

# **Parasitology Lab**

**CLS 417- Clinical Practice in Microbiology**

**Miss Zeina Alkudmani**

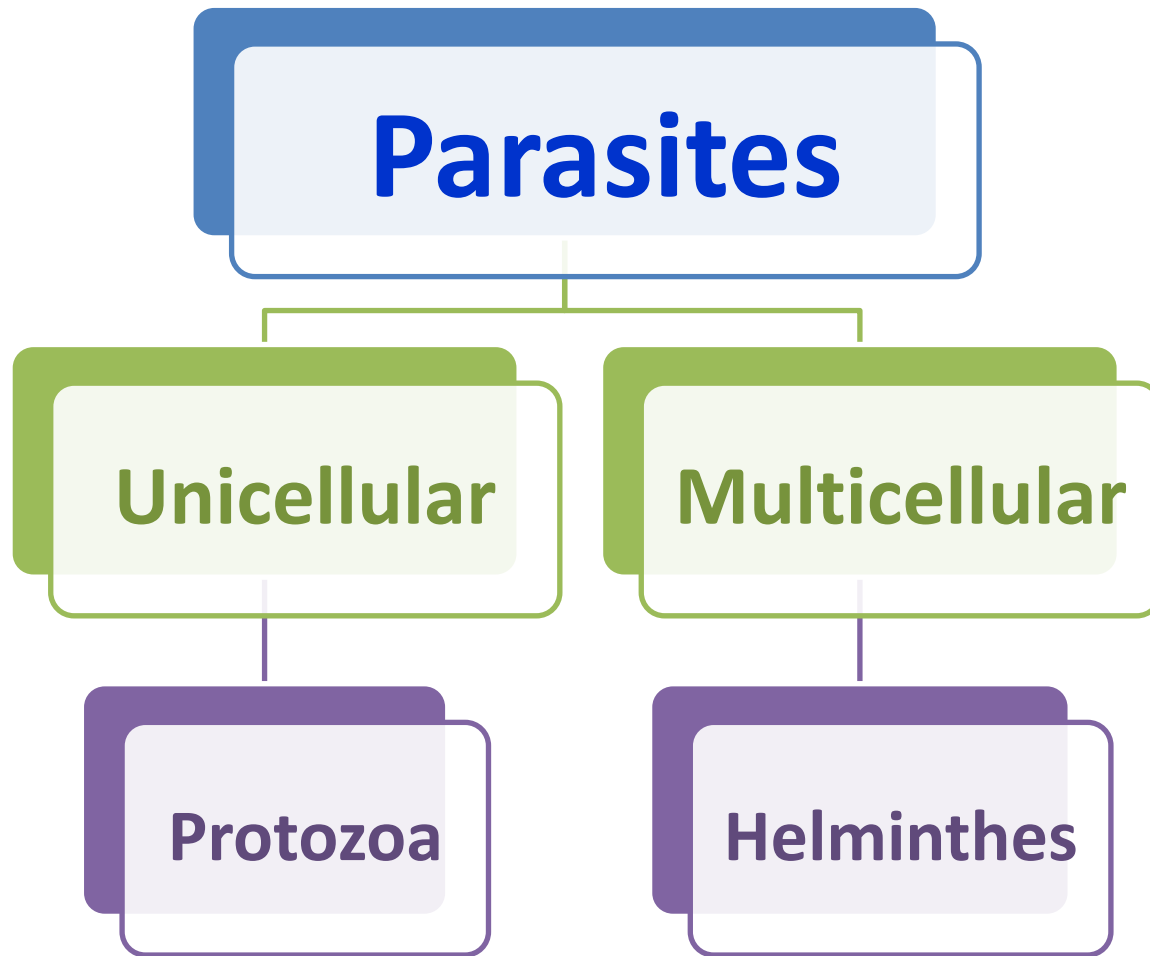
# Topics to be Covered in this Lecture:

- Types of specimens received in parasitology lab.
- Classification of parasites.
- Most common isolated parasites.
- Examination of stool sample: macroscopy, microscopy + stains.
- Lab Diagnosis of Most Common Parasitic Infections.
- Other methods for detecting parasitic infections.
- Occult blood test.

# Types of Specimen

- Stool for intestinal parasites.
- Urine for *Schistosoma* eggs.
- Blood for Malaria.
- Blood & CSF for *Trypanosomes*.
- Blood for Microfilaria.
- Bile of duodenal aspirate
- Corneal scraping, Contact lens and solution
- Pinworm or scotch tape preparation
- Skin fluid / Tissue juice
- Skin: *Onchocera microfilariae* / Tissue / Biopsy
- Worm / Insect
- Respiratory- sputum for *Paragonimus* eggs.

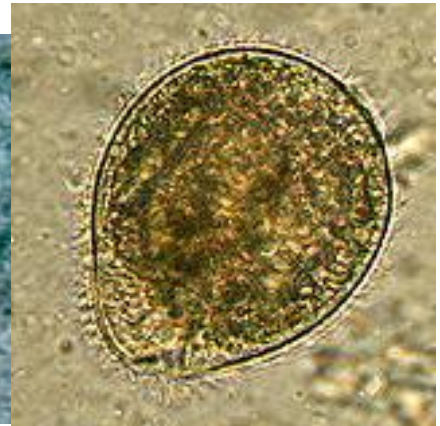
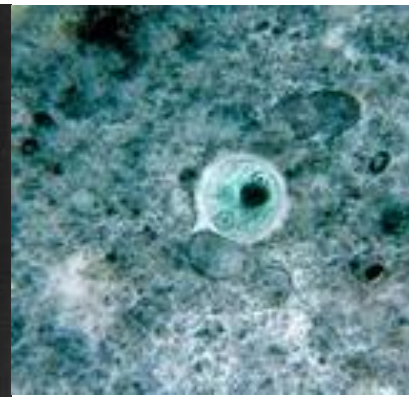
# Classification of Parasites



# Protozoa

Protozoa are classified according to the method of movement:

1. **Amoebae** (e.g. *Entamoeba histolytica*).
2. **Flagellates** (e.g. *Giardia lamblia*, *Trichomonas vaginalis*, *Trypanosoma* sp., *Leishmania* sp.).
3. **Sporozoans** (e.g. *Plasmodium* sp., *Toxoplasma gondii*).
4. **Ciliates** (e.g. *Balantidium coli*).



