

Diagnostic Parasitology

Medically Important Sporozoa

Disclaimer

- This presentation was meant to provide students with both didactic and laboratory skills as they apply to clinical parasitology. It is meant for educational purposes only and does not represent Cleveland Clinic views or practices.
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- Most of the information was adopted from the Textbook of Diagnostic Microbiology by Mahon & Lehman (see citation) but condensed for bite sized learning.

Protozoa

- Unicellular parasites
- Classified according to their motility organelles
 - Amebae move by pseudopodia
 - Flagellates move by flagella
 - Ciliates move by cilia
 - Sporozoa are nonmotile
- All reproduce asexually except for the sporozoa
 - Definitive host: host where sexual reproduction occurs
 - Intermediate host: host where asexual reproduction occurs

Cyst: nonmotile form resistance to environmental factors
Trophozoite: feeding motile form replicates in the host and responsible for causing damage

The Sporozoa a.k.a. Apicomplexa a.k.a Coccidia

Intestinal

- Three medically important members
 - *Cryptosporidium* species
 - *Cyclospora cayetanensis*
 - *Cystoisospora belli*
- Fecal oral transmission
- Oocysts are the infective form
- Modified acid-fast stain

Tissue

- One medically important members
 - *Toxoplasma gondii*
- Fecal oral transmission
- Oocysts are the infective form
- Serological testing

Blood

Two medically important genera

- *Plasmodium* species
- *Babesia* species
- Transmitted by vectors
- Sporozoite is the infective form
- Thick and thin Giemsa-stained blood smear

Intestinal Sporozoa: The 3 C's & Disease

Cryptosporidium species

- Leading cause of waterborne disease outbreaks in USA
- Occurs in summer when there is increased recreational water activity
- Cows can serve as reservoirs



Cyclospora cayetanensis

- Outbreaks from contaminated imported fruit and veggies



Cystoisospora belli

- Less common than cryptosporidium

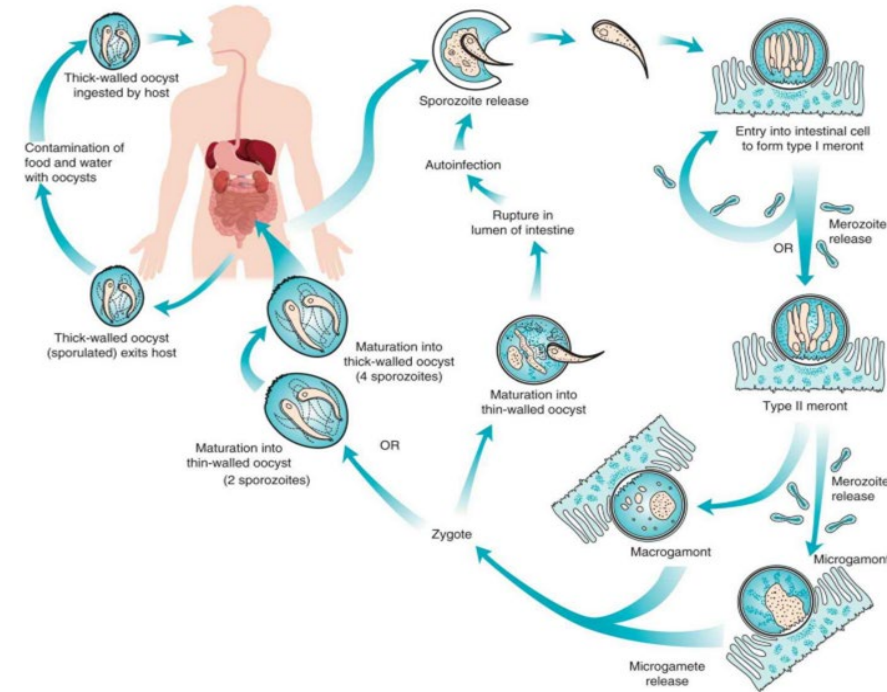
Hey!
I'm important
too!

• Disease

- Self limiting GI infection in immunocompetent hosts
- Can be life threatening in AIDs patients

Intestinal Sporozoa: Life Cycle of the 3 C's

- Life cycle
 - Sporogony
 - Ingestion of oocyst
 - Release of sporozoites
 - Enters intestinal cells
 - Matures to trophozoite
 - Forms a meront with merozoites
 - Meront ruptures and merozoites infect more cells
 - Schizogony
 - Merozoite transforms into micro/macrogamete
 - Macrogamete is fertilized and forms oocysts
 - Thin-walled oocyst: rupture within the intestine and result in autoinfection
 - Thick-walled: passed in feces and are infective

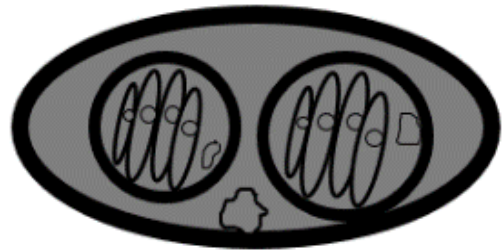


Cyclospora cayetanensis and *Cystoisospora belli*

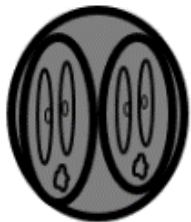
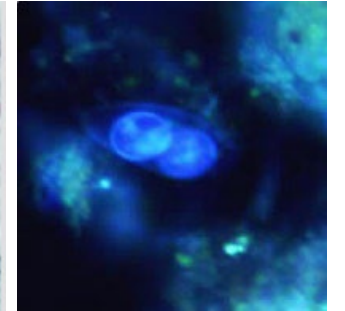
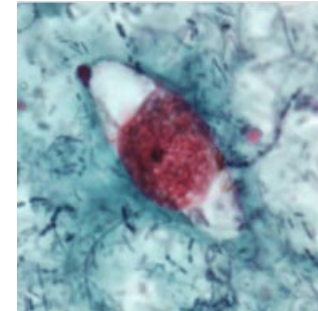
- No autoinfection
- Oocyst not infective when passed

Intestinal Sporozoa: 3 C's Microscopic Identification

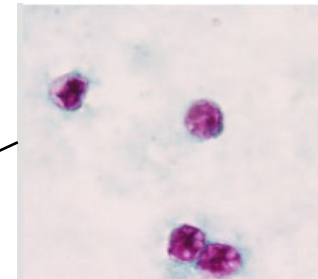
Oocysts of human Coccidia



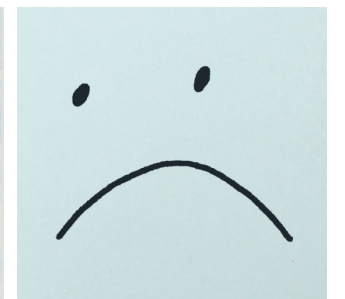
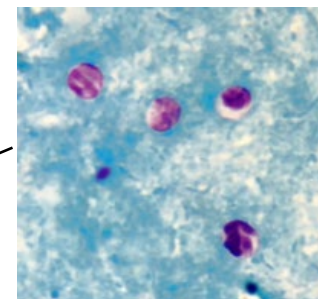
***Cystoisospora* (25-30 μm)**



***Cyclospora* (8-10 μm)**

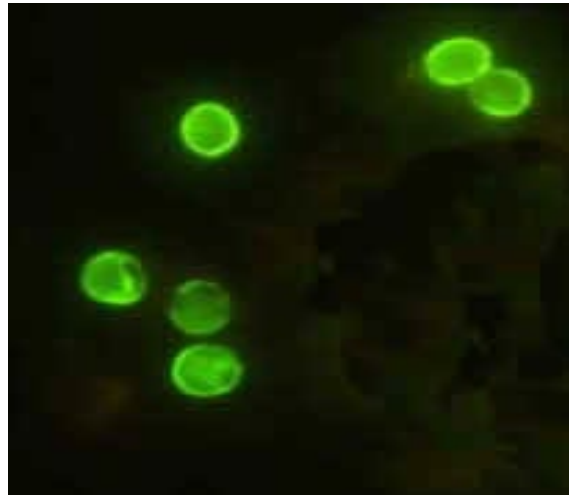


***Cryptosporidium* (4-6 μm)**



Intestinal Sporozoa: *Cryptosporidium* species

- Other tests for identification
 - Antigen detection tests
 - A. Direct fluorescent antibody test (DFA)
 - B. Enzyme immunoassay (EIA)
 - C. Lateral-flow immunochromatographic assays



A



B



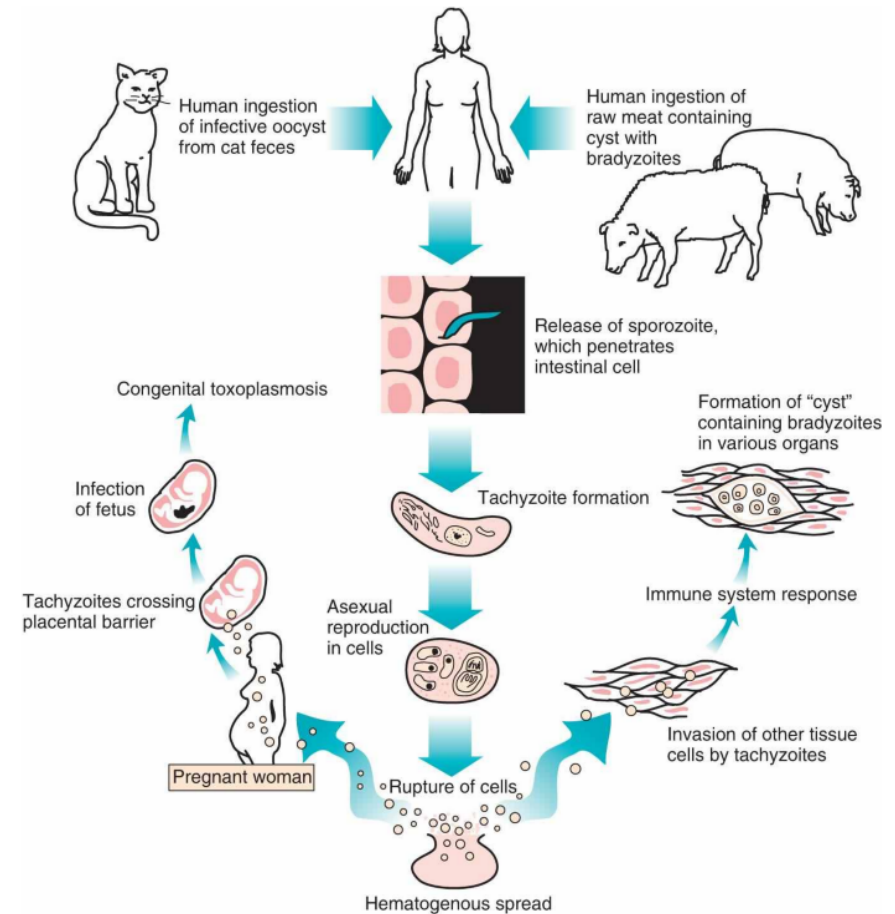
C

Tissue Sporozoa: *Toxoplasma gondii* & Disease

- Risk groups
 - HIV
 - Transplant recipients
 - Defects in T cell-mediated immunity
 - Pregnant
- Disease
 - Immunocompetent host: asymptomatic or mild flu-like
 - Immunocompromised host: fulminating encephalitis
 - Congenital: intellectual disability, microcephaly, seizure, hydrocephalus, & blindness
 - The earlier exposed the more severe

