Toxoplasma gondii & *Plasmodium sp*

Immunological Diagnosis

Parasitology (MA 301)

Medical Analysis Department

Summer School

Lab 4

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Toxoplasma gondii

Introduction

- Toxoplasma gondii is an obligate intracellular coccidian parasite
- Toxoplasma is now recognized as the most common protozoan parasite globally, with the widest range of hosts spread over 200 species of birds, reptiles, and mammals, including humans.

Morphology

T. gondii occurs in three forms:

1. Trophozoite (A)
2. Tissue cyst (B)
3. Oocyst (C)

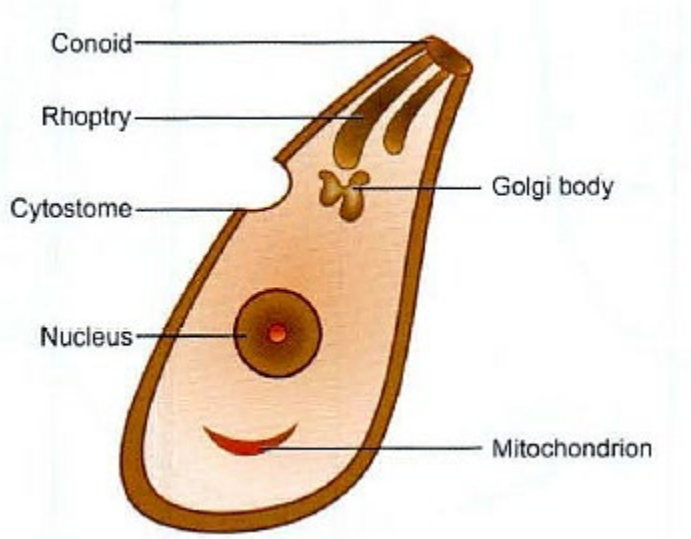
Morphology

- The trophozoite and tissue cyst represent stages in asexual multiplication (schizogony), while the oocyst is formed by sexual reproduction (gametogony or sporogony).
- All three forms occur in domestic cats and other felines, which are the definitive hosts and support both schizogony and gametogony.
- Only the asexual forms, trophozoites and tissue cysts are present in other animals, including humans and birds, which are the intermediate hosts.
- All three forms are infectious to man.

Morphology

Trophozoites (Tachyzoites)

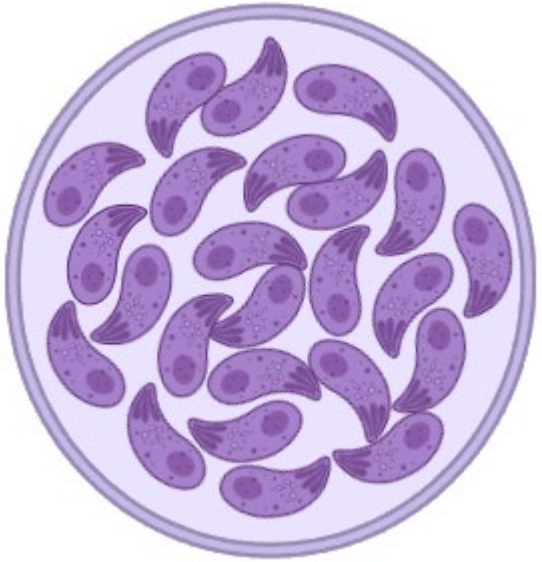
- The trophozoite is crescent-shaped, with one end pointed and the other end rounded.
- It measures 3-7 µm in length. The nucleus is ovoid and is situated at the blunt end of the parasite.
- The rapidly proliferating trophozoites in acute infection are called tachyzoites.

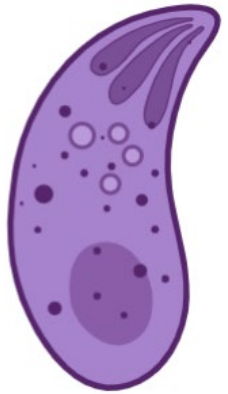


Morphology

Tissue cyst (Bradyzoite)

- Tissue cysts are the resting form of the parasite.
- They are found during chronic stage of the infection and can be found in the brain (most common site), skeletal muscles and various other organs.
- The cyst is round or oval, 10-20 µm in size and contains numerous bradyzoites. Cysts remain viable in tissue for several years.
- In immunologically normal hosts, the cysts remain silent, but in the immunodeficient subjects, they may get reactivated, leading to clinical disease.
- They reach various tissues and organs through blood and lymphatic dissemination.

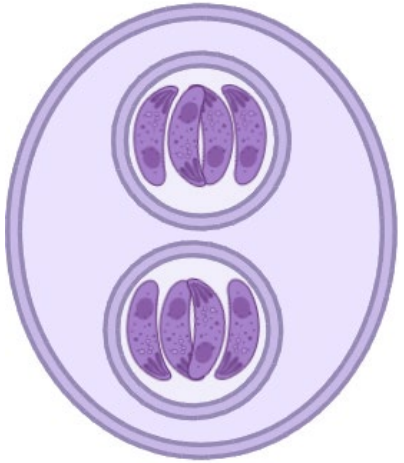




Morphology

Oocyst

- Oocysts develop only in definitive hosts in the intestine of cats and other felines, but not in humans.
- It is oval in shape and measures 10-12 µm in diameter. Each cyst is surrounded by a thick, resistant wall.
- The oocysts are formed by sexual reproduction (gametogony).
- Cats shed millions of oocysts which undergo sporulation in the soil with the formation of two sporocysts, each containing four sporozoites. The sporulated oocyst is infective.
- Oocyst is very resistant to environmental conditions and can remain infective in soil for about a year.
- When the infective oocyst is ingested, it releases sporozoites in the intestine, which initiate infection.

