بسم الله الرحمن الرحيم

كليـــــة نبتـــــه
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Medical Laboratory Sciences Program

Department of Parasitology

Student Practical Log Book

Semester 4

2018-2019

Name: ……

Student ID: ……

Batch: ……

Prepared by:
Us. Nagat Ibrahim
Us. Ekhlas ahmed

Instructions

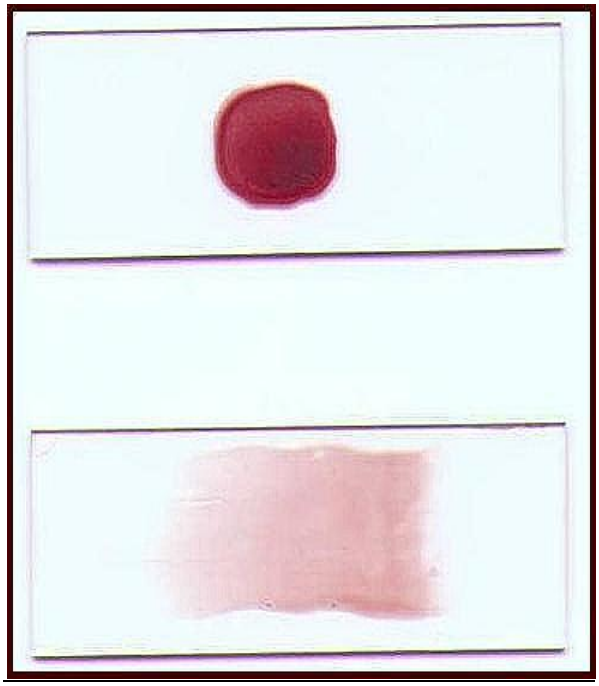
- **Wear gloves when in contact with body fluids, such as serum, plasma, urine or whole blood**
- **Wash your hands when gloves are removed or changed**
- **Perform procedures carefully to minimize aerosol formation**
- **Wear protective clothing such as laboratory coats or aprons when working with specimens**
- **Keep your hands away from your face**
- **Cover all superficial cuts before starting any work**
- **Dispose of specimens and other contaminated materials according to you laboratory's biohazard control procedure**
- **Keep your work area disinfected, disinfect tools and other items that have been in any contaminated area.**
- **Do not eat or drink or apply cosmetics while in the laboratory**

Malaria

Diagnosis of Malaria:

Blood Film For Malaria:

- Thick usually used to detect the malaria parasite. 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
- Thin is used to identify the malaria species.



Giemsa Stain

1. Stock 100× Giemsa Buffer 0.67 M

- Na_2HPO_4 59.24 g
- NaH_2PO_4H2O 36.38 g
- D.water 1000.00 ml

- Autoclave or filter-sterilize (0.2 µm pore). Sterile buffer is stable at room temperature for one year.

Giemsa :

- Buffer 0.0067M, pH 7.2 Stock Giemsa Buffer 10.0 ml Deionized water 990.0 ml Check pH before use. Should be 7.2. Stable at room temperature for one month
- Giemsa stain is available commercially, but the following formulation gives more constant results and does not expire
- Glass beads, 3.0 mm
- 250ml Absolute methanol,
- 250ml Glycerol
- Giemsa stain powder (certified) 3.0 g

Working solution:

RV/o

Calculate using the above formula to make working solution

The standard concentration of Giemsa for working 10%

Counting of malaria parasite

Thick smear:

Method NO (1)

+= 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.

Method NO (2)

$$\frac{\text{The number of parasite} \times 8000}{\text{number of leukocytes}} = \text{parasites per microlitres}$$

Find the proper volume of Giemsa for the different following volume:

1- 100 ml :

……
……
……..

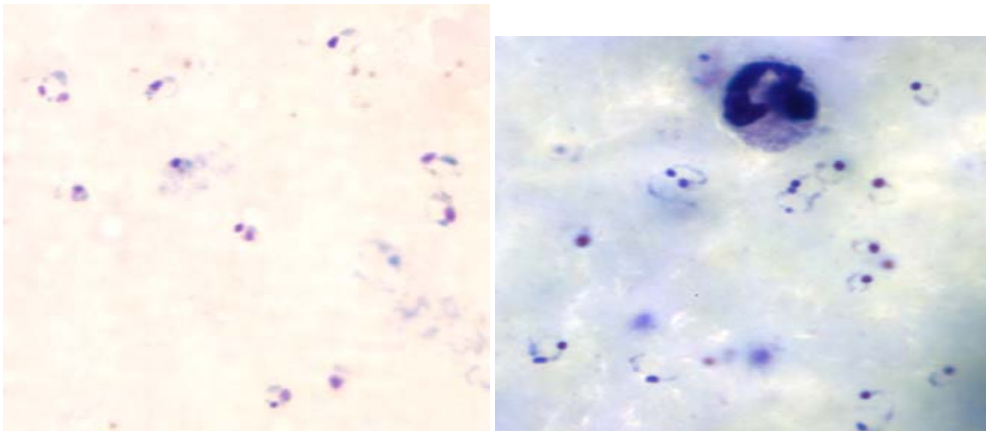
2- 250 ml:

……
……
…….

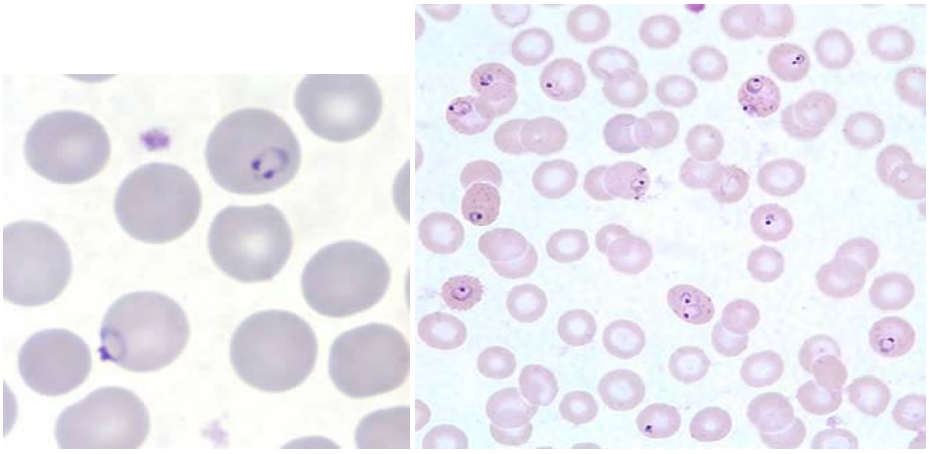
3- 25 ml

……
……
……..