# Helminthes

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graph TD; Helminthes[Helminthes] --> RoundWorms[Round Worms]; Helminthes --> FlatWorms[Flat Worms]; RoundWorms --> Nematodes[Nematodes]; Nematodes --> Ascaris[Ascaris lumbricoides]; Nematodes --> Enterobius[Enterobius vermicularis]; Nematodes --> Trichuris[Trichuris trichiura]; Nematodes --> Strongyloids[Strongyloids stercoralis]; FlatWorms --> Cestodes[Cestodes (tapeworms)]; FlatWorms --> Trematodes[Trematodes (Flukes)]; Cestodes --> Taenia[Taenia sp.]; Trematodes --> Schistosoma[Schistosoma];
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## Round Worms

### Nematodes

*Ascaris lumbricoides*  
*Enterobius vermicularis*  
*Trichuris trichiura*  
*Strongyloids stercoralis*

## Flat Worms

Cestodes  
(tapeworms)  
*Taenia sp.*

Trematodes  
(Flukes)  
*Schistosoma*

# 线虫

Nematode



似蚓蛔线虫（蛔虫）

*Ascaris lumbricoides*



毛首鞭形线虫（鞭虫）

*Trichuris trichiura*



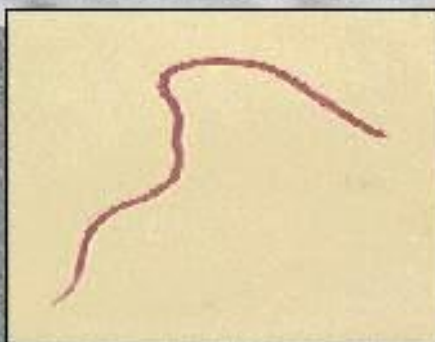
蠕形住肠线虫（蛲虫）

*Enterobius vermicularis*



钩虫

Hookworm



旋毛形线虫（旋毛虫）

*Trichinella spiralis*



丝虫

Filaria



# 绦 虫

Tapeworm (cestode)



曼氏迭宫绦虫

**Spirometra  
mansoni**



链状带绦虫  
(猪带绦虫)

**Taenia solium**



肥胖带绦虫  
(牛带绦虫)

**Taenia saginata**



微小膜壳绦虫

**Hymenolpis  
nana**



细粒棘球绦虫  
(包生绦虫)

**Echinococcus  
granulosus**



# 吸虫

Trematode



华枝睾吸虫  
(肝吸虫)

**Clonorchis  
sinensis**

布氏姜片虫  
(肠吸虫)

**Fasciolopsis  
buski**

卫氏并殖吸虫  
(肺吸虫)

**Paragonimus  
westermani**

斯氏狸殖吸虫

**Pagumogonimus  
skrjabini**

日本血吸虫  
(血吸虫)