Tissue Sporozoa: Life Cycle of *Toxoplasma gondii*

- Ingest oocyst or bradyzoite
- Release of sporozoite
- Infects intestinal cell
- Tachyzoite forms and multiplies
- Rupture of cell and infection of more tissues
- Cyst forms containing bradyzoites (dormant)



Tissue Sporozoa: *Toxoplasma gondii* Detection

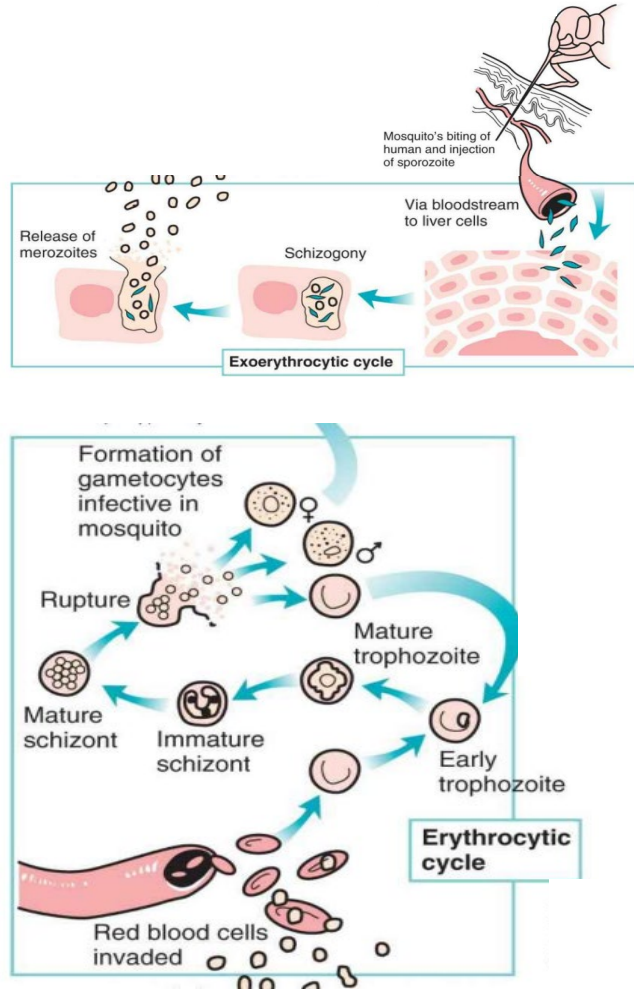
- The levels of antibodies to the organism show a rapid increase during infection, and tests for antibodies are most used for diagnosis.
 - Indirect fluorescent antibody tests and EIAs with *T. gondii* organisms as the antigen are routinely used for diagnosis.
 - An IgM-specific test may also be used to diagnose acute infections
 - Primary toxoplasmosis in pregnant women
 - Neonates

Blood Sporozoa: *Plasmodium* species

- *Plasmodium* species
 - *P. falciparum*
 - *P. malariae*
 - *P. ovale*
 - *P. vivax*
- Transmission
 - Bite of Anopheline mosquito
 - Blood transfusion
 - Infected needles
- Symptoms
 - Malarial paroxysm- repeated at regular intervals depending on the species
 - First phase (15-60 min.): shaking chill
 - Second phase (2-4 hr.): fever with headache, myalgia, and nausea
 - Third phase (2-4 hr.) fever breaks followed by profuse sweating and exhaustion

