria deaths are associated with *P. falciparum* infections.
- RBCs infected with the maturing forms of this parasite express parasite proteins called PfEMP-1 associated with morphological structures (“knobs”) that permit them to stick to endothelial cells lining the blood vessels and result in sequestration of these infected RBCs within the vascular bed of vital organs.
- When this occurs in the brain, the resulting cerebral malaria may lead to coma and death.
- Congenital malaria and infection of the placenta may result in stillbirth, low birth weight infants, or perinatal mortality.
- After the initiation of blood stage infection by the parasite, *the repeated infection of erythrocytes by merozoites results in exponential growth.* As a result, the parasitized RBCs accumulate in the capillaries and sinusoids of blood vessels, causing general congestion in the peripheral blood circulation. The congestion causes organomegaly, notably splenomegaly and possibly hepatomegaly.
• In vivax malaria, these processes occur rather acutely and the affected organs, particularly the spleen, become susceptible to rupture following trauma.
• In severe falciparum malaria, the kidneys may show punctate hemorrhages and tubular necrosis. Severe hemolysis and damage in the renal tubules results in hemoglobinuria or in its most severe form “blackwater fever”.

**Blackwater fever**

It is a severe manifestation of Falciparum malaria which occurs in a previously infected subject and is characterized by sudden intravascular hemolysis followed by fever and haemoglobinuria.

**Etiology:** It is most commonly observed in non-immune individuals infected with P.falciparum who had received inadequate doses of quinine.

**Pathogenesis:**
• In falciparum malaria, intravascular hemolysis occurs periodically at the time of schizogony.
• In this time, large amount of RBCs are ruptured and release a huge amount of oxy-haemoglobin into the circulation.
• This stimulates the RE system to form antibodies (like haemolysin).
• So, repeated attacks of malaria produce a hyper-sensitized state, which, upon administration of quinine or by a heavy infection by P.falciparum, release a huge amount of haemolysin into the circulation, causing rapid intravascular hemolysis.

**Morphology of internal organs:**
• Kidneys and spleen are large and dark in colour. (due to congestion and pigmentation)
• Histologically, DCTs are blocked by eosinophilic granular Hb casts.
• Liver is enlarged and soft. It is stained yellow because of the presence of haemosiderin.
• Histologically, necrotic changes are most marked in the central zone of liver lobule.

**Effect of intravascular hemolysis:**
• Methaemalbuminemia: Formed by combining of albumin and haematin.
• Hyperbilirubinemia: Bilirubin formed by RE system.
• Haemoglobinuria: Hb is excreted when haptoglobin can’t bind excess Hb.

**Parasite in blood:**
Practically the parasites are not detected in majority of cases as they are destroyed by intravascular haemolysis.
**Clinical features:** The attack begins with fever and rigor, followed by aching pains in the loins, icterus, bilious vomiting, acute renal failure and circulatory collapse.

**Treatment:**
- If parasites are detected in blood, antimalarial chemotherapy with chloroquine should be immediately instituted.
- Acute renal failure is treated with the help of artificial kidney or by peritoneal dialysis.

**Note:**
- For the survival of malarial parasites inside the RBC, the enzyme G6PD is necessary. So the patients with G6PD deficiency get protection against P.falciparum malaria.
- For the high amount of HbF and HbS, the individuals of Thalassaemia and Sickle cell anemia also confer protection against P.falciparum malaria.
- PABA is also necessary for the metabolism of malarial parasites. So deficiency of PABA in the mother’s breast milk is protective for the children for malaria.

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**-CLINICAL FEATURES OF MALARIA-**

Infection with plasmodia causes intermittent fevers which are known as malaria. Each of the 4 species of plasmodium causes a specific type of fever and malaria as follows:

<table>
<thead>
<tr>
<th>Species</th>
<th>Malaria caused</th>
<th>Incubation period</th>
<th>Cycle of fever and recurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td>P.falciparum</td>
<td>Malignant tertian</td>
<td>10-14 days</td>
<td>48 hours cycle, recurs on every 3rd day. But instead of being intermittent, it may be continuous/ remittent.</td>
</tr>
<tr>
<td>P.vivax</td>
<td>Benign tertian</td>
<td>10-14 days</td>
<td>24 hours cycle, recurs on alternate days.</td>
</tr>
<tr>
<td>P.malariae</td>
<td>Quartan</td>
<td>18-42 days</td>
<td>72 hours cycle, recurs every 4th day.</td>
</tr>
<tr>
<td>P.ovale</td>
<td>Ovale</td>
<td>10-14 days</td>
<td>24 hours cycle, recurs on alternate days.</td>
</tr>
</tbody>
</table>
• After being bitten by a malaria-infected *Anopheles* mosquito, the first symptoms appear after an incubation period ranging from 10 to 42 days.
• Typical symptoms include fever, chills, sweats, rigors, headache, nausea and vomiting, body aches and general malaise. These symptoms are seen in all types of malaria.