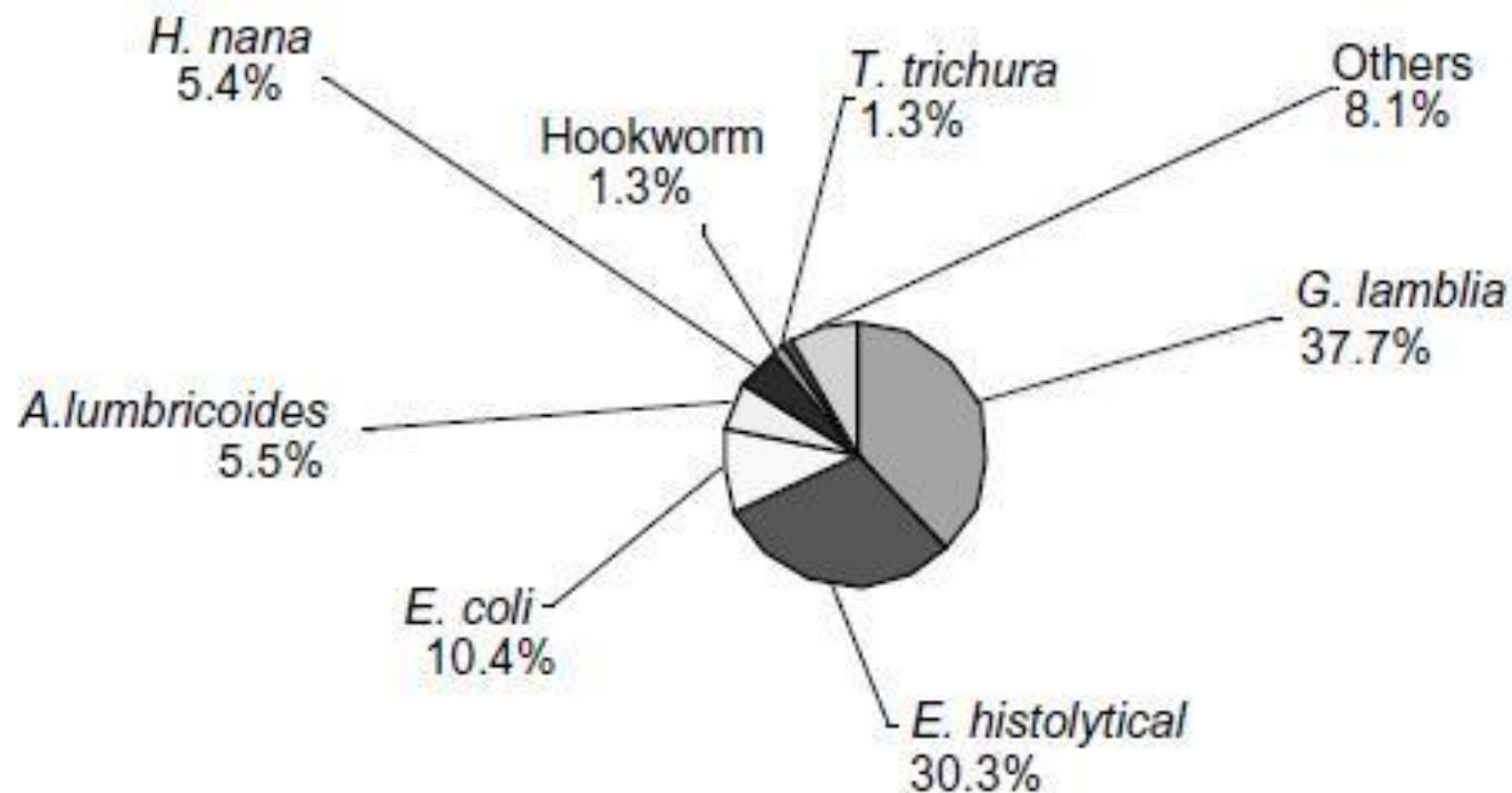
**Schistosoma  
japonicum**

# Most Common Parasites Isolated by Ministry of Health

- Bilharzia
- Leishmaniasis
- Malaria
- Toxoplasmosis

# Most Common Parasites Isolated from Stool in KKUH 2006

1. *Giardia lamblia*
2. *Hook worm*
3. *Trichuris trichiura*
4. *Ascaris lumbricoides*
5. *Entamoeba histolytica*
6. *Enterobius vermicularis*
7. *Hymenolepis nana*
8. *Strongyloids stercoralis*
9. *Schistosoma mansoni*



**Figure 1** Types of intestinal parasites among infected persons in Riyadh, Saudi Arabia 1999.

# Examination of Stool

- Macroscopic Examination
- Concentration techniques: sedimentation vs. floatation
- Microscopic- Wet Preparation
- Iron haematoxylin
- Modified Acid Fast for coccidia

# Macroscopic Examination of Stool

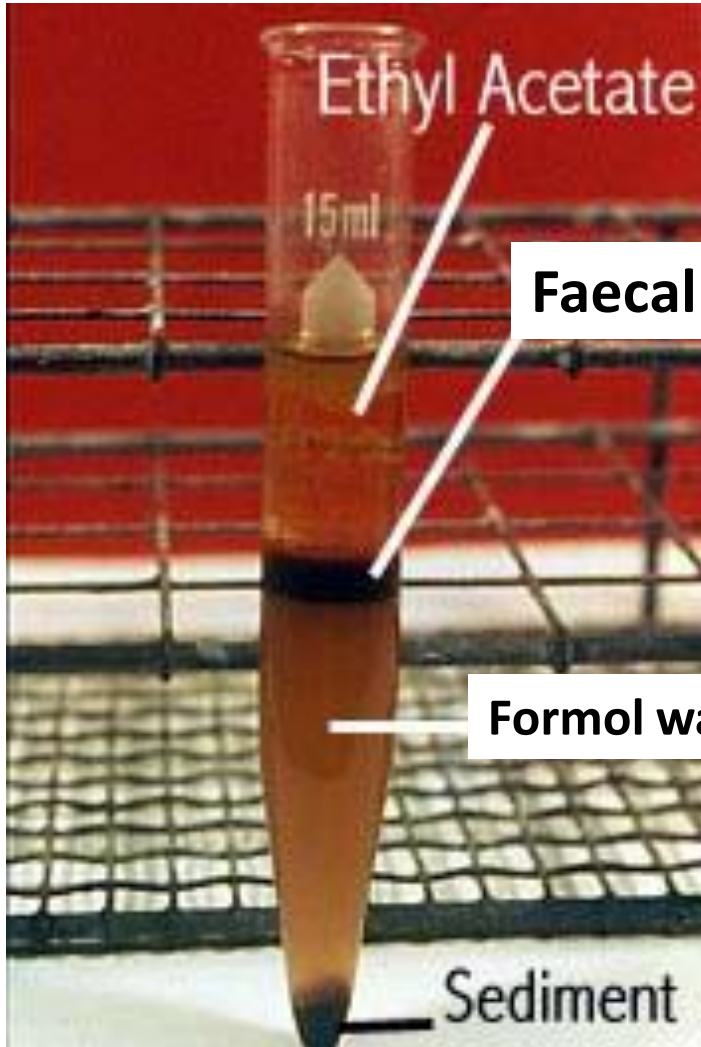
## Describe the appearance of the specimen:

- ✓ Color of the specimen.
- ✓ Consistency: formed, semifformed, unformed, or watery (fluid).
- ✓ Presence of blood, mucus or pus.
- ✓ Presence of fat: Giardiasis infection.
- ✓ Presence of worms.

# Concentration Technique: Formalin-Ether

- Use stool in sodium acetate formalin (SAF) preservative.
- Strain to remove large faecal particles.
- Repeat: centrifuge and washing with saline.
- Use sediment for permanent stain smears.
- Add formalin, ether (or ethyl acetate) to the remaining sediment, centrifuge, make thin smear, and examine 10x and 40x.