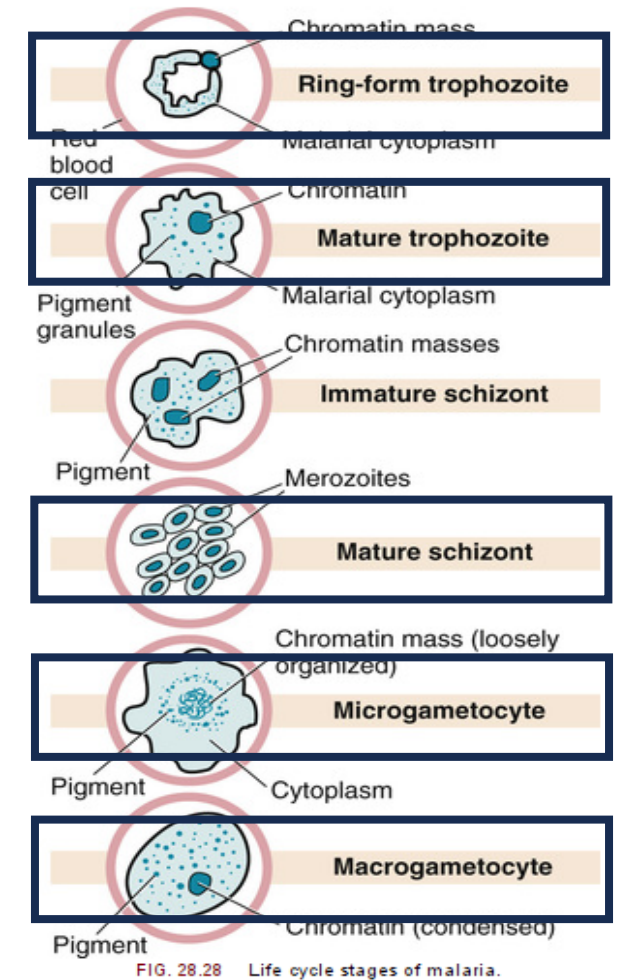


Blood Sporozoa: *Plasmodium* species

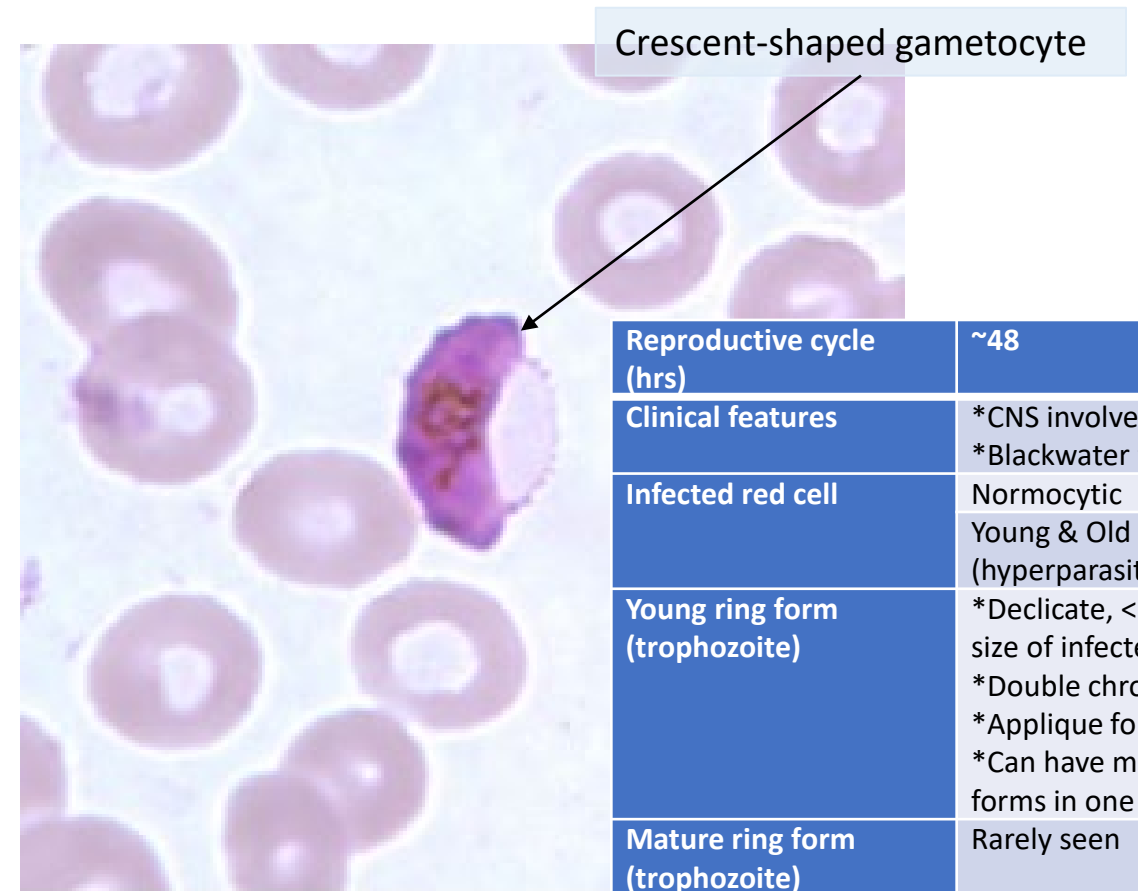
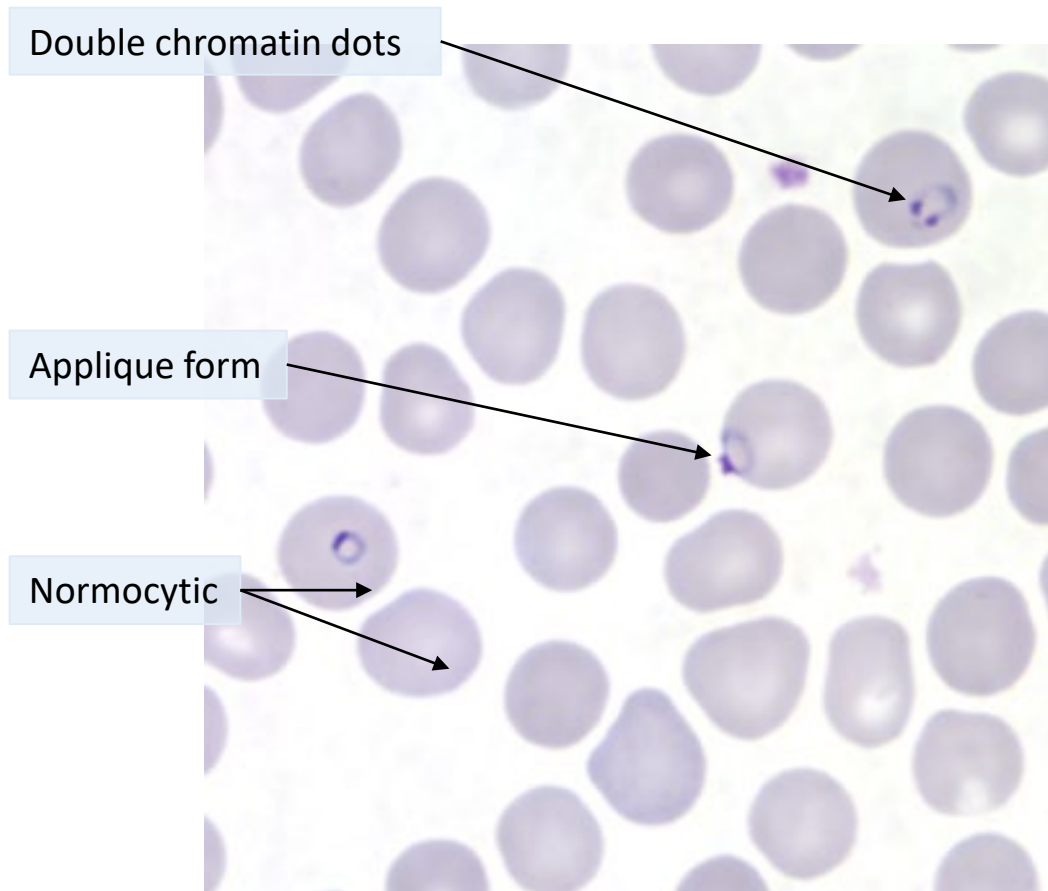


- **Schizogony (asexual)**
 - **Exoerythrocytic phase (liver)**
 - Sporozoites are injected
 - Penetrate parenchymal cells
 - Matures to trophozoite
 - Matures to schizont with merozoites
 - Rupture of parenchymal cells
 - Release of merozoites into blood stream
 - **Erythrocytic phase (RBCs)**
 - Merozoite invades RBCs
 - Matures to trophozoite
 - Matures to schizont with merozoites
 - Rupture of RBCs
 - Release of merozoites into blood stream

Plasmodium vivax and *ovale* can remain latent in the liver for up to three years

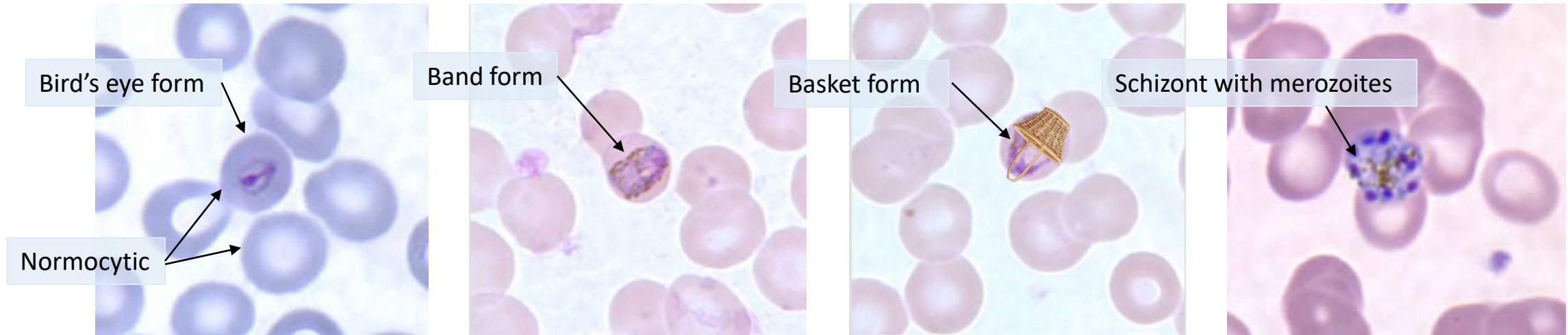


Blood Sporozoa: *Plasmodium falciparum*



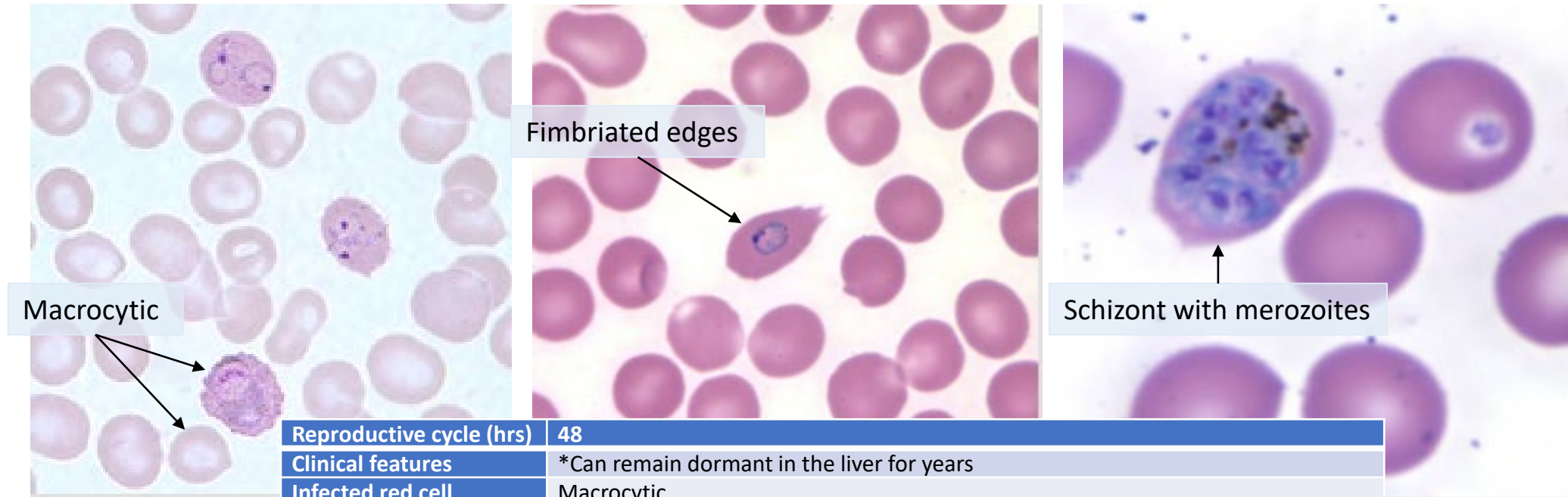
Reproductive cycle (hrs)	~48
Clinical features	*CNS involvement *Blackwater fever
Infected red cell	Normocytic Young & Old (hyperparasitemia)
Young ring form (trophozoite)	*Delicate, <1/3 the size of infected RBC *Double chromatin dots *Applique forms *Can have multiple ring forms in one RBC
Mature ring form (trophozoite)	Rarely seen
Schizont	Rarely seen
Gametocyte	Crescent-shaped gametocyte
Cytoplasmic pigment (red dots)	Maurer clefts