Plasmodium falciparum



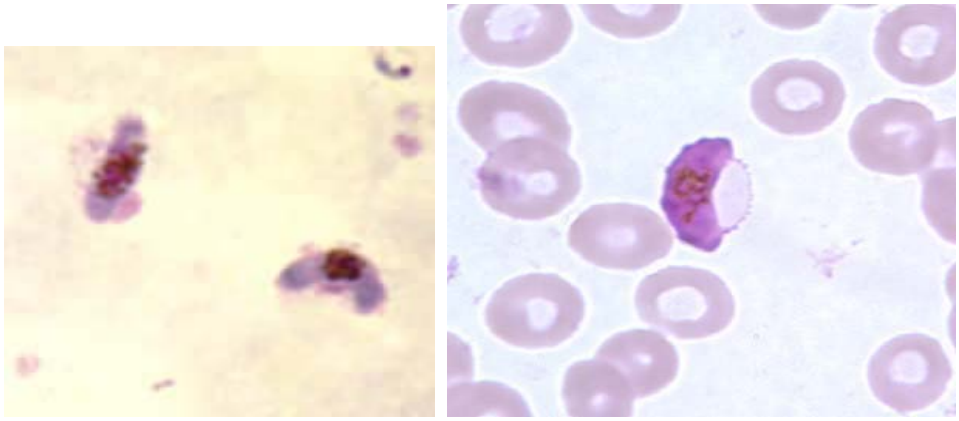
Ring stage of Olasmodium flaciparum in thick blood film



A B

A:Thin, delicate rings in a thin blood smear. Note the double chromatin dot in the infected rbc at top, and the appliqué form in the infected rbc at bottom.

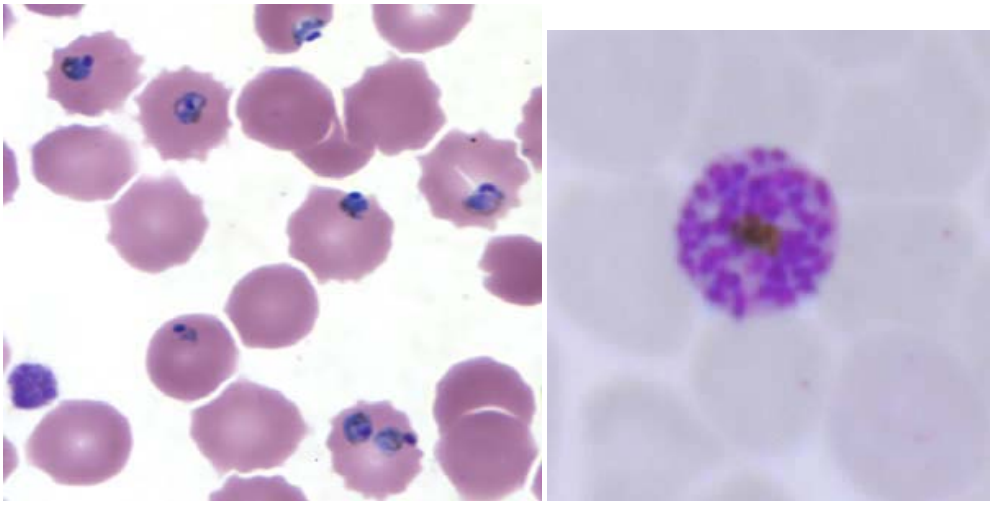
B: Rings and some developing trophozoites seen in thin smears, Note also the presence of Maurer's clefts, which are often seen in older ring forms. Maurer's clefts stain best with an alkaline pH of 7.2—7.6.



A B

A: Gametocytes in a thick blood smear.

B: Gametocyte in a thin smear showing the membrane of the RBCs

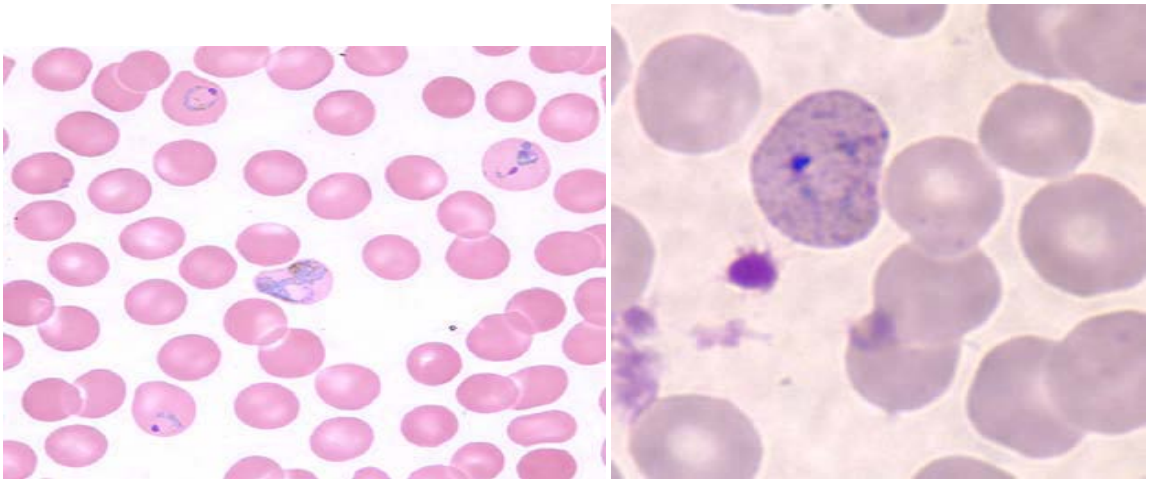


A B

A: Compact trophozoites in a thin blood smear.