# Floatation Techniques

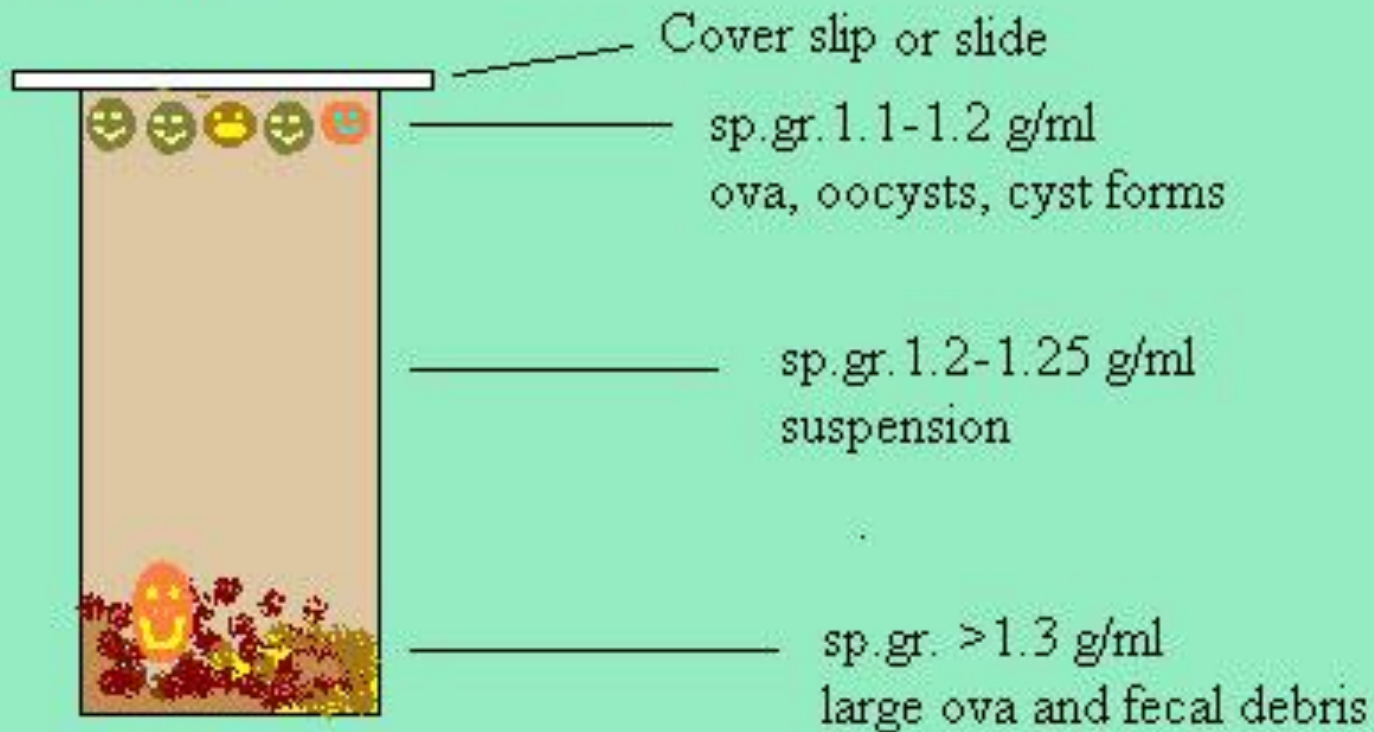
- Zinc Sulphate.
- Saturated Sodium Chloride.

## Principle

The solution used has a specific gravity. Part of the stool specimen is emulsified in the solution and left undisturbed for the eggs and cysts to float to the surface. Then they are collected by cover glass or glass slide.

# Zinc Sulphate Floatation

## Fecal flotation



# Wet Preparation

1. Place one drop of **saline (0.85% NaCl)** on the left side of the slide and one drop of **iodine** on the right side of the slide.
  2. Take a small amount of fecal sediment and thoroughly emulsify the stool in saline and iodine using an applicator stick. The sample should be spread thinly.
  3. Cover slip, scan the entire area with the 10x objective. Then switch to 40X objective to look for eggs or protozoa.
- **If the specimen is watery or unformed, don't add saline & examine directly. Then use eosin instead of iodine.**

# Saline-Iodine Wet Smear

Fig. 1

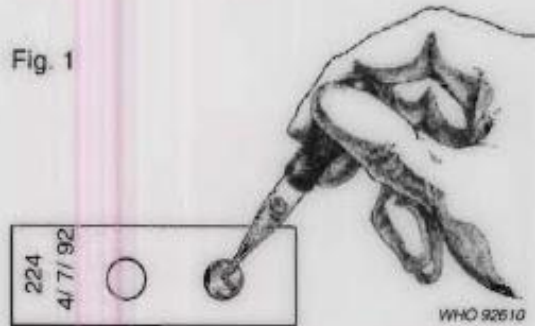


Fig. 2

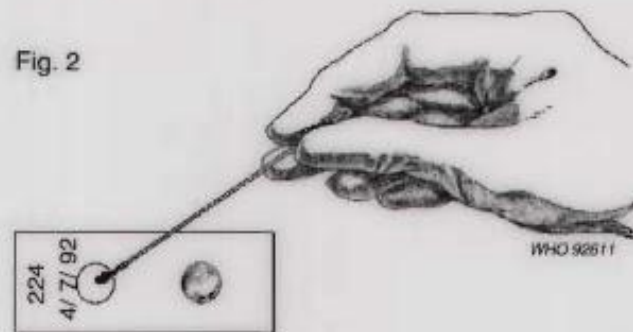


Fig. 3

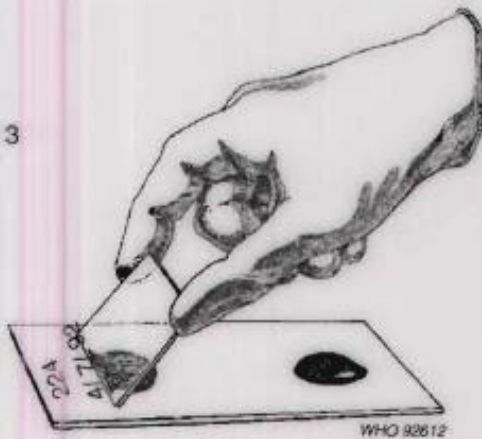
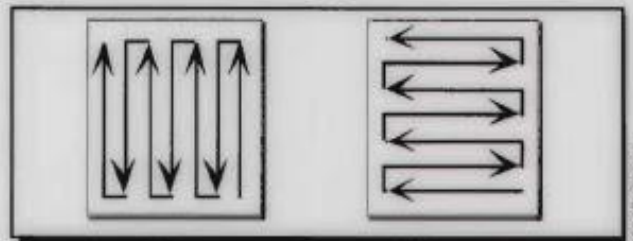