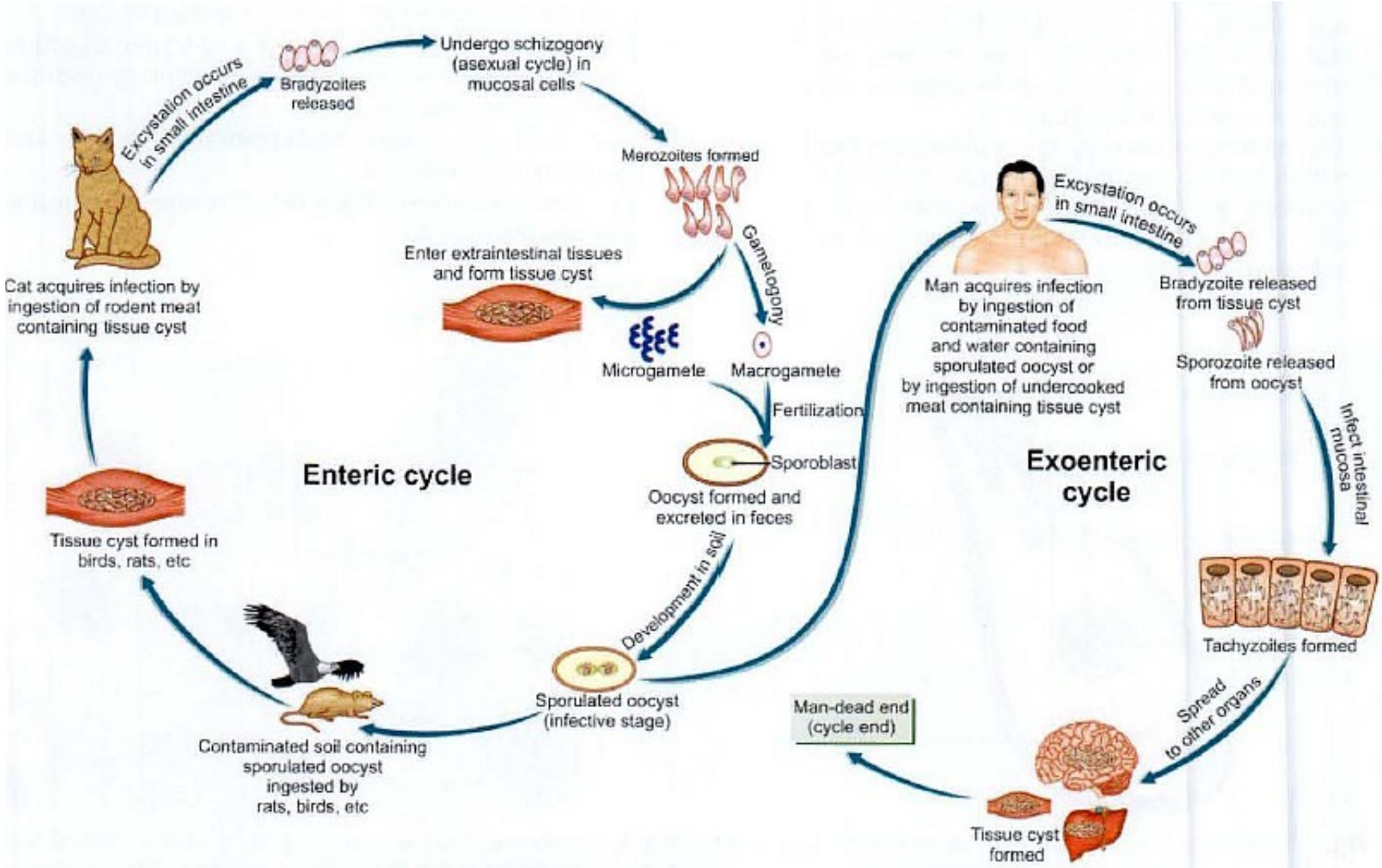


Life cycle

Host: T. gondii completes its life cycle in two hosts.

- Definitive hosts: Cats and other felines, in which both sexual and asexual cycles take place.
- Intermediate hosts: Man and other mammals, in which only the asexual cycle takes place.
- T. gondii has two types of life cycles:

1. Enteric cycle
2. Exoenteric cycle.


Enteric cycle
Cat acquires infection by ingestion of rodent meat containing tissue cyst
Excystation occurs in small intestine
Bradyzoites released
Undergo schizogony (asexual cycle) in mucosal cells
Merozoites formed
Enter extraintestinal tissues and form tissue cyst
Gametogony
Microgamete
Macrogamete
Fertilization
Sporoblast
Oocyst formed and excreted in feces
Development in soil
Sporulated oocyst (infective stage)
Contaminated soil containing sporulated oocyst ingested by rats, birds, etc
Tissue cyst formed in birds, rats, etc
Exoenteric cycle
Man acquires infection by ingestion of contaminated food and water containing sporulated oocyst or by ingestion of undercooked meat containing tissue cyst
Excystation occurs in small intestine
Bradyzoite released from tissue cyst
Sporozoite released from oocyst
Infect intestinal mucosa
Tachyzoites formed
Spread to other organs
Man-dead end (cycle end)
Tissue cyst formed

Laboratory Diagnosis

The diagnosis of acute toxoplasmosis is made mainly by demonstration of trophozoites and cysts in tissue and body fluids and by serology

1. Microscopy

- Tachyzoites and tissue cysts can be detected in various specimens like blood, sputum, bone marrow aspirate, cerebrospinal fluid (CSF), amniotic fluid, and biopsy material from lymph node, spleen and brain.
- Smear made from earlier specimens is stained by Giemsa.