Blood Sporozoa: *Plasmodium malariae*



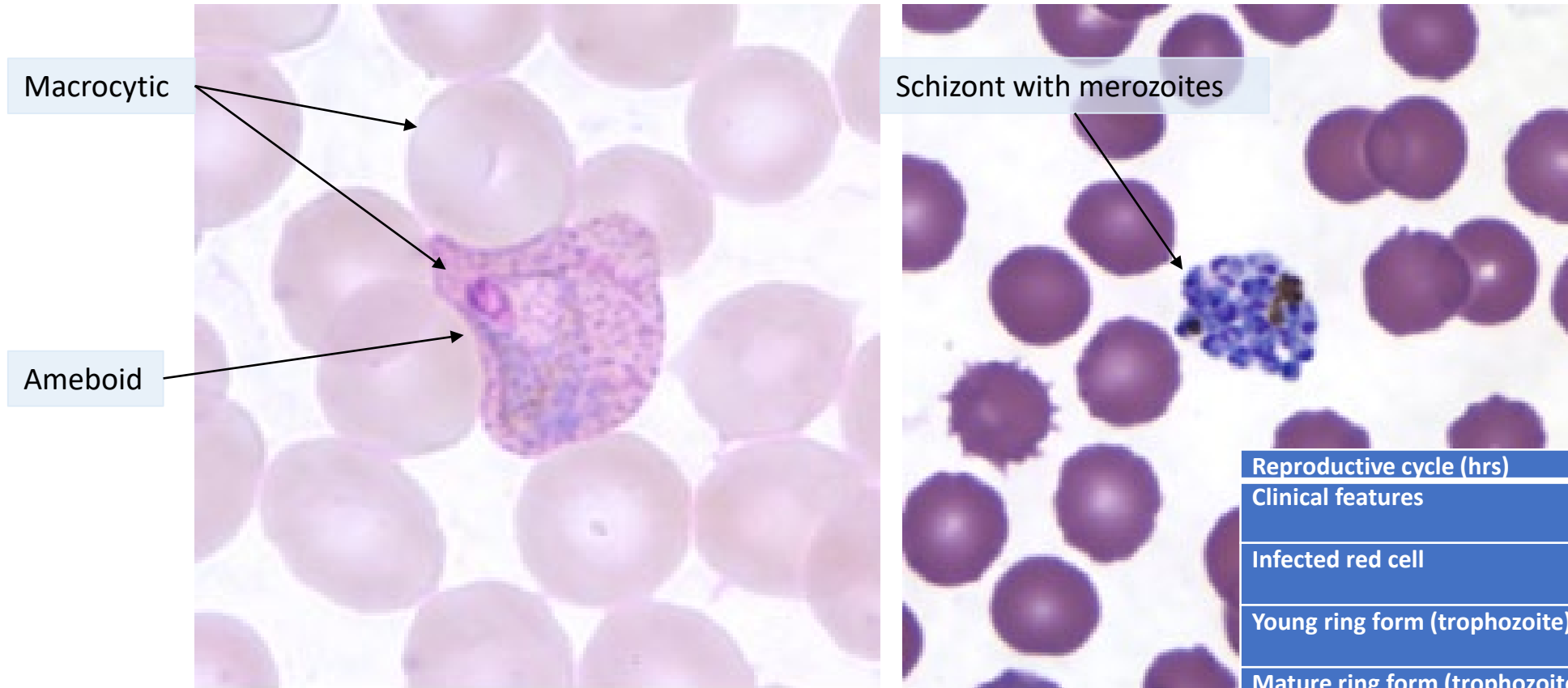
Reproductive cycle (hrs)	72
Clinical features	*Nephrotic syndrome
Infected red cell	Normocytic
	Old
Young ring form (trophozoite)	*Thick, 1/3 the size of infected RBC *bird's eye forms
Mature ring form (trophozoite)	*Band forms *Basket forms *Dark brown pigment
Schizont	*Average of 8 merozoites in daisy petal arrangement around clumped pigment
Gametocyte	Oval
Cytoplasmic pigment (red dots)	Ziemann dots

Blood Sporozoa: *Plasmodium ovale*



Reproductive cycle (hrs)	48
Clinical features	*Can remain dormant in the liver for years
Infected red cell	Macrocytic
	Young
Young ring form (trophozoite)	* $\geq 1/3$ size of the infected RBC
Mature ring form (trophozoite)	*Fimbriated edge of infected RBCs
	*Golden brown pigment
Schizont	*Average of 8 merozoites
Gametocyte	Oval
Cytoplasmic pigment (red dots)	Schuffners dots

Blood Sporozoa: *Plasmodium vivax*



Reproductive cycle (hrs)	48
Clinical features	*Can remain dormant in the liver for years
Infected red cell	Macrocytic Young
Young ring form (trophozoite)	*Can have multiple ring forms in one RBC
Mature ring form (trophozoite)	*Amoeboid form *Golden brown pigment
Schizont	*16 merozoites
Gametocyte	Oval
Cytoplasmic pigment (red dots)	Schuffners dots

Blood Sporozoa: *Plasmodium* species

Characteristics	<i>Plasmodium falciparum</i>	<i>Plasmodium malariae</i>	<i>Plasmodium ovale</i>	<i>Plasmodium vivax</i>
Reproductive cycle (hrs)	~48	72	48	
Clinical features	*CNS involvement *Blackwater fever	*Nephrotic syndrome	*Can remain dormant in the liver for years	
Infected red cell	Normocytic		Macrocytic	
	Young & Old (hyperparasitemia)	Old	Young	
Young ring form (trophozoite)	*Delicate, <1/3 the size of infected RBC *Double chromatin dots *Applique forms *Can have multiple ring forms in one RBC	*Thick, 1/3 the size of infected RBC *bird's eye forms	*≥1/3 size of the infected RBC *Can have multiple ring forms in one RBC	
Mature ring form (trophozoite)	Rarely seen	*Band forms *Basket forms *Dark brown pigment	*Fimbriated edge of infected RBCs *Golden brown pigment	*Amoeboid form *Golden brown pigment
Schizont	Rarely seen	*Average of 8 merozoites in daisy petal arrangement around clumped pigment	*Average of 8 merozoites	*16 merozoites
Gametocyte	Crescent-shaped gametocyte	Oval		
Cytoplasmic pigment (red dots)	Maurer clefts	Ziemann dots	Schuffners dots	

Blood Sporozoa: *Plasmodium* species

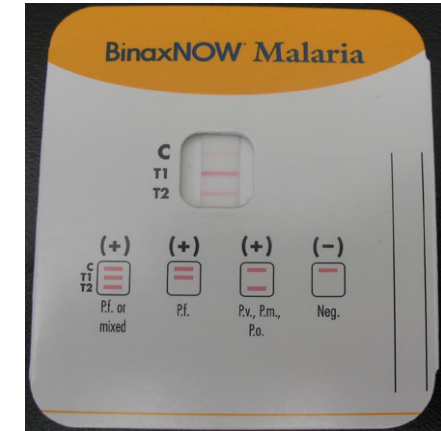
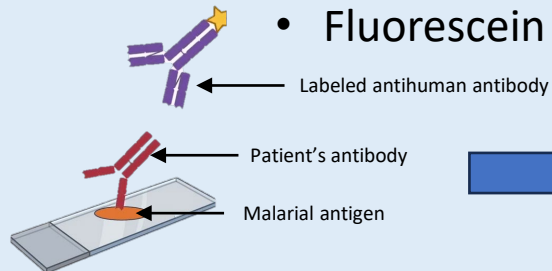
- Other tests for identification

- A. Rapid Diagnostic Tests (RDTs)

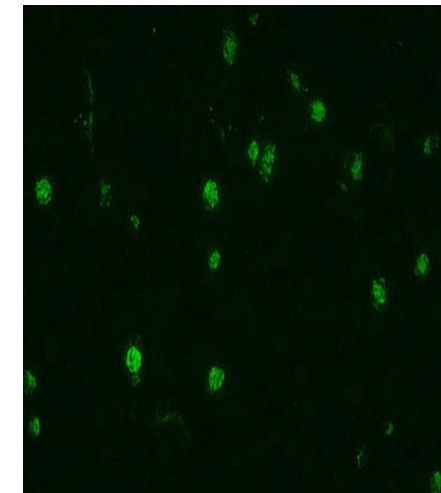
- Immunoassays
 - Immunochromatographic principle to detect soluble proteins from malarial organisms in blood

- B. Serology

- Indirect fluorescent antibody test (IFA)
 - Plasmodium species schizonts are used as antigen
 - Patient serum containing antibodies will bind
 - Fluorescein –labeled antihuman antibody is added



A



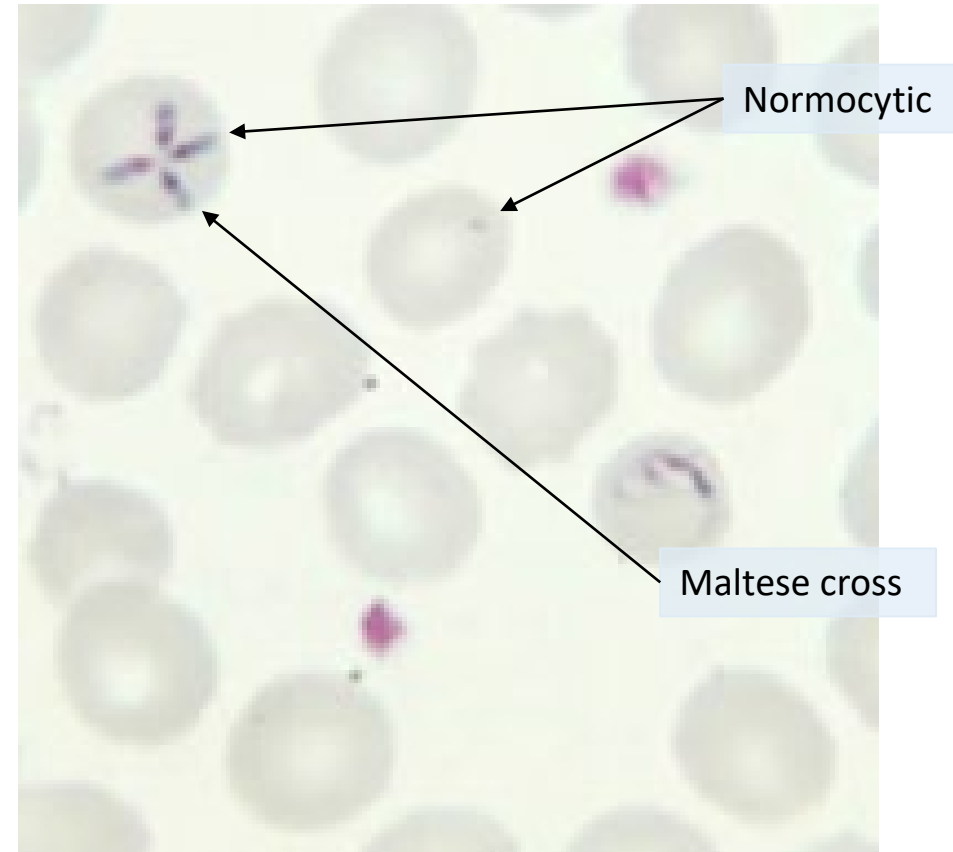
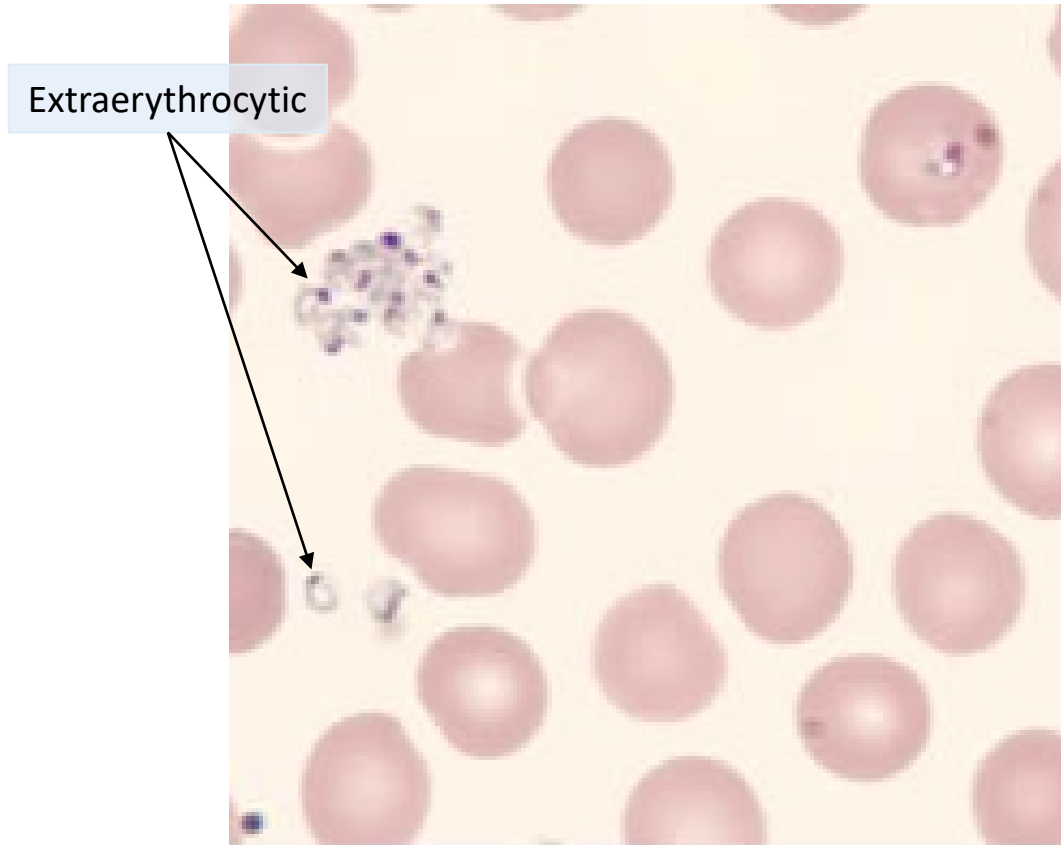
B