Fig. 4



**12-C THE ATLANTA CONSTITUTION, Thu**

<p><b>Unfurn. Apts., Duplexes 635</b></p> <p>MARIETTA - New duplex, 3 BR., 1 1/2 BA., fully equipped, dishwasher, disposal, washer-dryer, full attic, 425 month. FARMER McCREARY, 304-473 weekdays, after 5:00, weekends 422-6011</p> <p><b>Unfurn. Apts., Duplexes 635</b></p>	<p><b>Unfurn. Apts., Du</b></p> <p><b>Mablet Villag</b></p> <p>1 AND 2 BR. - Adj. shag carpet, full elec. Adjacent to Hawthorn Center. 948-6451</p> <p><b>MT. LEON APTS. - 1</b> Rd., N.E., 2 BR., carpet</p>
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**What Makes Quail Creek Quail Creek?**

# Reporting Stool Wet Smear

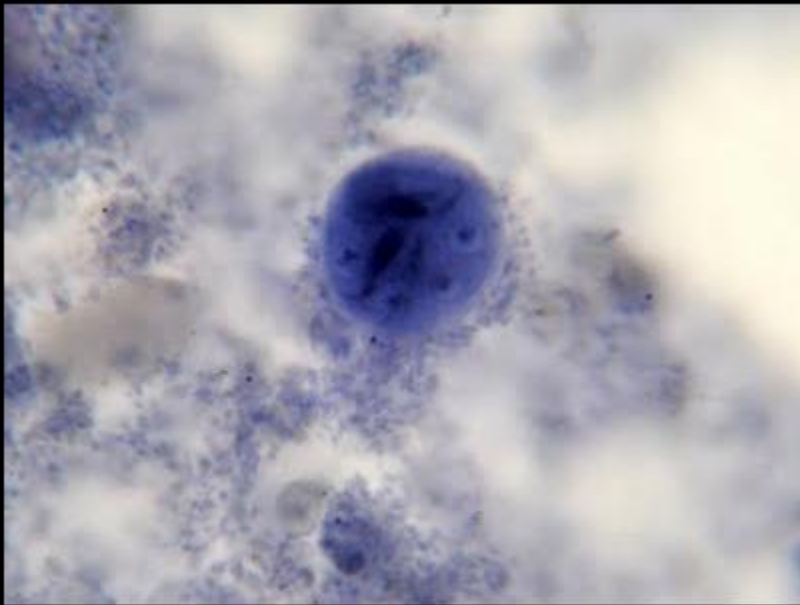
- ✓ Examine microscopically for:
  - **Motile parasites:** *E. histolytica*, *G. lamblia*.
  - **Eggs of helminthes.**
  - **Cycts and oocysts of protozoa.**
- ✓ Report the no. of larvae and each species of egg found in the entire saline as follows:
  - **Scanty:** 1-3 /preparation
  - **Few:** 4-10 / prep.
  - **Moderate:** 11-20 / prep.
  - **Many:** 21-40 /prep.
  - **Very high:** > 40 / prep.

# Permanent Stain Smears Method

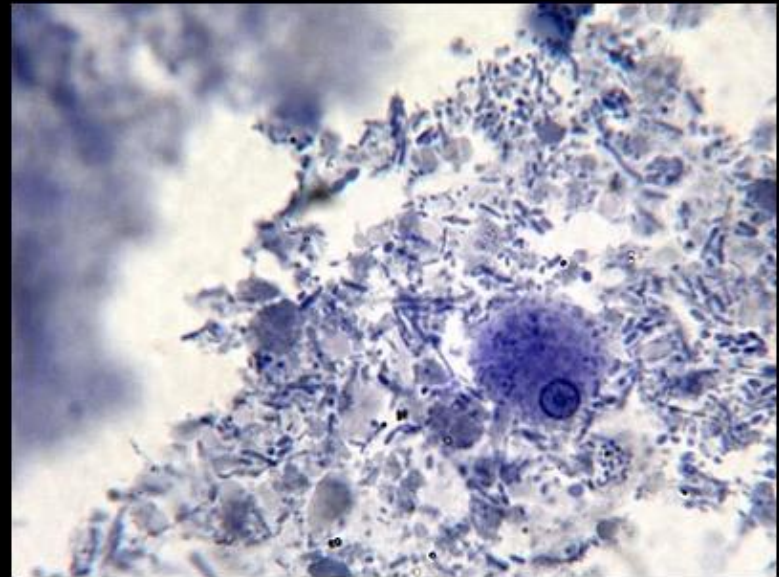
- Permanent stain smears are used primarily for the identification of trophozoites, occasionally cysts, and for the confirmation of species.
- Small organisms missed by other examinations may be found on stain smears.
- Although experienced microscopists can identify certain organisms on wet prep most identification should be considered tentative until confirmed by a permanent stained slide.
- **Permanent stains include:**
  - ✓ **Iron hematoxylin:** intestinal protozoa
  - ✓ **Modified acid fast:** coccidia oocysts including *Cryptosporidium*, *Isospora* and *Cyclospora* species.