Laboratory Diagnosis

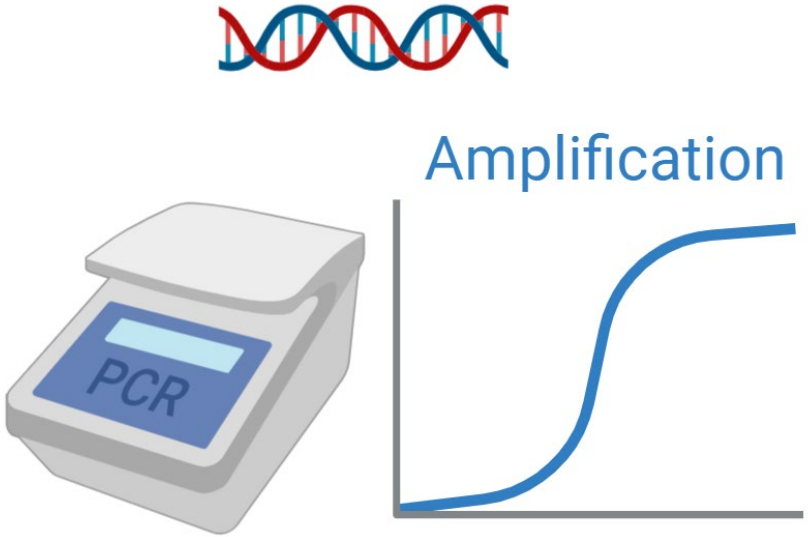
2. Serology

- **Antibody detection:** Diagnosis of acute infection with T. gondii can be made by detection of the simultaneous presence of IgM and IgG antibodies.

Laboratory Diagnosis

3. Molecular Method

- Deoxyribonucleic acid (DNA) hybridization techniques and polymerase chain reaction (PCR) are increasingly used to detect Toxoplasma from different tissues and body fluids.
- B, gene of T. gondii can be detected by PCR of the amniotic fluid in case of congenital toxoplasmosis.

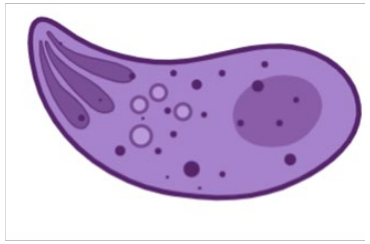


Laboratory Diagnosis

Imaging

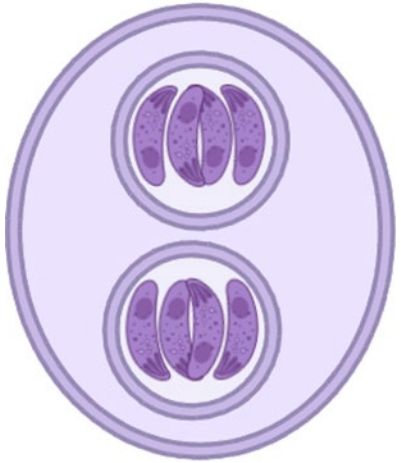
Magnetic resonance imaging (MRI) and computed tomography (CT) scan are used to diagnose toxoplasmosis with central nervous system involvement.

Ultrasonography (USG) of the fetus in utero at 20-24 weeks of pregnancy is useful for diagnosis of congenital toxoplasmosis.

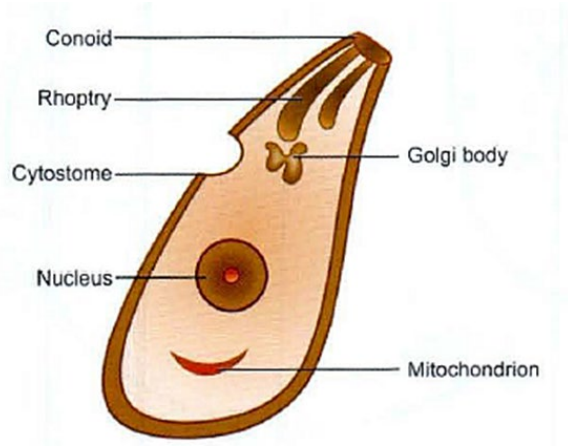


Toxoplasma gondii
Tissue cyst (bradyzoite)

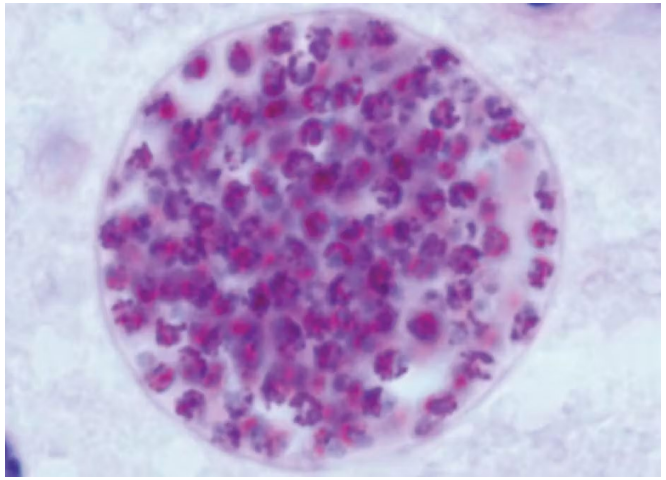
Trichomonas vaginalis
(trophozoite)



Toxoplasma gondii
Oocyst



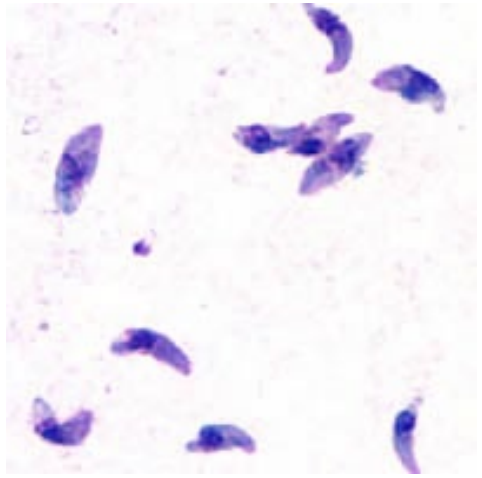
Toxoplasma gondii
Trophozoite (tachuzoite)



Toxoplasma gondii
Tissue cyst (bradyzoite)

Trichomonas vaginalis
(trophozoite)

Toxoplasma gondii
Oocyst



Toxoplasma gondii
Trophozoite (tachuzoite)

Plasmodium sp.