Blood Sporozoa: *Babesia* species

- Transmission
 - Bite of a Ixodes tick
 - White-footed mice and white-tailed deer are reservoirs
- Disease
 - Often asymptomatic, but immunosuppressed may have symptoms like malaria
 - Fever is not cyclic
- Life cycle
 - Sporozoites infect RBCs
 - Once inside sporozoites become trophozoites
 - Trophozoites reproduce asexually to form merozoites
 - RBC lyses



Blood Sporozoa: *Babesia* species



Blood Sporozoa: *Babesia* species

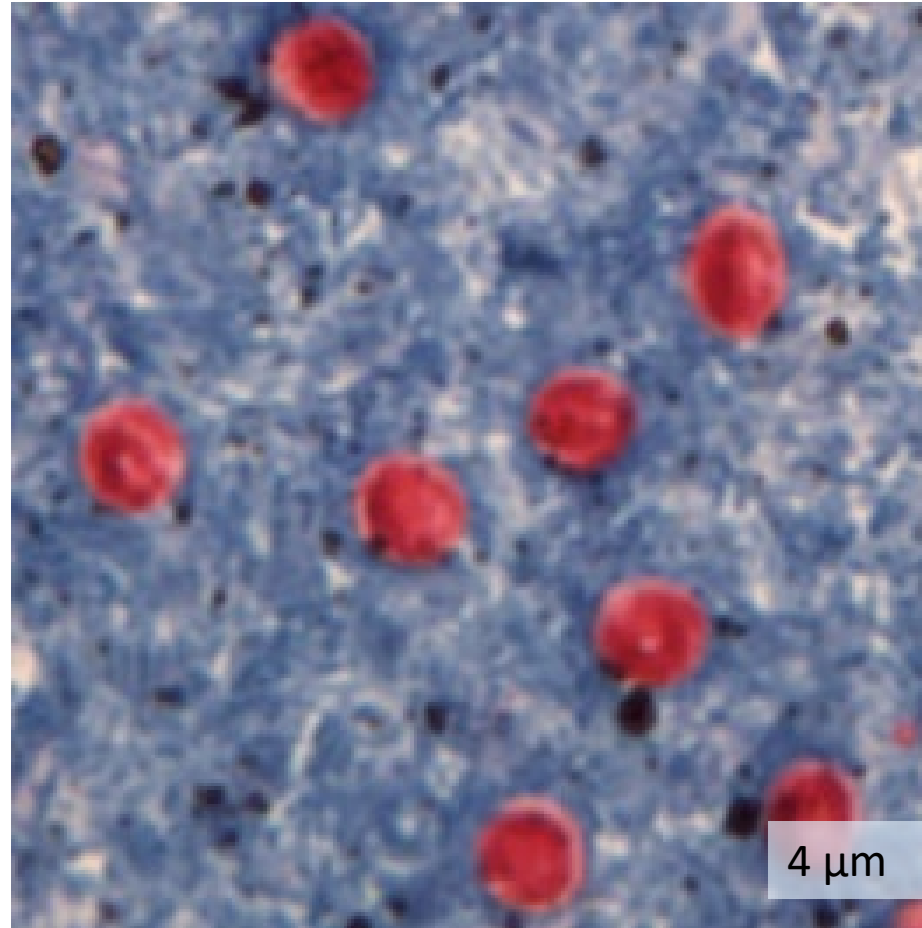
- Other tests for identification
 - Serology
 - Immunofluorescence assays
 - IgG titers greater than 1 :1024 indicative of recent or active infection
 - Molecular methods
 - High suspicion but smears remain negative

Microsporidia (fungus)

- Contains atleast 9 genera
- Risk
 - HIV
 - Organ transplant recipients
 - Older adults
 - Patients with traveler's diarrhea
- Symptoms
 - Can infect a wide variety of organs
 - Intestinal- diarrhea cramps, lost of appetite, and fatigue
 - *Enterocytozoon bienersi*
 - *Encephalitozoon intestinalis*
- Identification
 - Modified trichrome stain