B: Mature schizont in a thin blood smear

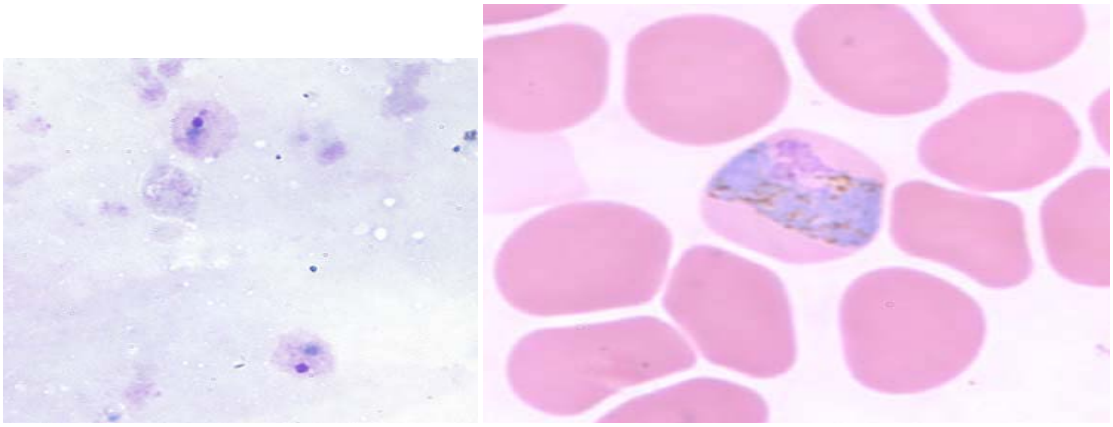
Plasmodium vivax



A B

A: Rings and a trophozoites in a thin blood smear.

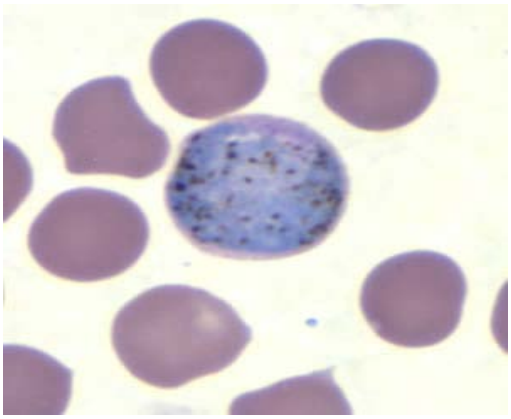
B: Ameboid ring in an enlarged and distorted infected rbc. Schüffner's dots are visible.

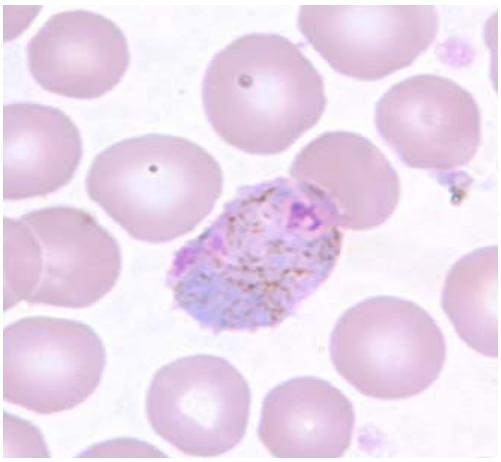


A B

A: Trophozoites in thick blood smears

B: Trophozoite (Band Form)

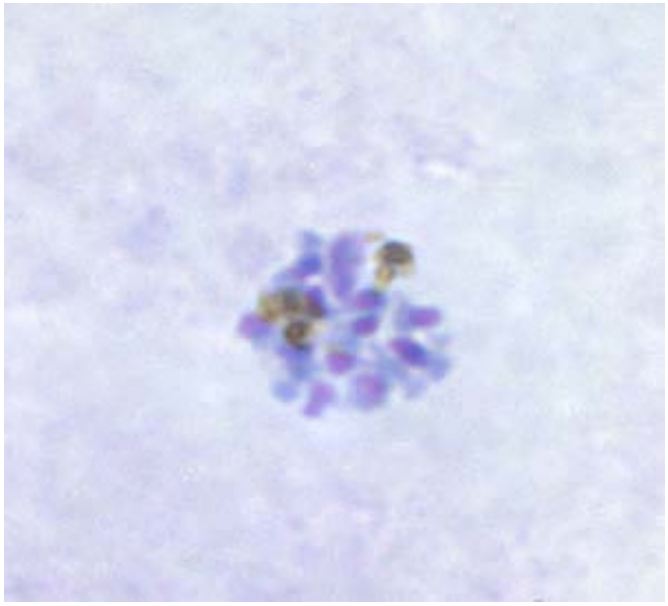




A B

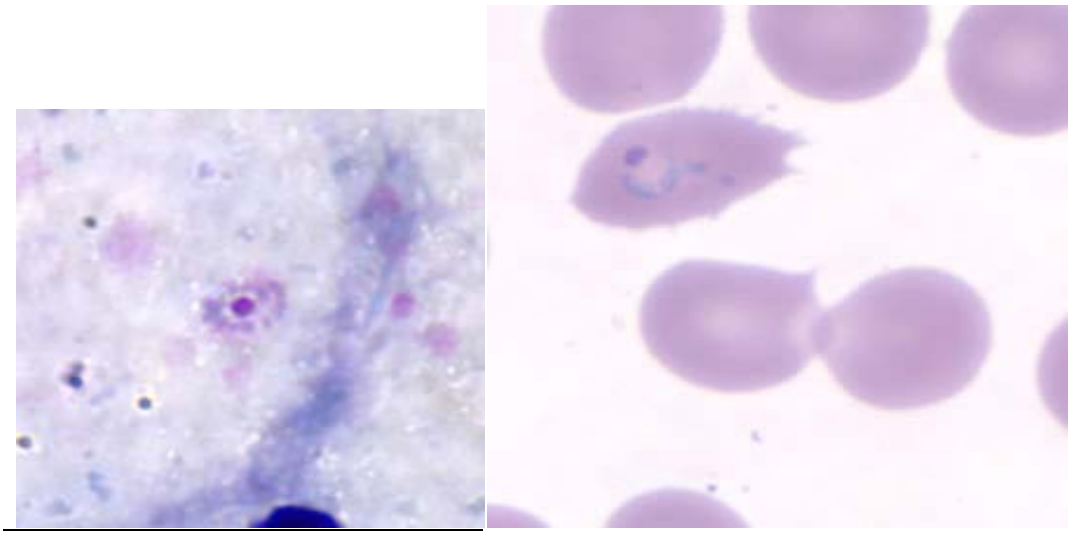
A: P. vivax gametocytes are round to oval with scattered brown pigment and may almost fill the rbc. Schüff-ner's dots may appear more fine in comparison to those seen in P. ovale.

B: Gametocytes in thin blood smears. Note the enlargement of the infected rbc and the scattered pigment.