Introduction

- They live intracellularly, at least during part of their life cycle.
- At some stages in their life cycle, they possess a structure called the apical complex, by means of which they attach to and penetrate host cells.
- These protozoa are therefore grouped under the Phylum Apicomplexa.
- The disease they cause is called Malaria

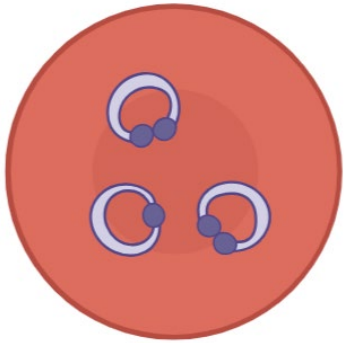
Causative agents of human malaria

- *Plasmodium vivax*: Benign tertian malaria
- *Plasmodium falciparum*: Malignant tertian malaria
- *Plasmodium malariae*: Benign quartan malaria
- *Plasmodium ovale*: Benign tertian malaria.

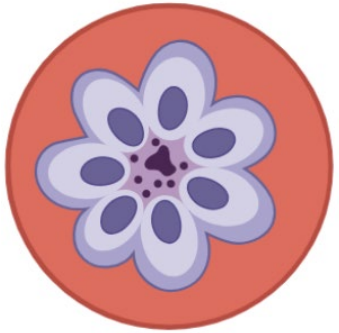
Morphology

The followings are the stages of plasmodium sp,

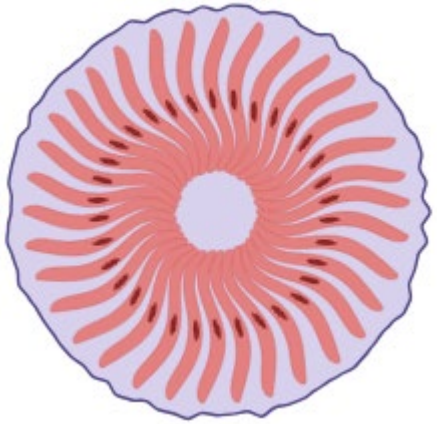
Sporozoite