**Febrile paroxysm of malaria:**
- The malarial paroxysm starts generally in the **early afternoon** but may start at any time.
- Each paroxysm shows 3 stages:
  1. A **cold stage**: lasting 20 minutes- 1 hour.
  2. A **hot stage**: lasting 1-4 hours.
  3. A **sweating stage**: lasting 2-3 hours.
- *The febrile paroxysm synchronizes with the erythrocytic schizogony of the malarial parasite.*
- *The malaria paroxysm is typically accompanied by sudden shaking chills.* This may last for 10 to 15 minutes or longer.
- *During this stage, the patient complains of feeling extremely cold, despite a steady elevation of body temperature.* Chills may be followed by severe frontal headache and myalgia (muscular pain) in the limbs and back. This stage lasts 2-6 hours in *P. vivax* and *P. ovale* infections, 6 hours or more in *P. malariae* infection and considerably longer in falciparum malaria.
- *Finally, the patient starts to sweat profusely for several hours and usually begins to feel better until the onset of the next paroxysm.*

• Special features of fever caused by different species of plasmodium:

<table>
<thead>
<tr>
<th>Species</th>
<th>Feature of fever</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. falciparum</em> (malignant tertian malaria)</td>
<td>In falciparum malaria, the fever is typically not intermittent, but may be continuous (after the first paroxysm, the temperature may be at the same level) or remittent (after the first paroxysm, the temperature may show a fall), and it sometimes does not show the typical 3 stages of febrile paroxysm.</td>
</tr>
<tr>
<td><em>P. vivax</em> (Benign tertian malaria)</td>
<td>Here the initial pyrexia may be continuous, remittent or quotidian (24 hour cycle), but the characteristic intermittent periodic fever comes only at the later stages.</td>
</tr>
<tr>
<td><em>P. malariae</em> (Quartan malaria)</td>
<td>The fever is of typical quartan variety, that is a 72 hours cycle with recur at each 4th day.</td>
</tr>
<tr>
<td><em>P. ovale</em> (Ovale malaria)</td>
<td>It also produces alternate day fever and is clinically similar to vivax malaria.</td>
</tr>
</tbody>
</table>
Differentiating between relapse and recrudescence

- **Relapsing disease implies the reappearance of parasitemia in sporozoite-induced infection, following adequate antiblood stage therapy.**
- In the case of *P. vivax* and *P. ovale*, the development of exo-erythrocytic forms allows the parasite to remain dormant within the hepatocyte. These dormant parasites are called hypnozoites.
- Accordingly, despite eradication of parasites from the peripheral circulation with conventional antimalarial drugs, a fresh wave of exo-erythrocytic merozoites can emerge from the hepatocytes and reinitiate the infection. The hypnozoites can remain quiescent in the liver for more than five years. *So, in order to achieve radical cure, therefore, it is necessary to destroy not only the blood circulating parasites, but also the hypnozoites.*
- *P. falciparum* and *P. malariae* do not develop hypnozoites and do not cause relapsing disease.
- **Recrudescence is the recurrence of symptoms of malaria after a subclinical or asymptomatic level of parasitemia for a certain period of time.** This recrudescence likely occurs in cases where the blood stages of malaria are maintained at very low levels **after inadequate drug treatment.** Such parasites may become drug resistant. All malaria species can cause recrudescence.
- In the case of *P. falciparum*, the parasites can recrudesce after one or two days, whereas *P. malariae* can do so for up to 30 years.

**Anemia**
After a few paroxysms, a microcytic/ normocytic hypochromic type of malaria develops as a result of destruction of RBCs.

**Splenomegaly**
After a few paroxysms and usually by the 2nd week, the splenic enlargement becomes palpable.
LABORATORY DIAGNOSIS OF MALARIA

MICROSCOPICAL EXAMINATION

• A microscopical examination of a blood film forms one of the most important diagnostic procedures in malaria.

• A careful examination of thin film will show plasmodia provided that no antimalarial drugs have been administered prior to the taking of blood films.

• It is a good practice to examine both thin and thick film of blood, because examination of thick film will help quickly identifying the parasite and then examination of thin film will help identifying the species.

• Difficulty in identifying the species may ensue when only some ring forms are observed. In these cases, the blood should be examined some hours after to reach a diagnosis.

• Difficulties in detecting the malarial parasites when:
  1. Blood films are taken after administration of an antimalarial drug.
  2. In all cases of primary infection, during the first 2-3 days.

RAPID DIAGNOSTIC TOOLS

• These tests are done when microscopic examination of parasites can’t be done.

• The tests are named: Dip-stick test/ Test-strip test.

• These tests are based on detection of antibodies to 2 malarial antigens in the blood: HRP2 (Histidine rich protein 2) and parasite LDH (Lactate dehydrogenase).

• The antibodies are denoted by PfHRP2 and PFLDH respectively.

• PfHRP2 is specific for P.falciparum, and it is cleared from blood very slowly and takes almost 1 month after acute infection.

• PFLDH is nonspecific for all plasmodium species and it is cleared rapidly, within days after initiating the treatment.

Other methods having limited value in diagnosing malaria are:

✓ Cultural methods
✓ Blood counting methods
✓ Serological tests
✓ Immunofluorescence test
✓ Gel precipitation test
✓ Sternal puncture etc.