Schizont in Thick Blood Film

Plasmodium ovale

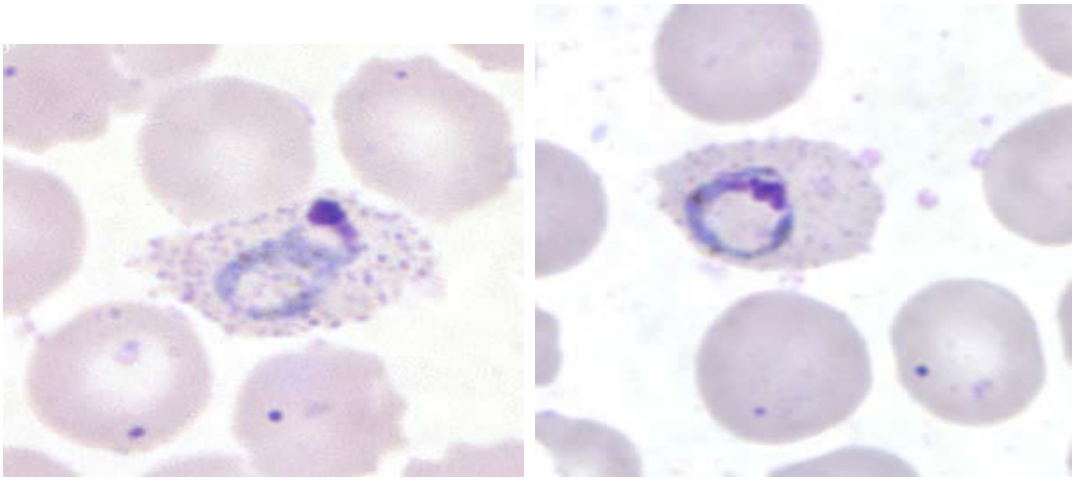


A B

A: Ring in a thick blood smear.

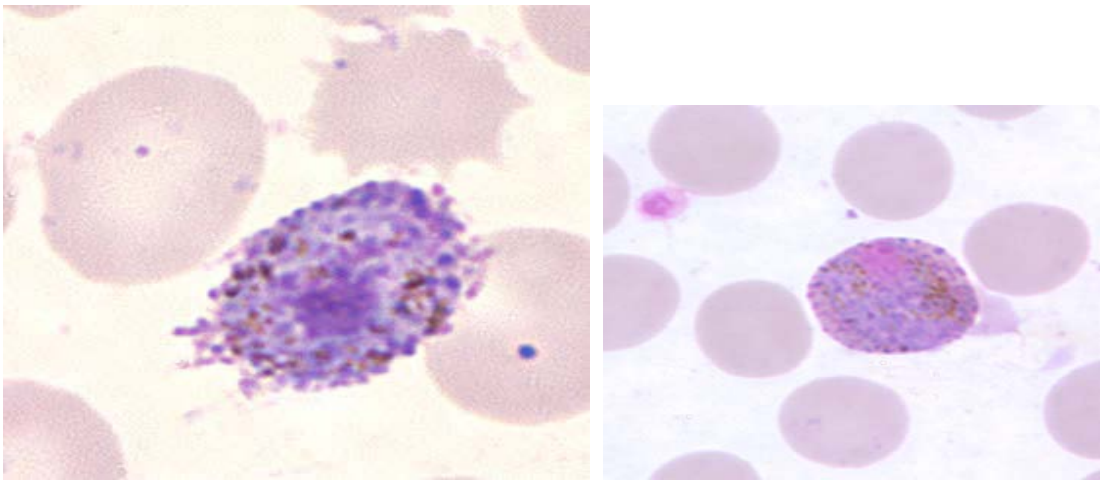
B: Rings in fimbriated RBCs in thin blood smears.

Rings



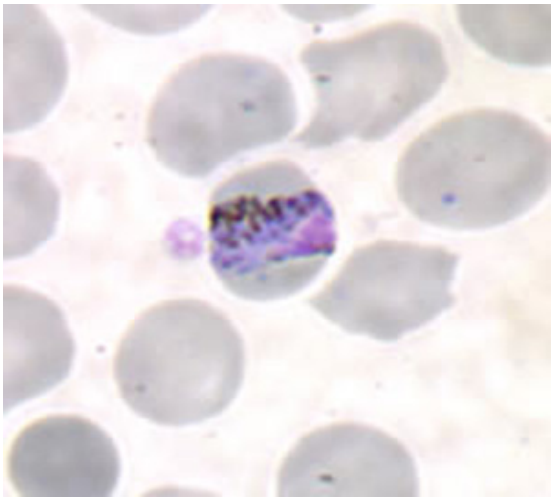
A: Compact trophozoites showing Schüffner’s dots. The image on the left also shows prominent fimbriation.

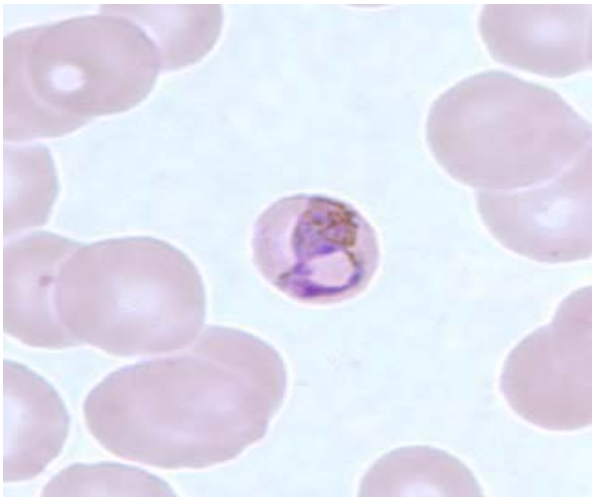
B: Compact trophozoite in a fimbriated rbc in a thin blood smear. Schüffner’s dots are also visible.



Gametocyte in a thin blood smear.