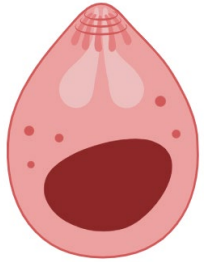
Ring stage



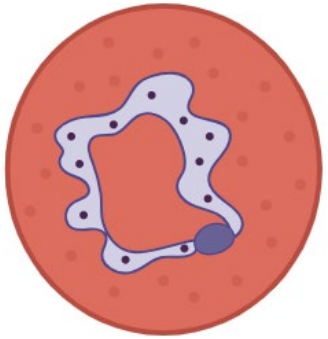
Schizont



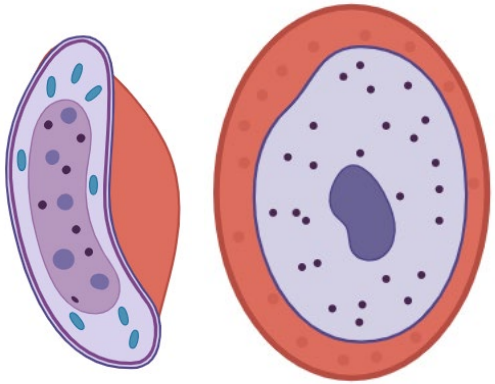
Oocyst



Merozoite

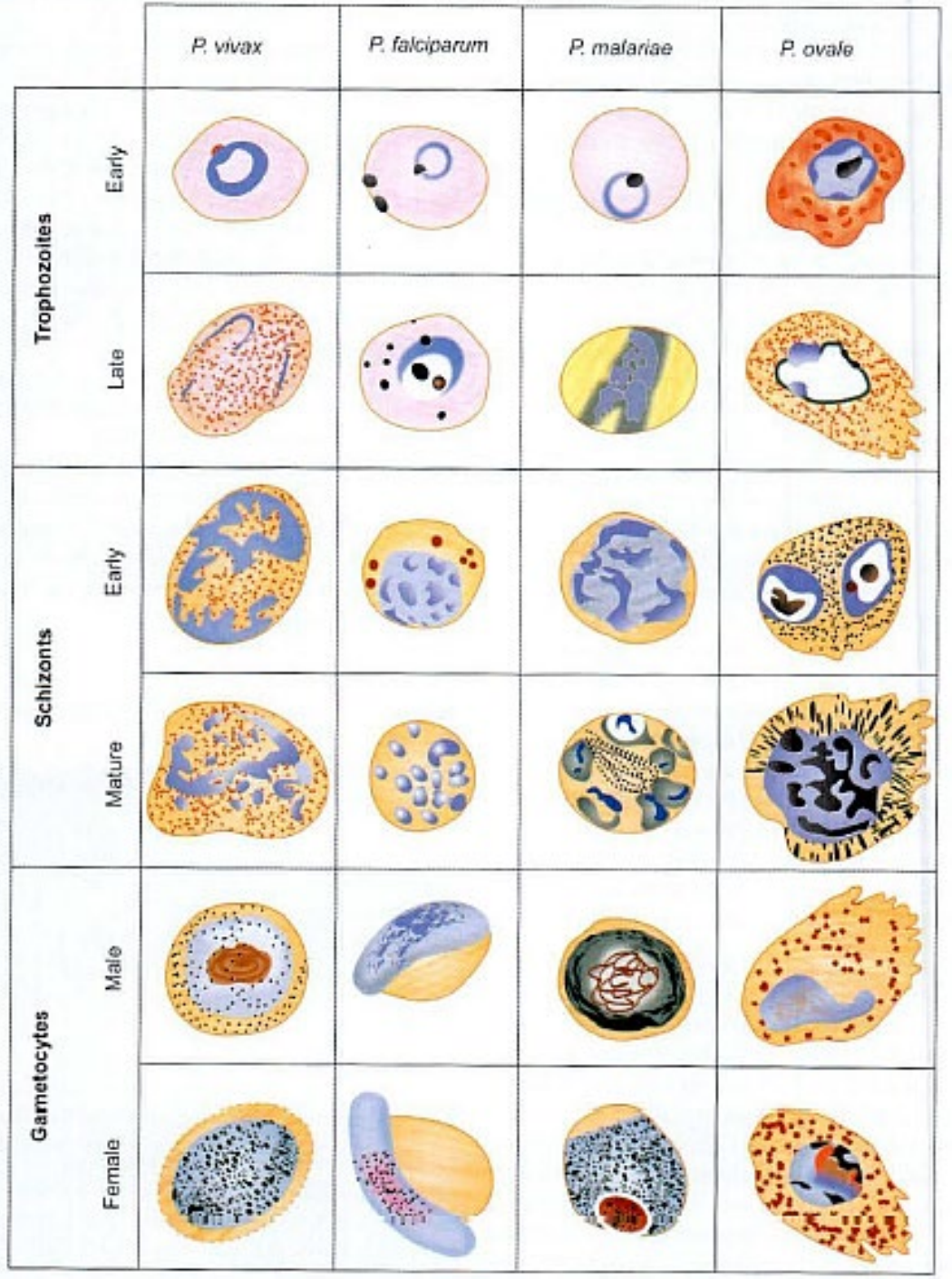


Trophozoite



Gametocyte

Morphology


P. vivax
P. falciparum
P. malariae
P. ovale
Trophozoites
Early
Late
Schizonts
Early
Mature
Gametocytes
Male
Female

Vector

Human malaria is transmitted by over 60 species of female Anopheles mosquitoes.

The male mosquito feeds exclusively on fruits and juices, but the female needs at least two blood meals before the first batch of eggs can be laid.

Life Cycle

Malaria parasite passes its life cycle in two hosts:

1. Definitive host:

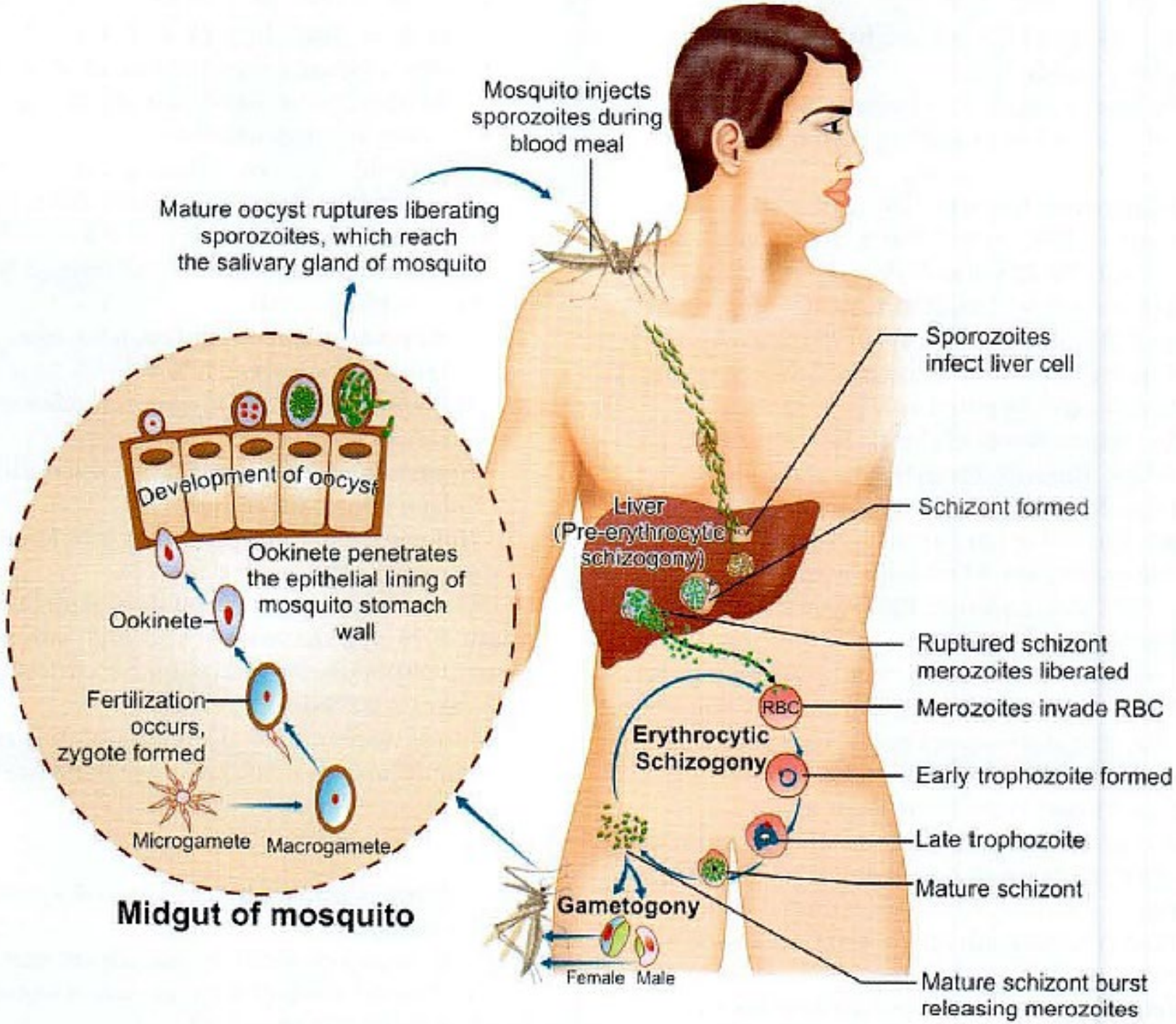
Female Anopheles mosquito.