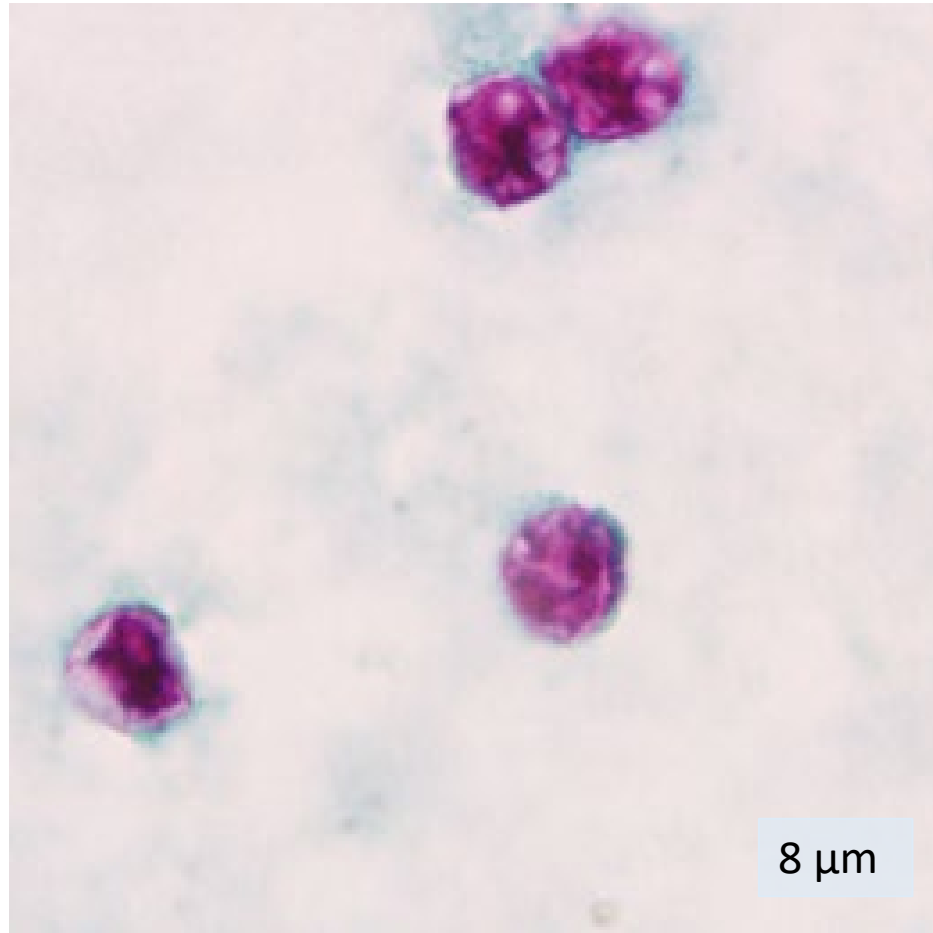
Modified Acid-fast



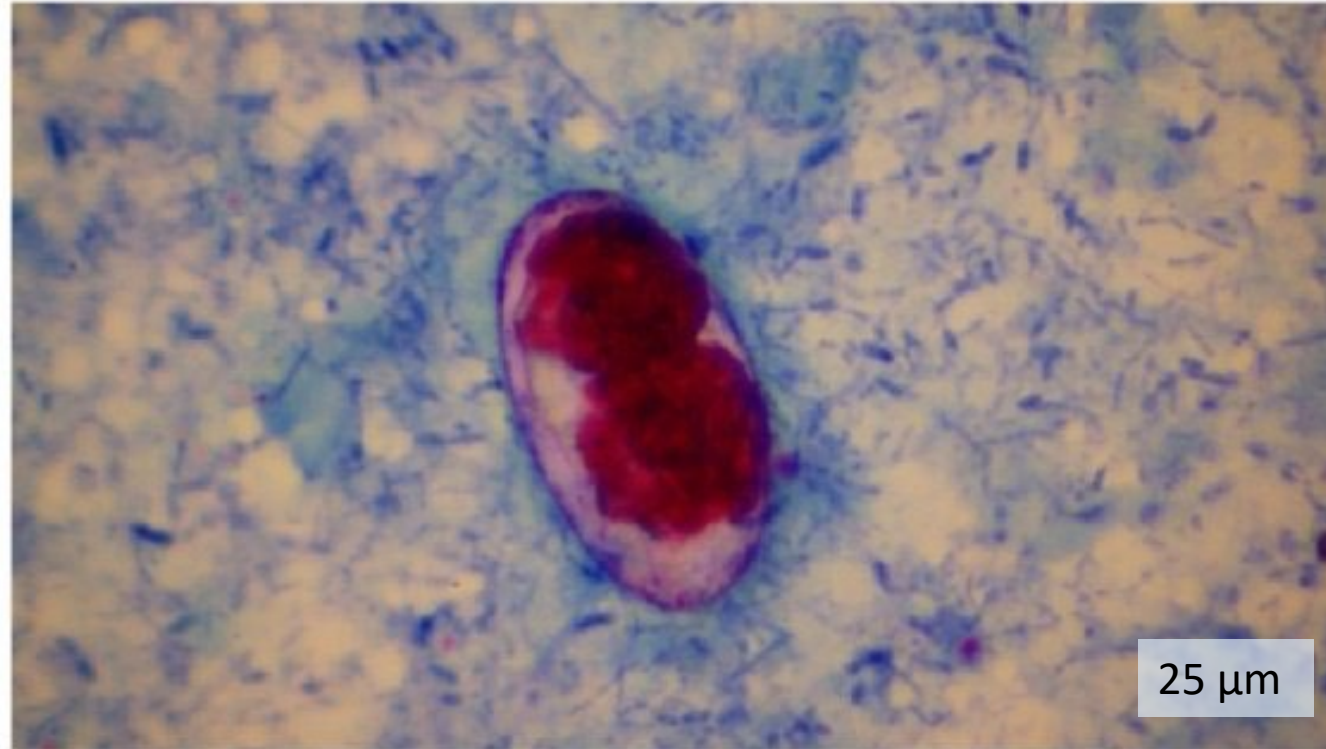
Cryptosporidium sp.



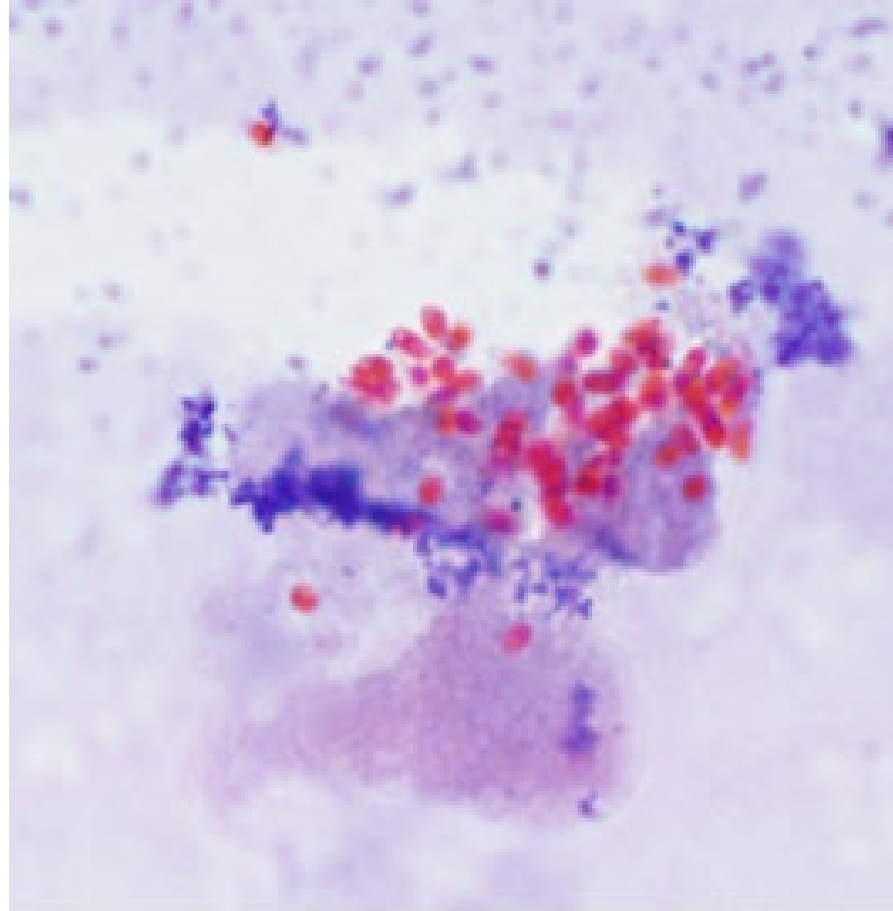
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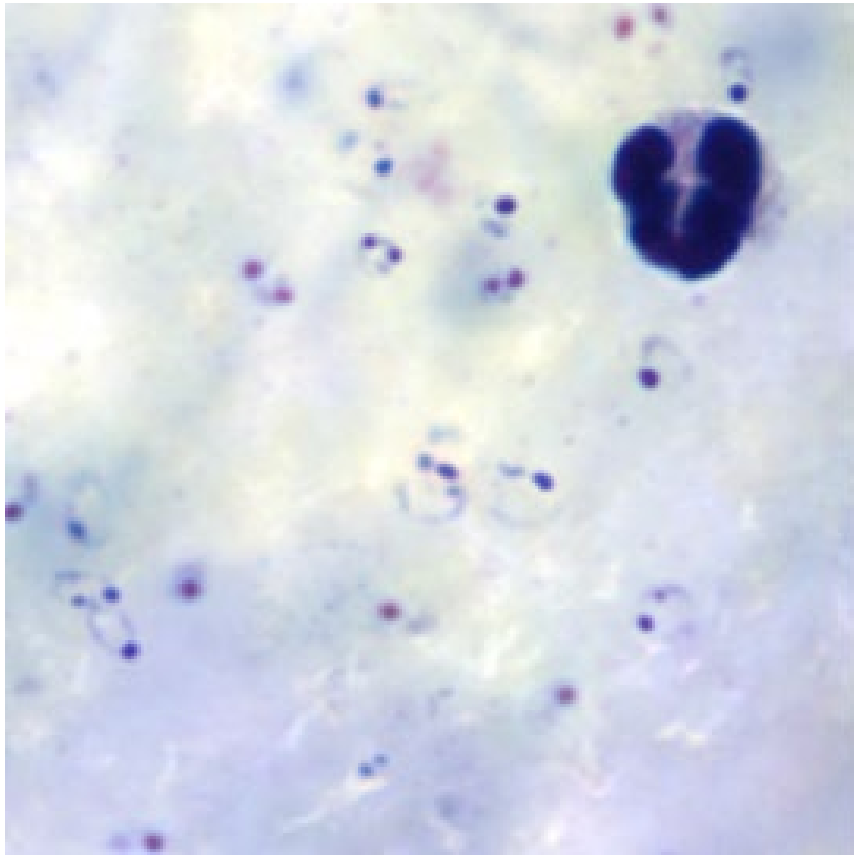
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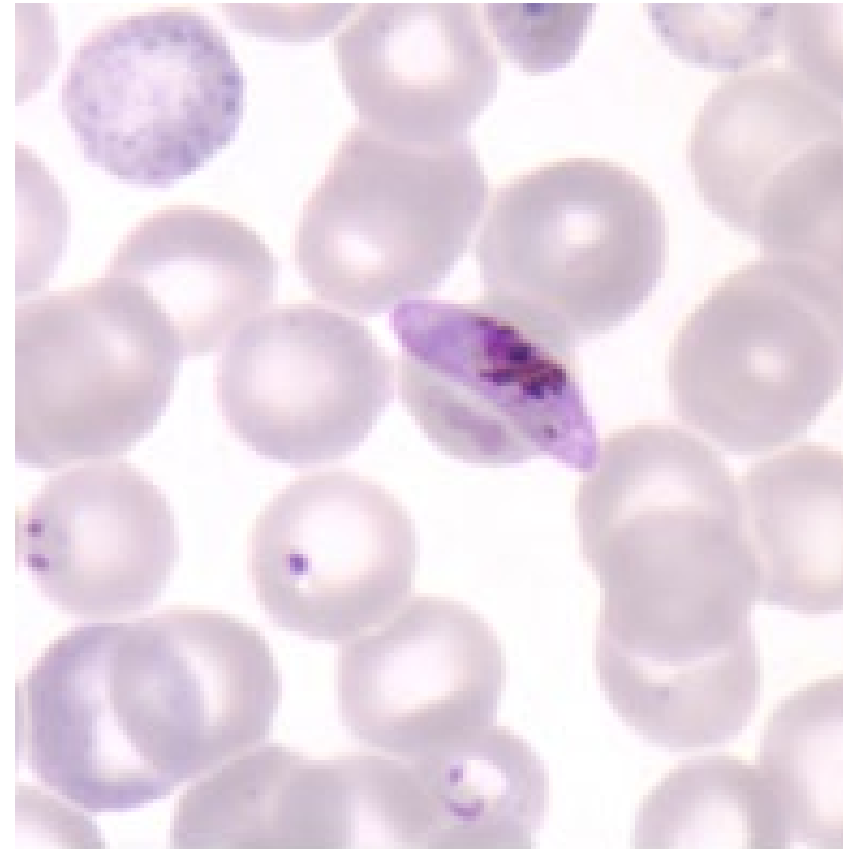
Modified Trichrome