Plasmodium malariae

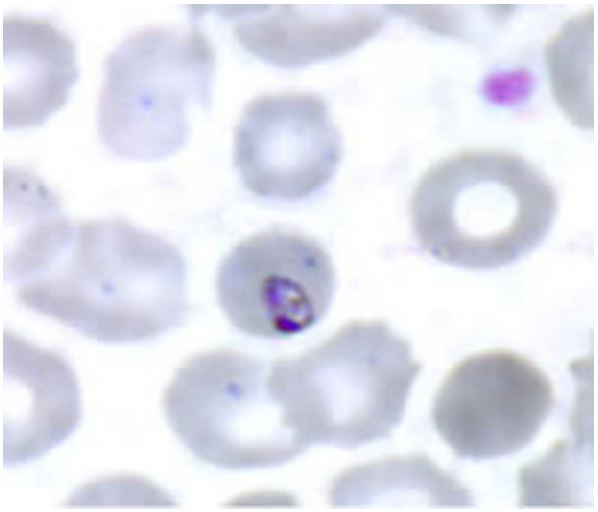


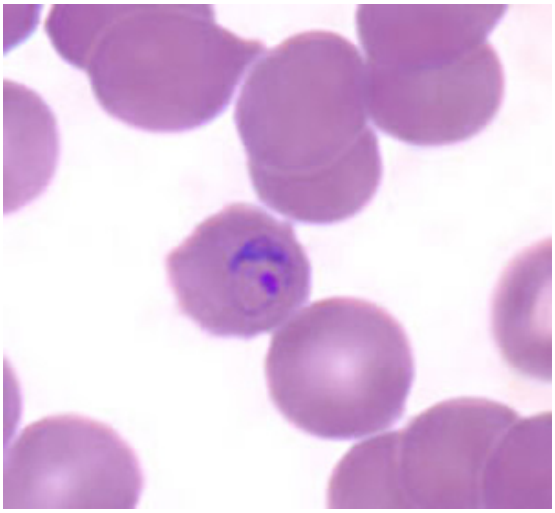


A B

A: Trophozoite of *P.malariae* (Band form).

B: Basket-form" trophozoites in a thin smear.

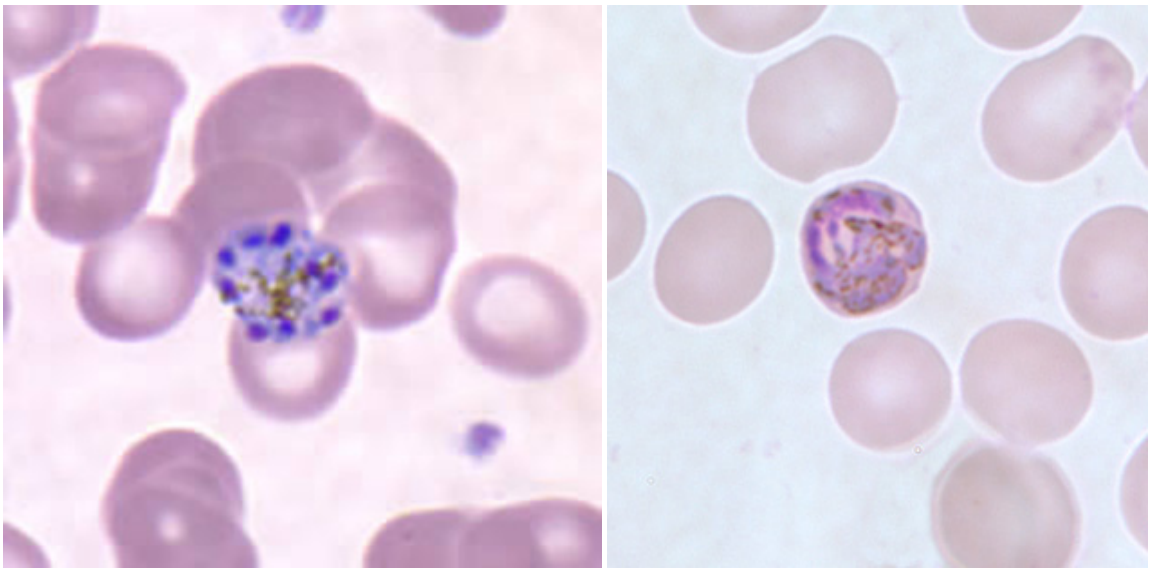




A B

A: Rings in thin blood smears

B: Rings in thin blood smears (Bird eye)

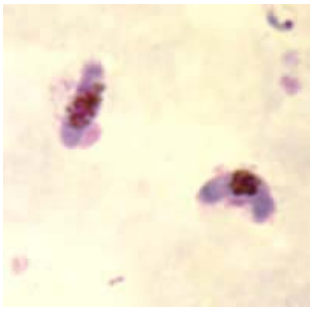


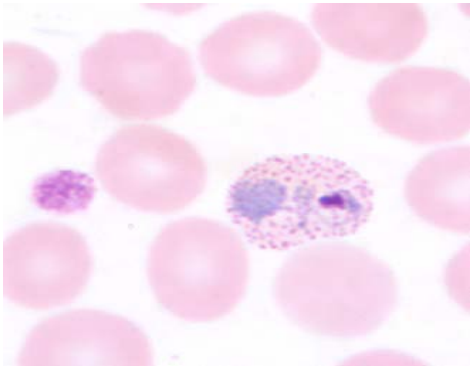
A B

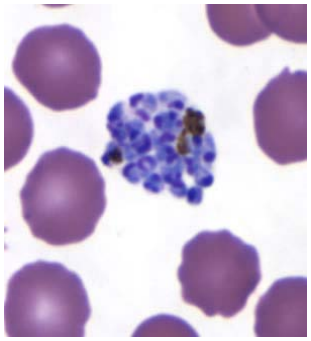
A: Schizonts in thin blood smears. The schizont on the left has the appearance of a rosette pattern.

B: Gametocytes in thin blood smears.

Identify the following parasites:







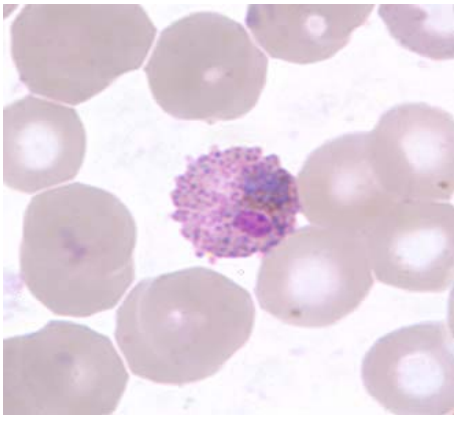
A B C

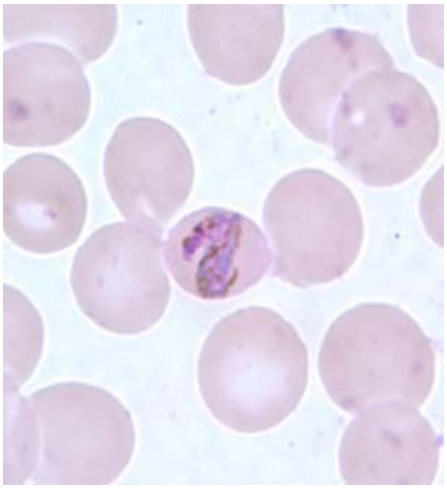
A……

B……

C……

Identify the following parasites:





A B

A……

B……

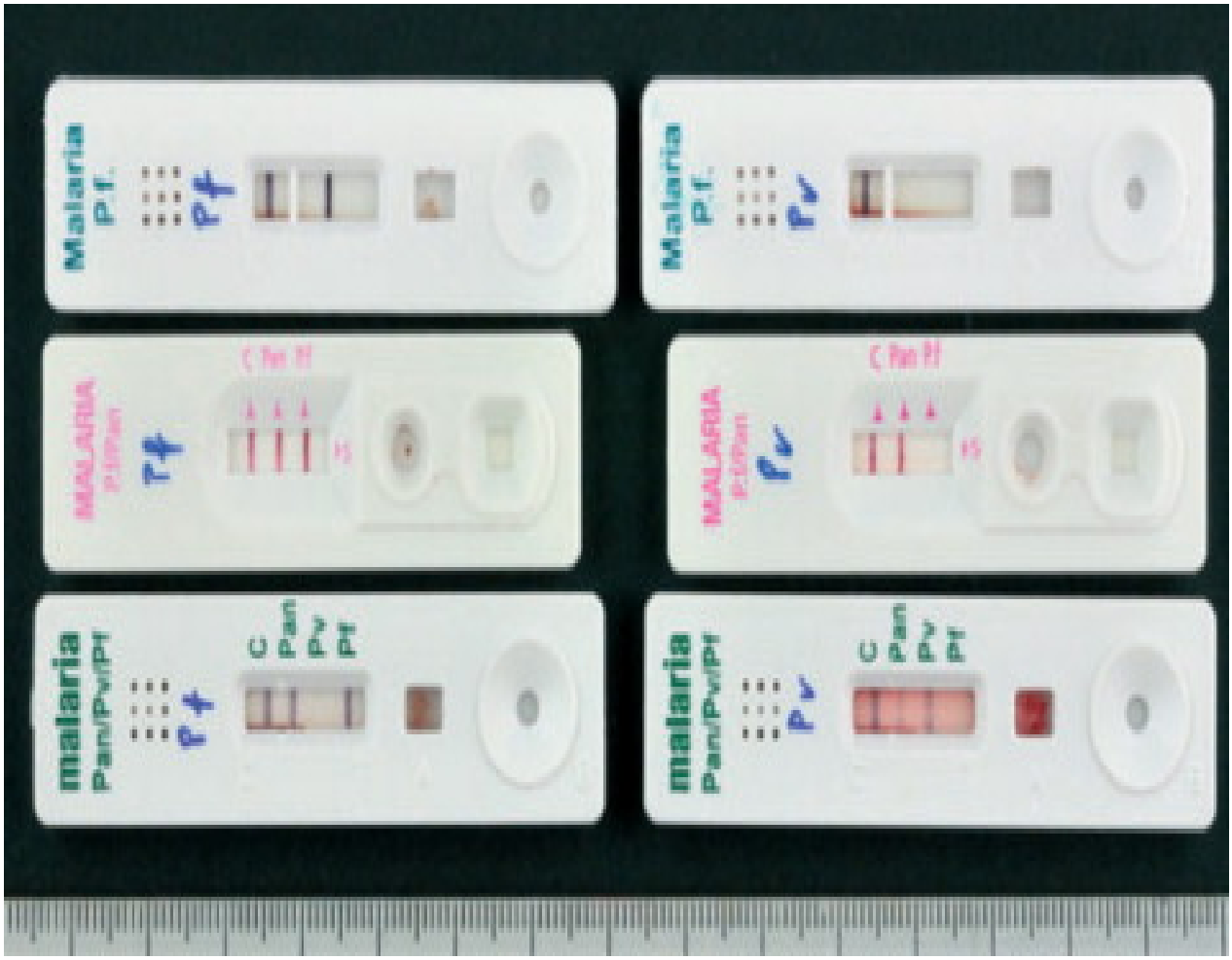
2- **Antigen Detection**

Immuno-chromatographic Test:

- Serological tests most often use a dipstick or cassette format, and provide results in 2-15 minutes.
- Immunochromatographic tests are based on the capture of the parasite antigens from the peripheral blood using either monoclonal or polyclonal antibodies against the parasite antigen targets.

Immunochromatographic tests can target

- The histidine-rich protein 2 of *P. falciparum.*
- A pan-malarial Plasmodium aldolase.
- The parasite specific lactate dehydrogenase.



Leishmaniasis

Cutaneous leishmaniasis

Lab-Diagnosis

1-Leishmanin Skin Test(LST):

Principal:

- Immunological reaction (cellular immunity)
- 0.1-0.2 leishmania antigen injected intradermally.
- After 48-72 will be read.
- Swelling & readness
- Result ≥ 5 → Positive
- Result : Less than 5 → Negative

Write the following LST results:

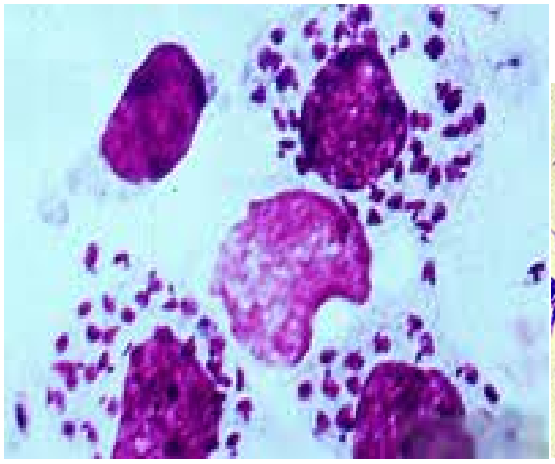
1-6.0 mm……

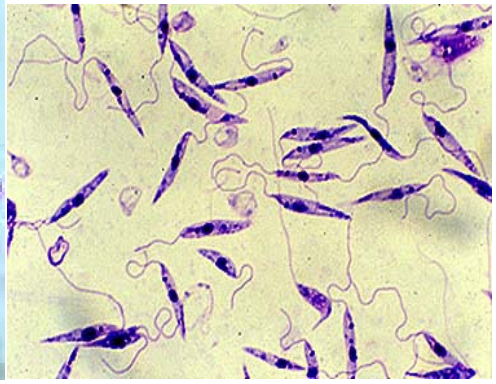
2- 3.0 mm……..