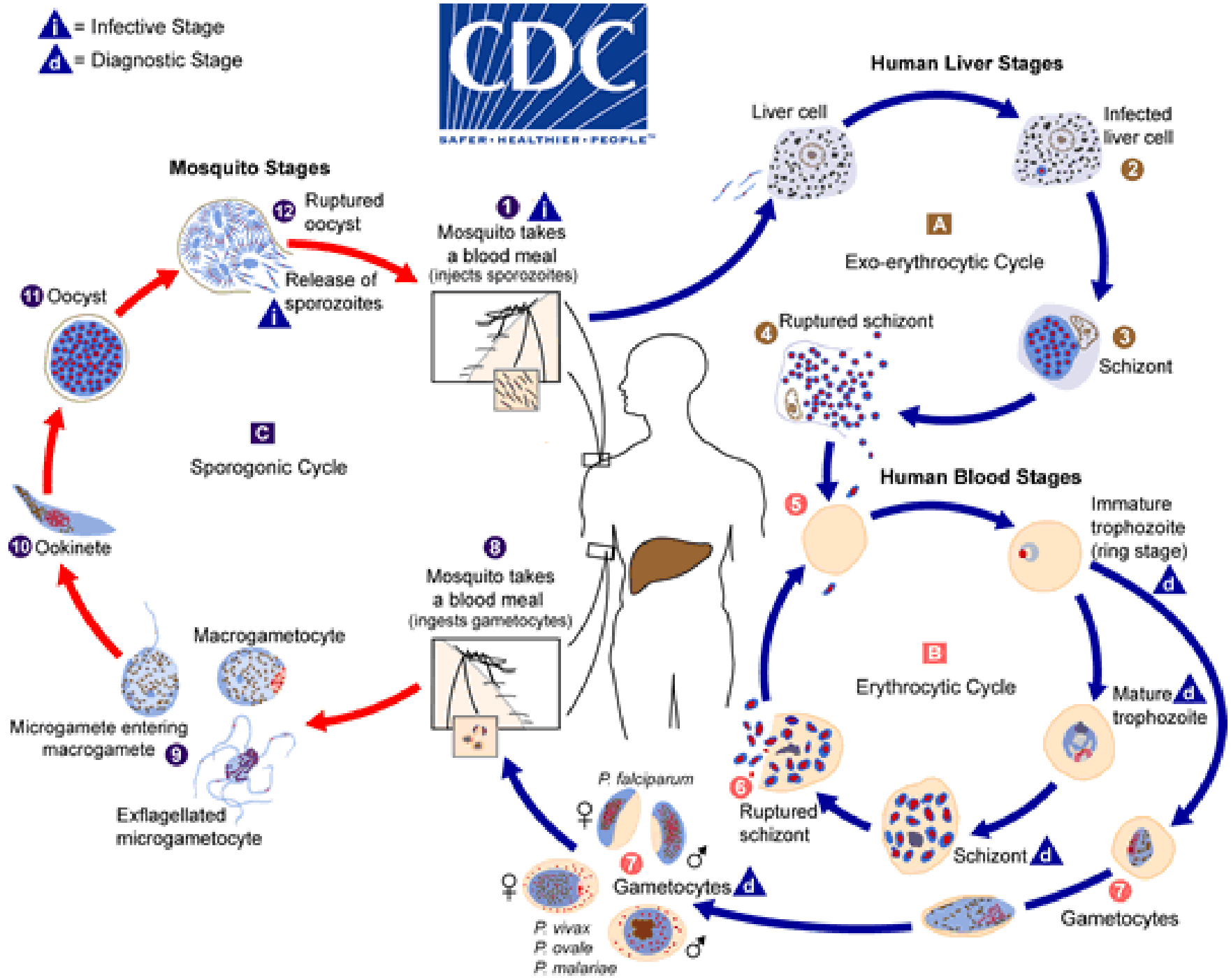
2. Intermediate host:.

Human

Life Cycle

- When an infected Anopheles mosquito bites a human, it injects sporozoites into the bloodstream.
- The sporozoites travel to the liver, invade liver cells, and multiply into merozoites.
- Merozoites are released into the blood, where they infect red blood cells. They multiply to develop to the Ring stage, then the Trophozoite stage, after that, schizonts and then, and then cause the cells to rupture, producing fever and chills.
- Some parasites in red blood cells develop into male and female gametocytes instead of merozoites.
- When another mosquito bites the infected person, it ingests these gametocytes.
- In the mosquito’s gut, the gametocytes fuse to form a zygote, which develops into an ookinete, then an oocyst.
- The oocyst releases new sporozoites that migrate to the mosquito’s salivary glands, ready to infect another human host.


Mosquito injects sporozoites during blood meal
Mature oocyst ruptures liberating sporozoites, which reach the salivary gland of mosquito
Development of oocyst
Ookinete penetrates the epithelial lining of mosquito stomach wall
Ookinete
Fertilization occurs, zygote formed
Microgamete
Macrogamete
Midgut of mosquito
Sporozoites infect liver cell
Liver (Pre-erythrocytic schizogony)
Schizont formed
Ruptured schizont merozoites liberated
RBC
Merozoites invade RBC
Erythrocytic Schizogony
Early trophozoite formed
Late trophozoite
Mature schizont
Gametogony
Female
Male
Mature schizont burst releasing merozoites


i = Infective Stage
d = Diagnostic Stage
CDC
SAFER·HEALTHIER·PEOPLE™
Human Liver Stages
Liver cell
Infected liver cell
2
A
Exo-erythrocytic Cycle
3
Schizont
4
Ruptured schizont
Human Blood Stages
5
Immature trophozoite (ring stage)
d
Mature trophozoite
d
B
Erythrocytic Cycle
6
Ruptured schizont
Schizont
d
7
Gametocytes
Gametocytes
d
P. falciparum
P. vivax
P. ovale
P. malariae
8
Mosquito takes a blood meal (ingests gametocytes)
Macrogametocyte
Microgamete entering macrogamete
9
Exflagellated microgametocyte
10
Ookinete
11
Oocyst
12
Ruptured oocyst
Release of sporozoites
Mosquito Stages
1
Mosquito takes a blood meal (injects sporozoites)
C
Sporogonic Cycle