Blood Parasite



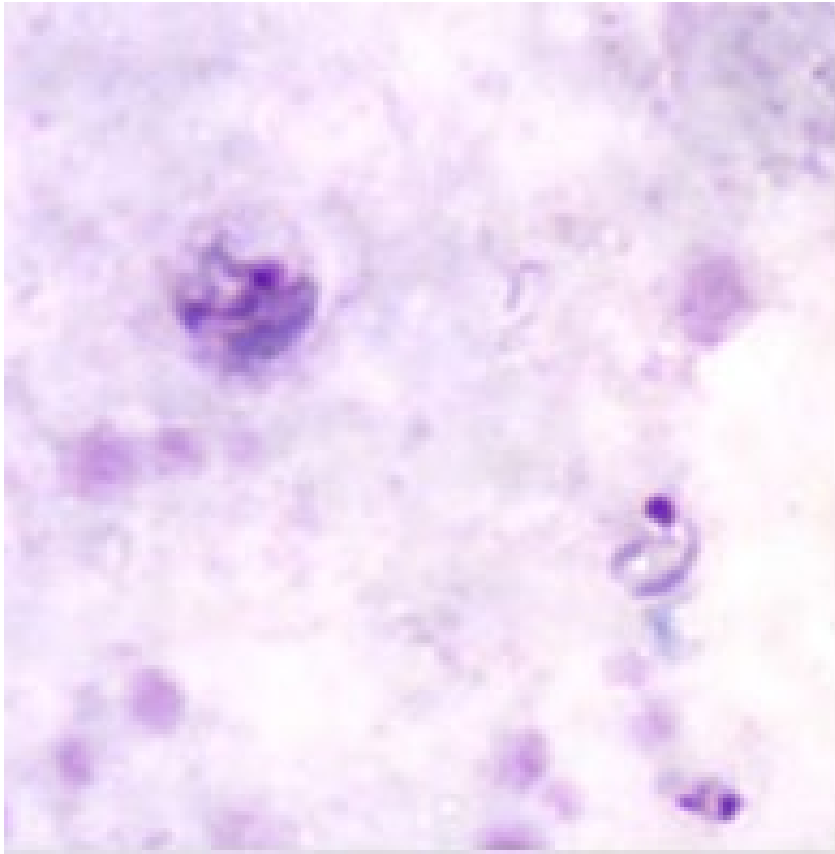
Thick Smear



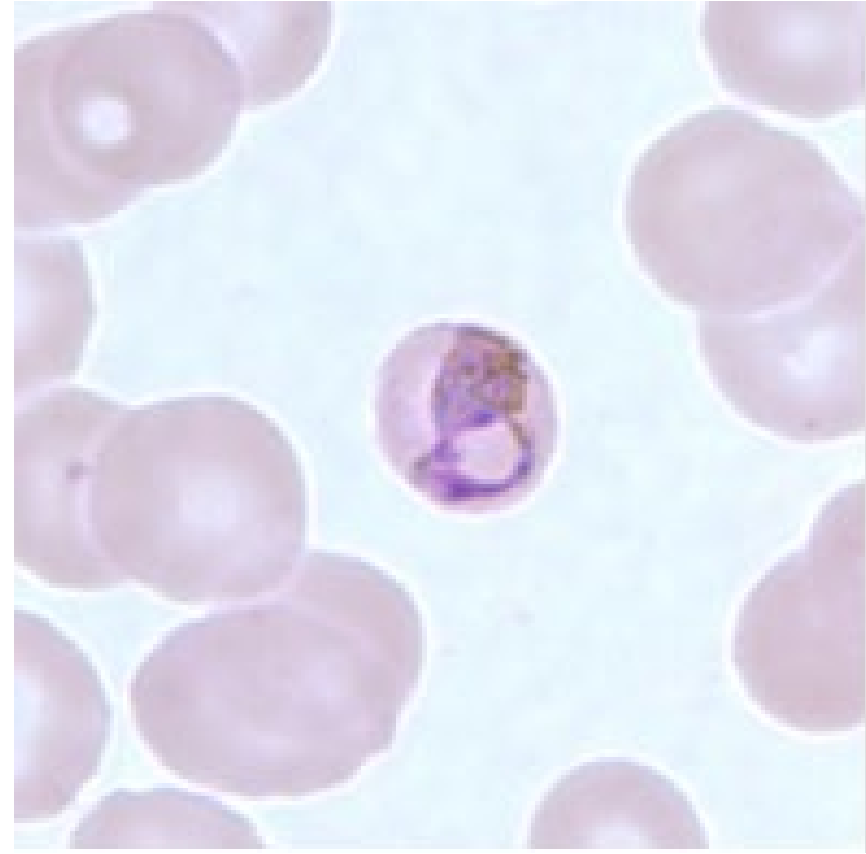
Thin Smear



Blood Parasite



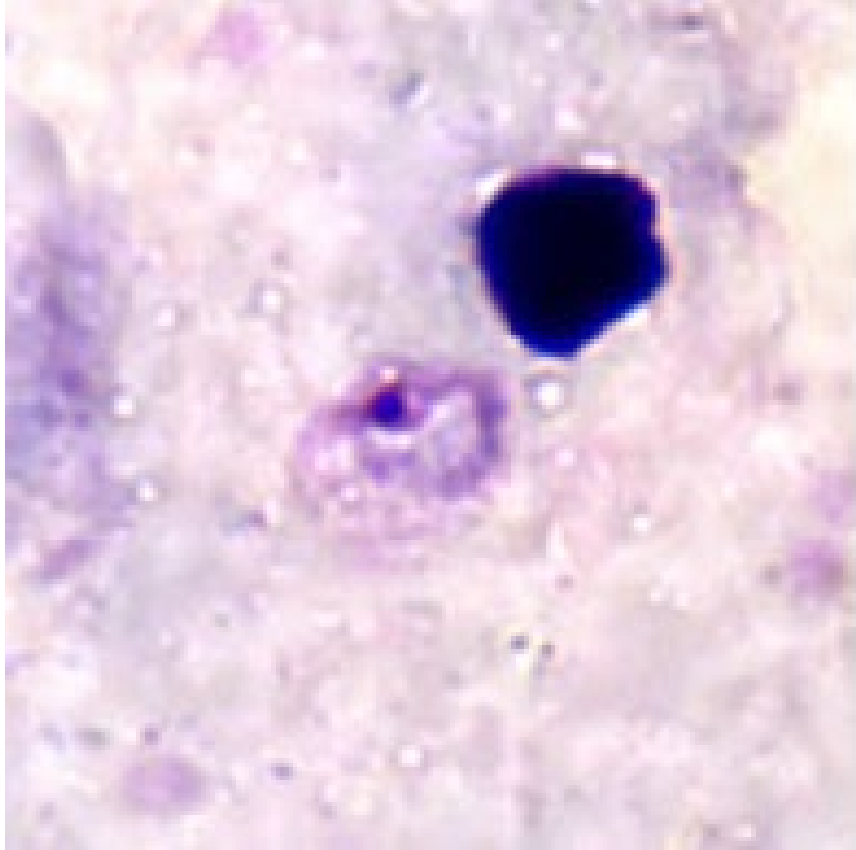
Thick Smear



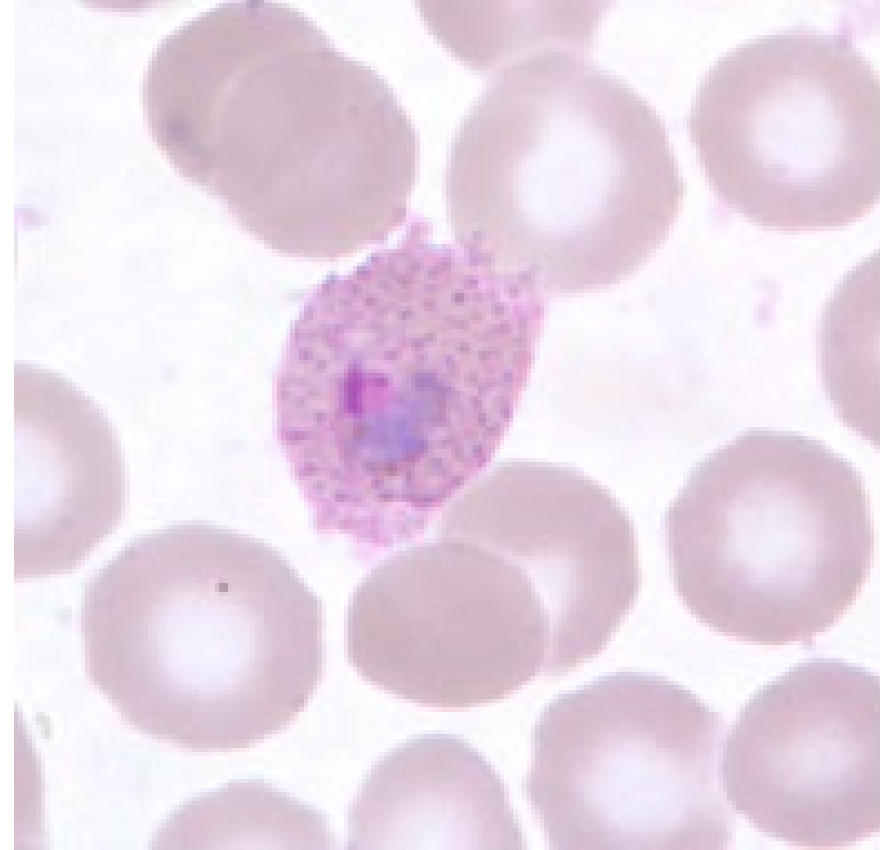
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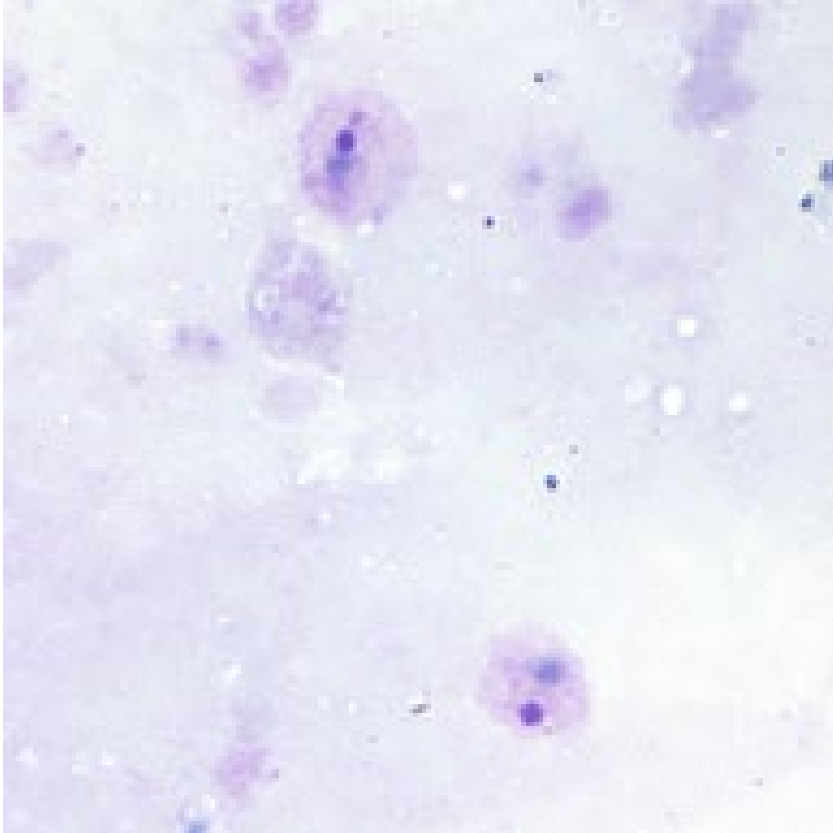
Thick Smear