# *Iron Haematoxylin Stain*

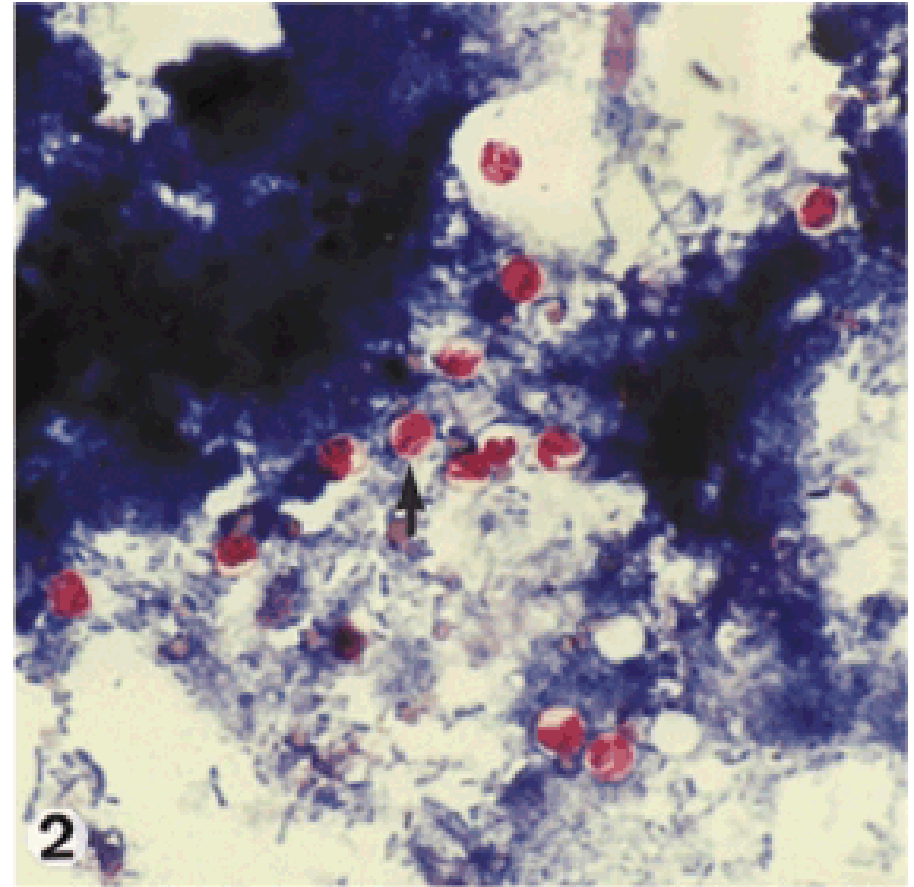
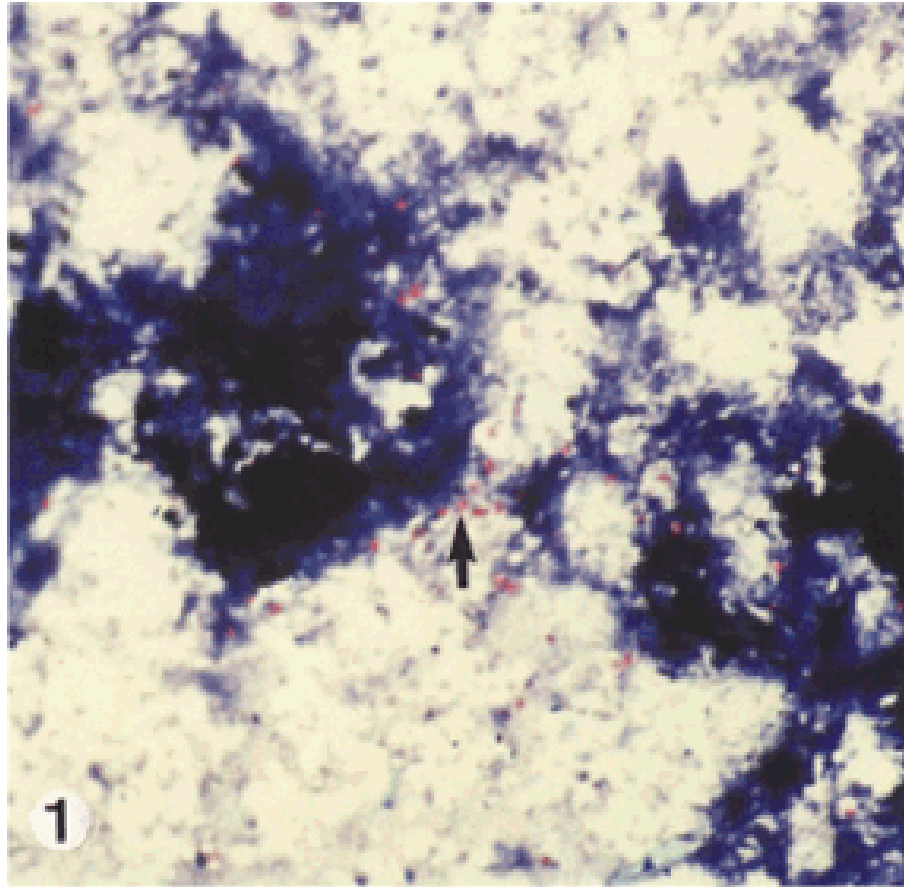


*E. histolytica* cyst showing 4 nuclei and chromatoidal bars with rounded ends.