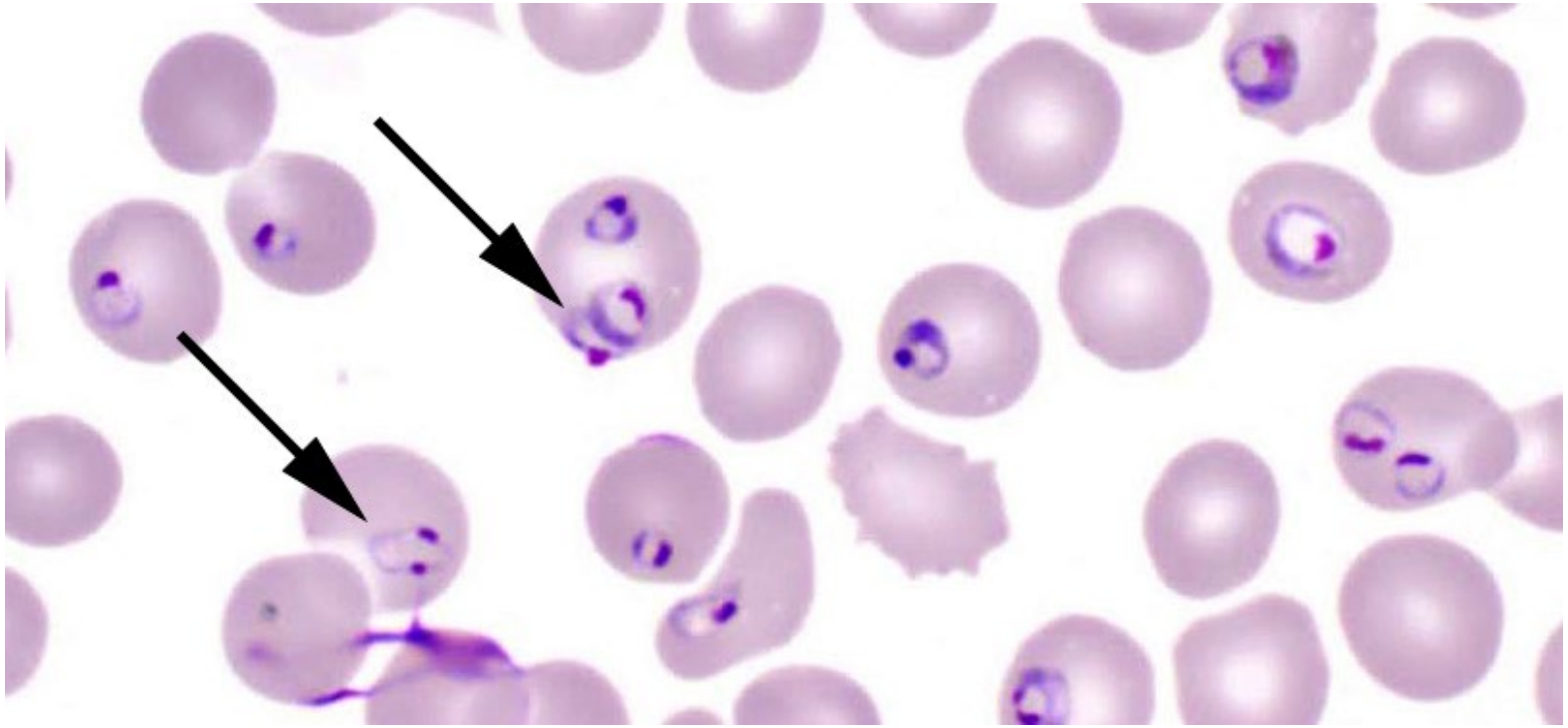
Laboratory Diagnosis

Demonstration of Parasite by Microscopy

Diagnosis of malaria can be made by demonstration of malarial parasite in the blood.

Two types of smears are prepared from the peripheral blood. One is called thin smear and the other is called thick smear.

Blood smear showing *Plasmodium* sp.



Demonstration of Parasite by Microscopy

Thin smears:

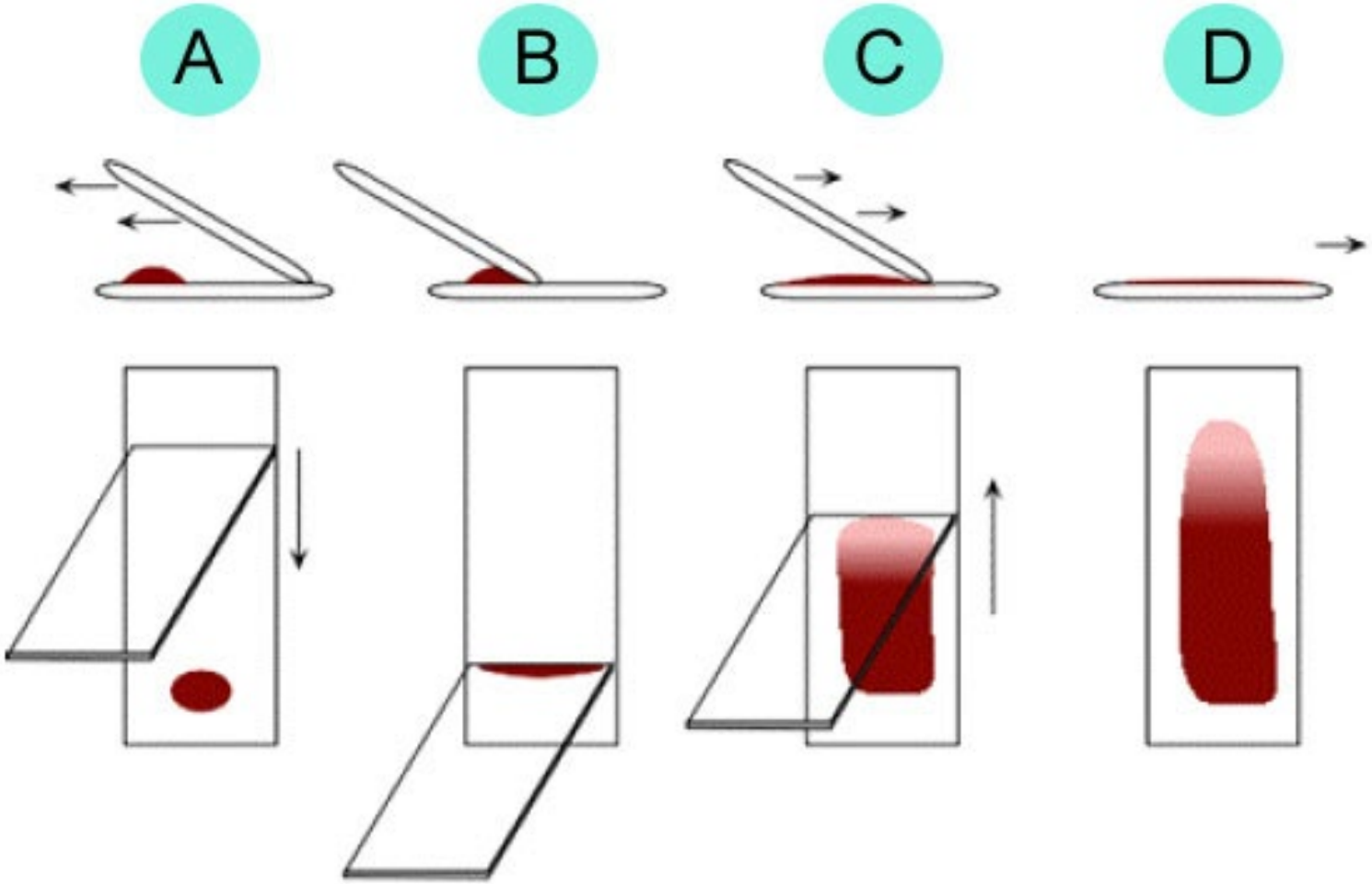
They are prepared from capillary blood of finger tip and spread over a good quality slide by a second slide held at an angle of 30-45° from the horizontal such that a tail is formed. Then stain it with Giemsa.