3-7.5 mm …….

2- Scraping from lesion or ulcer.

- (i) Direct examination.
- (ii) Culture (NNN media) or RPMI



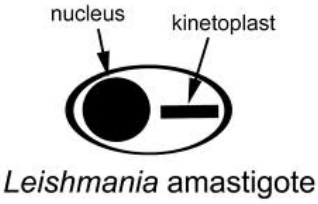


A B

A: Amastigote (Direct smear)

B: Promastigote (Culture)

Amastigote of leishmania



Leishmania amastigote

NNN medium

A B

A: RPMI 1640 for culture of Leishmaniasis

B: Subculture in culture flasks.

Fill the gaps using these words (Solid, liquid,amastigote, promastigote):

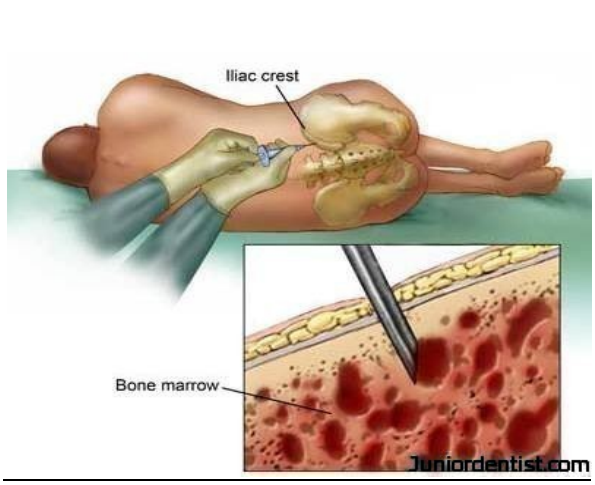
1- RPMI is…….culture medium
2- NNN is …….culture medium
3- From direct sample of bone marrow we can detect……
4- From culture medium we can detect……..

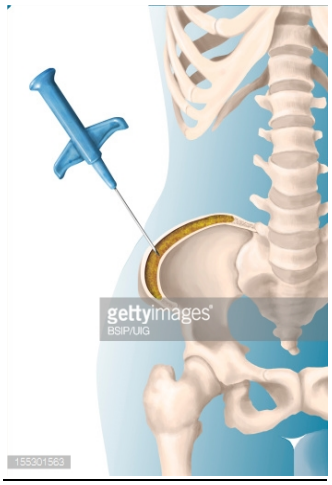
Visceral leishmaniasis

Lab diagnosis

1-Bone marrow Aspiration:

(i) Direct smear
(ii) Culture in NNN or RPMI





2- Serological Test

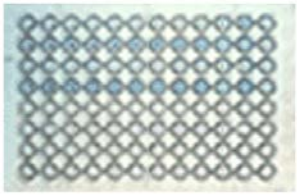
(i) Direct Agglutination Test (DAT):

Direct agglutination test

- Direct agglutination test (DAT) based on agglutination of the **trypsinized whole promastigotes** is useful in endemic regions. Its sensitivity ranges from 91-100% and specificity from 72 to 100%.

(ii)Enzymed Linked Immune Sorbent Assay (ELISA)

DAT test



ELISA test

Answer all of the following questions:

1- What is the principal of DAT

.....
.....