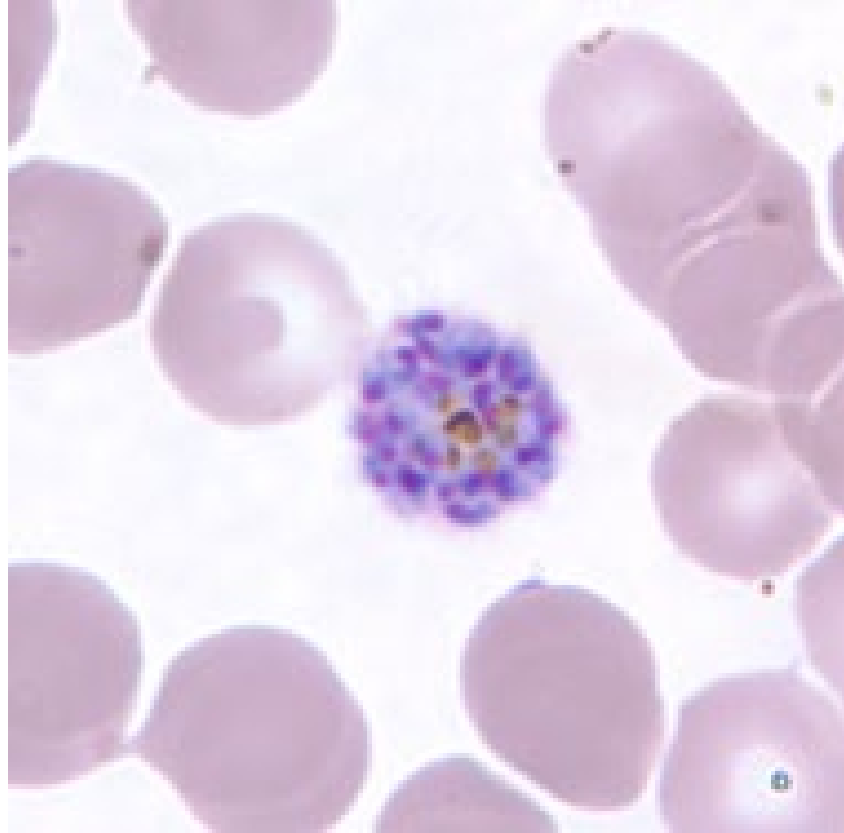
Thin Smear



Blood Parasite



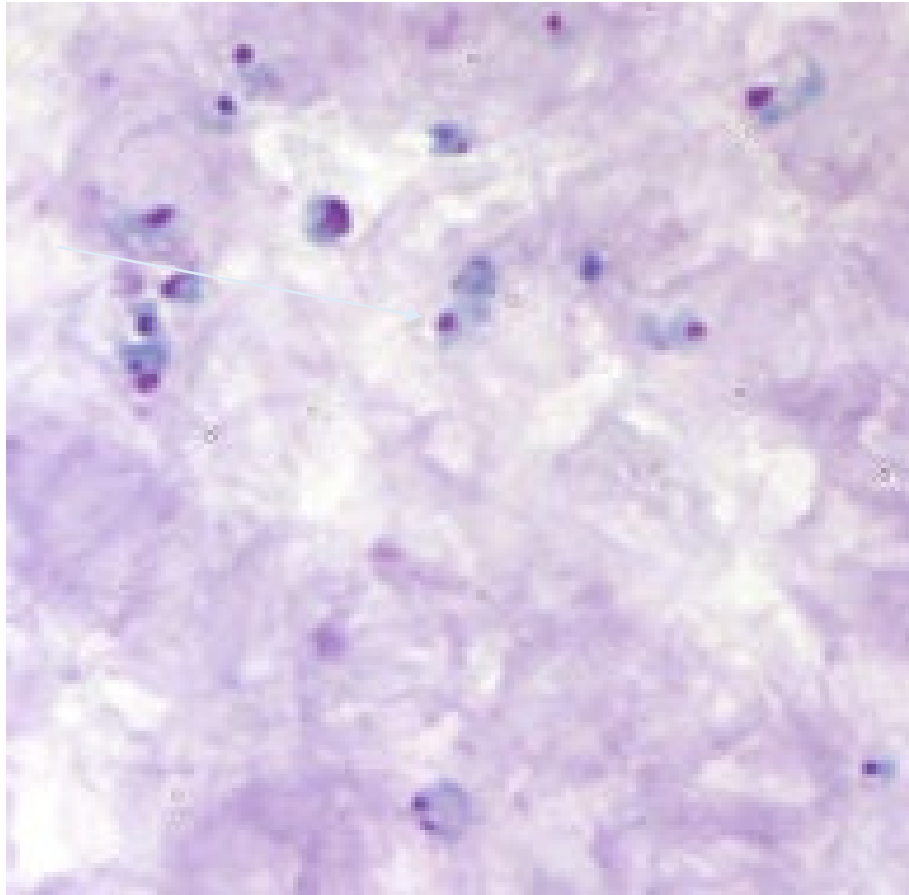
Thick Smear



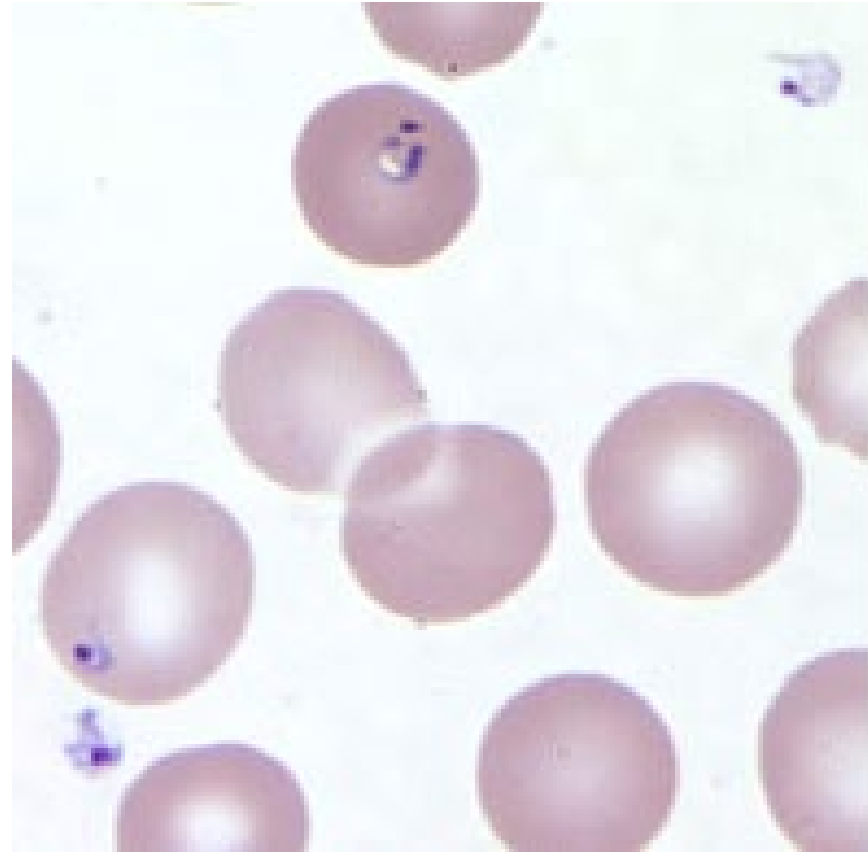
Thin Smear



Blood Parasite



Thick Smear



Thin Smear



Resources

- Mahon, Connie R.; Mahon, Connie R.; Lehman, Donald C.; Lehman, Donald C.. Textbook of Diagnostic Microbiology - E-Book. Elsevier Health Sciences. Kindle Edition.
- Centers for Disease Control. 2019, November 20. *CDC-DPDx-Parasites A-Z Index*. Centers for Disease Control and Prevention.
<https://www.cdc.gov/dpdx/az.html>