- Thin film is examined first at the tail end

Purpose

- single layer of red cells should be formed
- determining the species.

Thin blood smear



Demonstration of Parasite by Microscopy

Thick smears:

- In a thick film, usually **three drops** of blood are spread over a small area (about 10 mm). Then stain it with Giemsa.
- The amount of blood in a thin smear is about **1- 1.5 µL,** while in a thick smear it is **3-4 µL.**
- If parasites are not detected in the thin film, then the thick film should be examined.

Laboratory Diagnosis

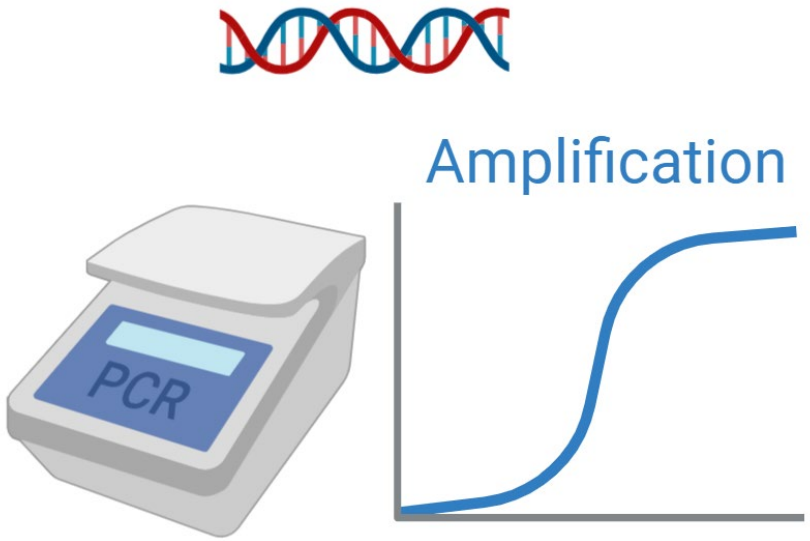
Fluorescence microscopy:

Kawamoto technique: Fluorescent dyes like acridine orange or benzothiocarboxy purine are used, which stain the parasites entering the RBCs but not white blood cells (WBCs).

Laboratory Diagnosis

3. Molecular Method

- DNA detection of the parasite



Immunological Diagnosis in Parasitology

Introduction

Immunological diagnosis plays a crucial role in detecting parasitic infections, especially when parasites are difficult to visualize microscopically or when parasite load is low.

These techniques rely on identifying parasite-specific antigens or host antibodies produced in response to infection.

Antibody Detection

These tests detect host-produced antibodies against parasites.

They are useful when:

1. The parasite load is low or absent in peripheral samples
2. The infection is chronic
3. The parasite resides in deep tissues (e.g., Toxoplasma, Leishmania, Echinococcus)

Antibody Detection

Example of Parasites

1. Toxoplasma gondii – IgM/IgG serology
2. Leishmania donovani / Leishmania infantum – rK39 antibody test
3. Trypanosoma cruzi – ELISA for Chagas disease
4. Echinococcus granulosus – hydatid cyst antibody tests
5. Schistosoma spp. – antibody detection in low-intensity infections
6. Trichinella spiralis – ELISA for IgG

Samples used to detect Antibody

1. Blood (Serum)
2. Cerebrospinal fluid (CSF)

Antigen detection

Antigen tests identify parasite molecules (proteins) present in clinical samples such as stool, blood, urine.

Antigen Detection

Example of Parasites:

1. Giardia lamblia – stool antigen ELISA or rapid test
2. Entamoeba histolytica – stool antigen detection
3. Cryptosporidium parvum – coproantigen tests
4. Plasmodium falciparum – HRP-II and pLDH rapid diagnostic test
5. Strongyloides stercoralis – coproantigen detection (in some labs)
6. Taenia solium – circulating antigen detection in neurocysticercosis

Samples used to detect Antigen

1. Stool
2. Urine
3. Blood

References

- Paniker, C. K. J. & Ghosh, S. 2021. *Paniker's textbook of medical parasitology,* New Delhi, Jaypee Brothers Medical Publishers.