2-Name the Microscope that used to detect RPMI subculture flask

……
……
…

Draw amastige

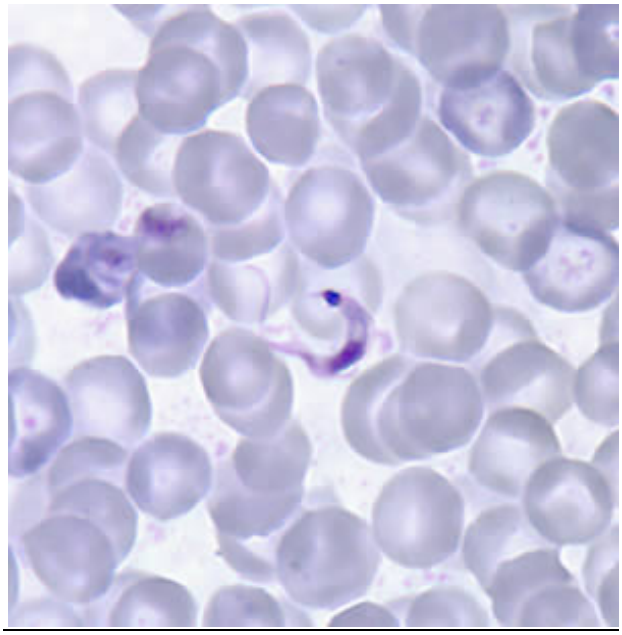
Draw promastigote

Trypanosomiasis

African trypanomiasis

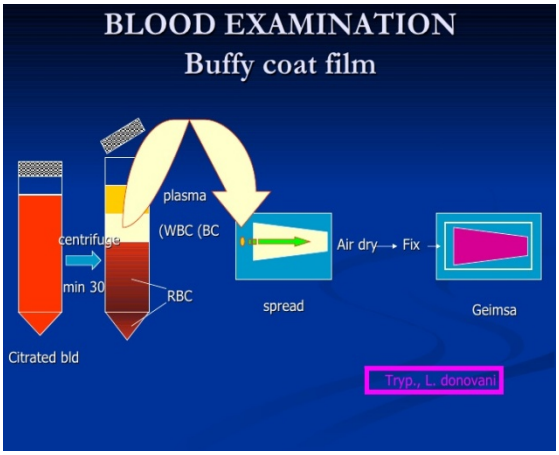
1- Blood Examination

(i) Direct examination

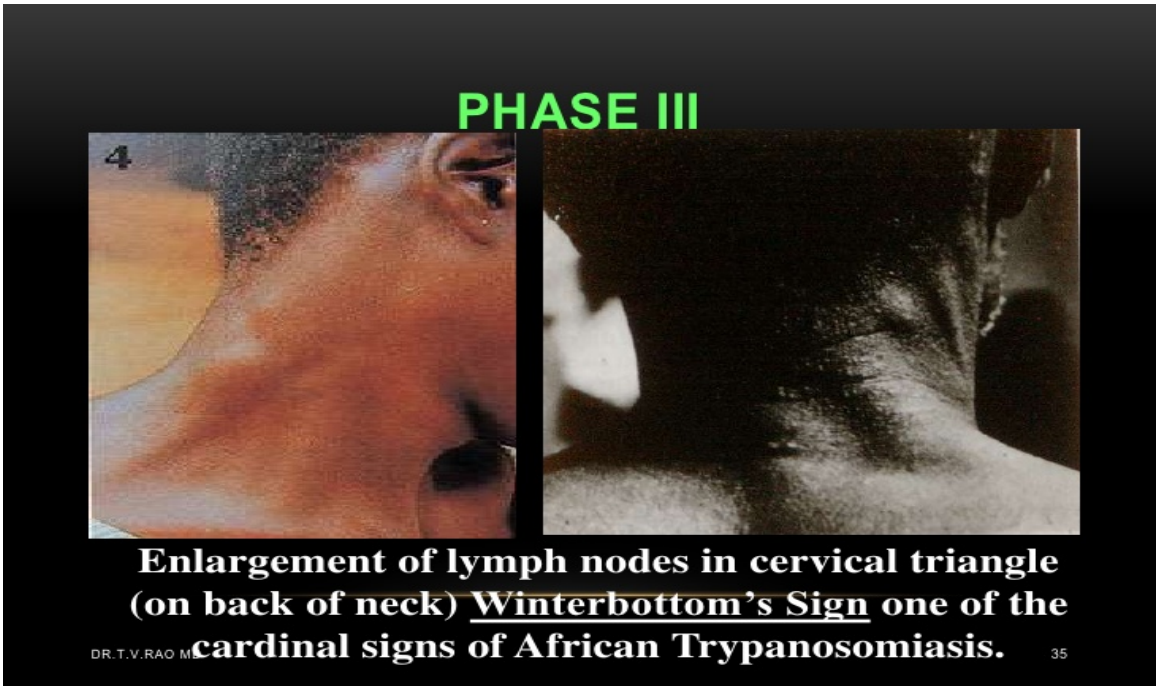


T. cruzi trypomastigote in a thin blood smear stained with Giemsa. Note the typical C-shape of the trypomastigote that characterizes T. cruzi in fixed blood smears.

(i) Concentration technique: capillary method



2- Lymph node Aspiration:



3- Cerebrospinal fluids (CSF).

Fill the gabs:

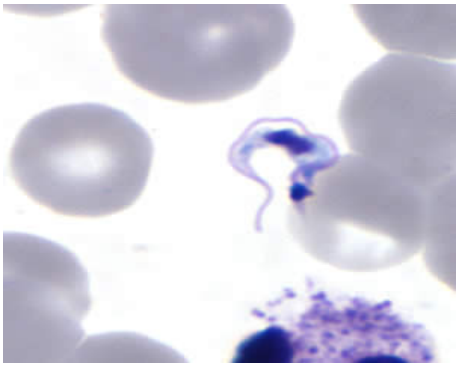
In CSF examination we find the glucose……..and protein……

IgM is……..

American trypanosomiasis

Lab diagnosis

1- Blood examination to detect Trypomastigote



2-Bone marrow aspiration , Lymph node Aspiration to detect amastigote

Trypanosoma cruzi amastigotes in heart tissue. The section is stained with hematoxylin and eosin (H&E)

3-Xenodiagnosis test:

Diagnosis (Xenodiagnosis)

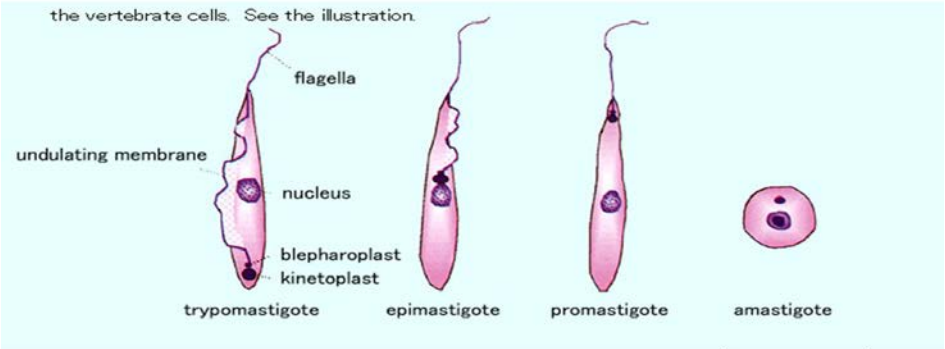
Highly efficient – demonstrate low level of parasite in blood

Method:

A Laboratory bred winged bug is starved for 2 weeks then fed on suspected patient's blood – 30 days later, it faeces & gut examined for trypanosomes.

Dr. RAAFAT T. MOHAMED

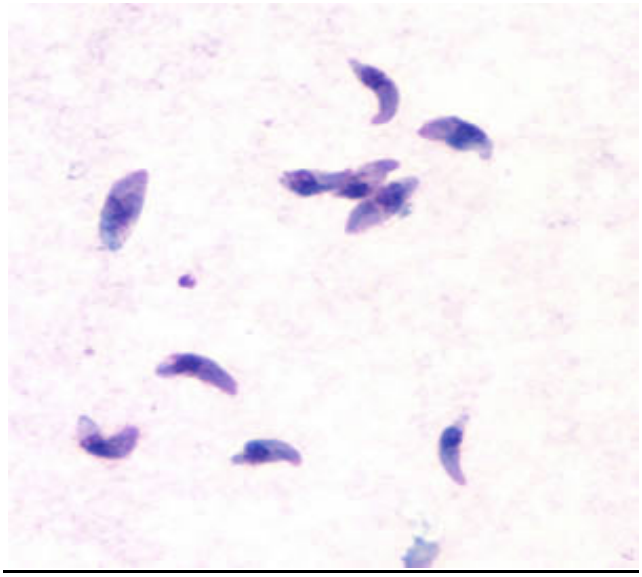
Different stages of Haemoflagellates



Toxoplasmosis

Lab diagnosis

1- Lymph nodes Aspiration or peritoneal fluid



Toxoplasma gondii tachyzoites, stained with Giemsa, from a smear of peritoneal fluid obtained from a laboratory-inoculated mouse

2- Serological test

(i)Complement Fixatin test

(ii) Sabin Feldmann Dye Test

Draw Tachyzoite

Draw bradyzoite