*E. histolytica* trophozoite; Iron Hematoxylin Stain (1000X)

# *Modified AF Staining*



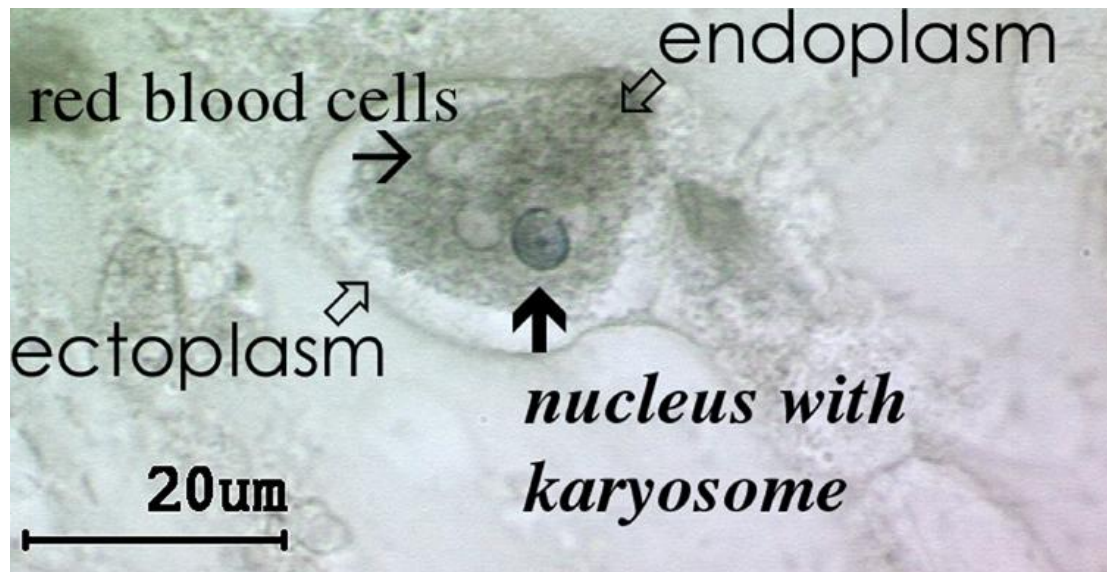
**Fig. 1 and 2 - *Cryptosporidium* spp oocysts in fecal smear. Acid-fast staining using alcohol-chloride acid solution(250x and 1000x).**

# **Lab Diagnosis of Most Common Parasitic Infections**

# *Entamoeba histolytica*

Lab diagnosis of amoebic dysentery is by finding *E. histolytica* trophozoites in fresh bloody stool sample or rectal scrape.

Red cells in trophozoites

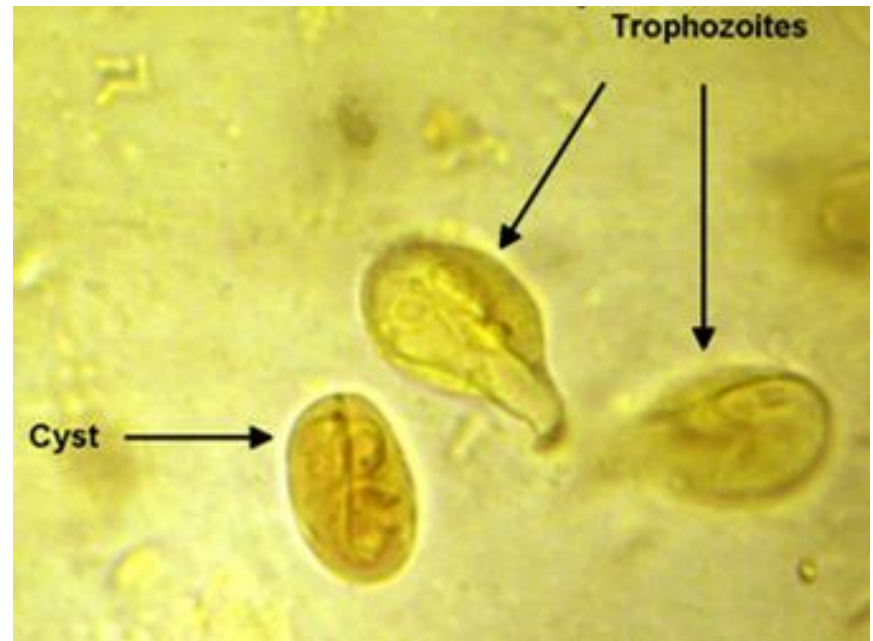


1-4 nuclei in cyst



# *Giardia Lamblia*

- Pale, fatty, and float in water stool sample.
- Lab diagnosis of giardiasis is by finding:
  - ✓ *G. Lamblia* trophozoites in fresh diarrhoeic sample specifically in mucus.
  - ✓ *G. Lamblia* cyst in formed specimens after concentration technique.

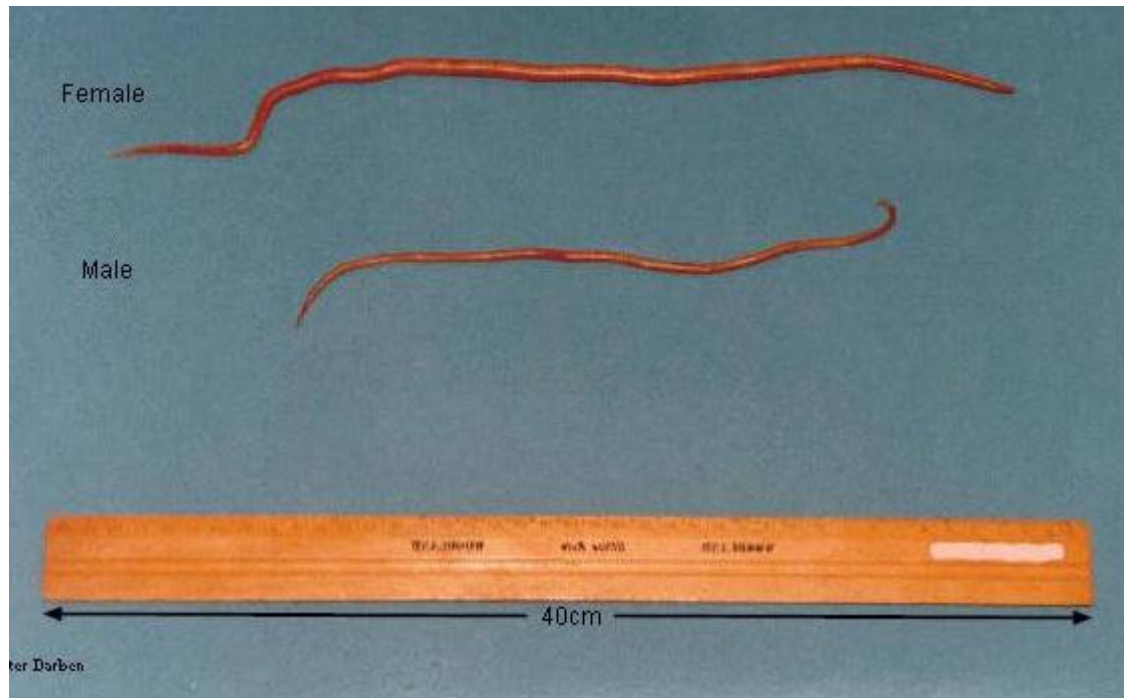
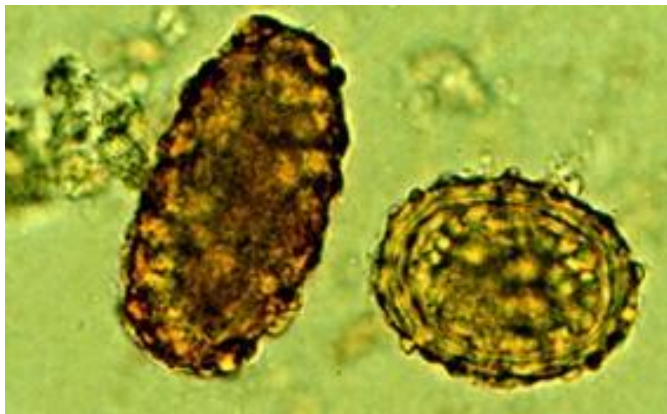




# *Ascaris lumbricoides*

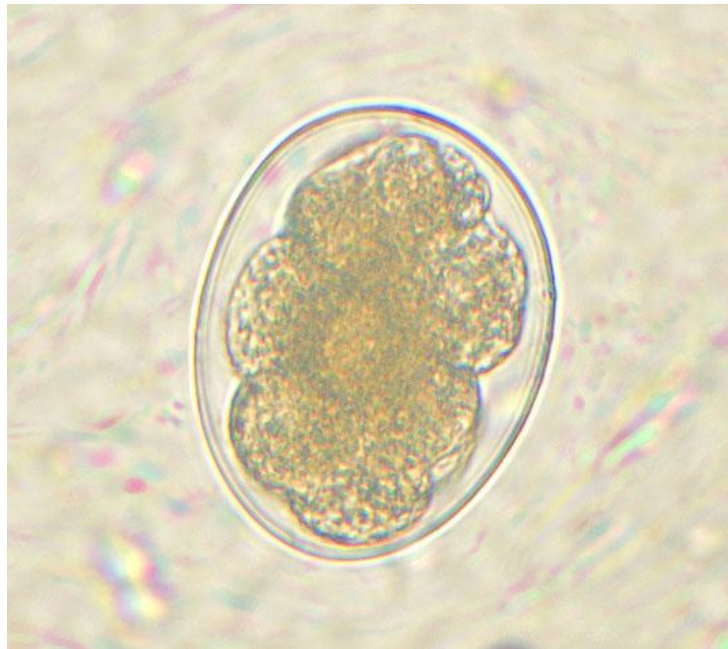
Lab diagnosis of Ascaris is by finding:

- Eggs in wet preparation of stool sample
- Worms expelled from mouth or anus.



# ***Hookworms***

Lab diagnosis of hookworm infection is by finding eggs in stool sample. If required, concentration techniques can be used.

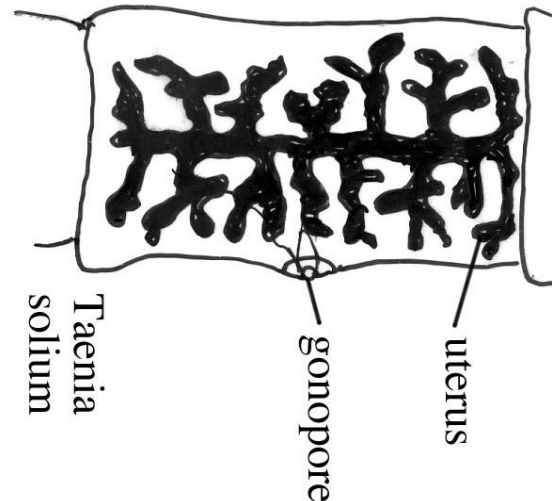
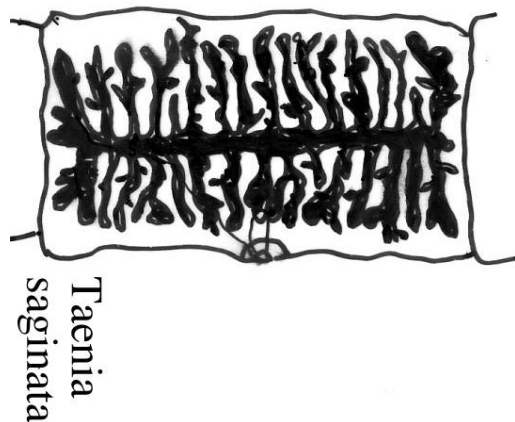




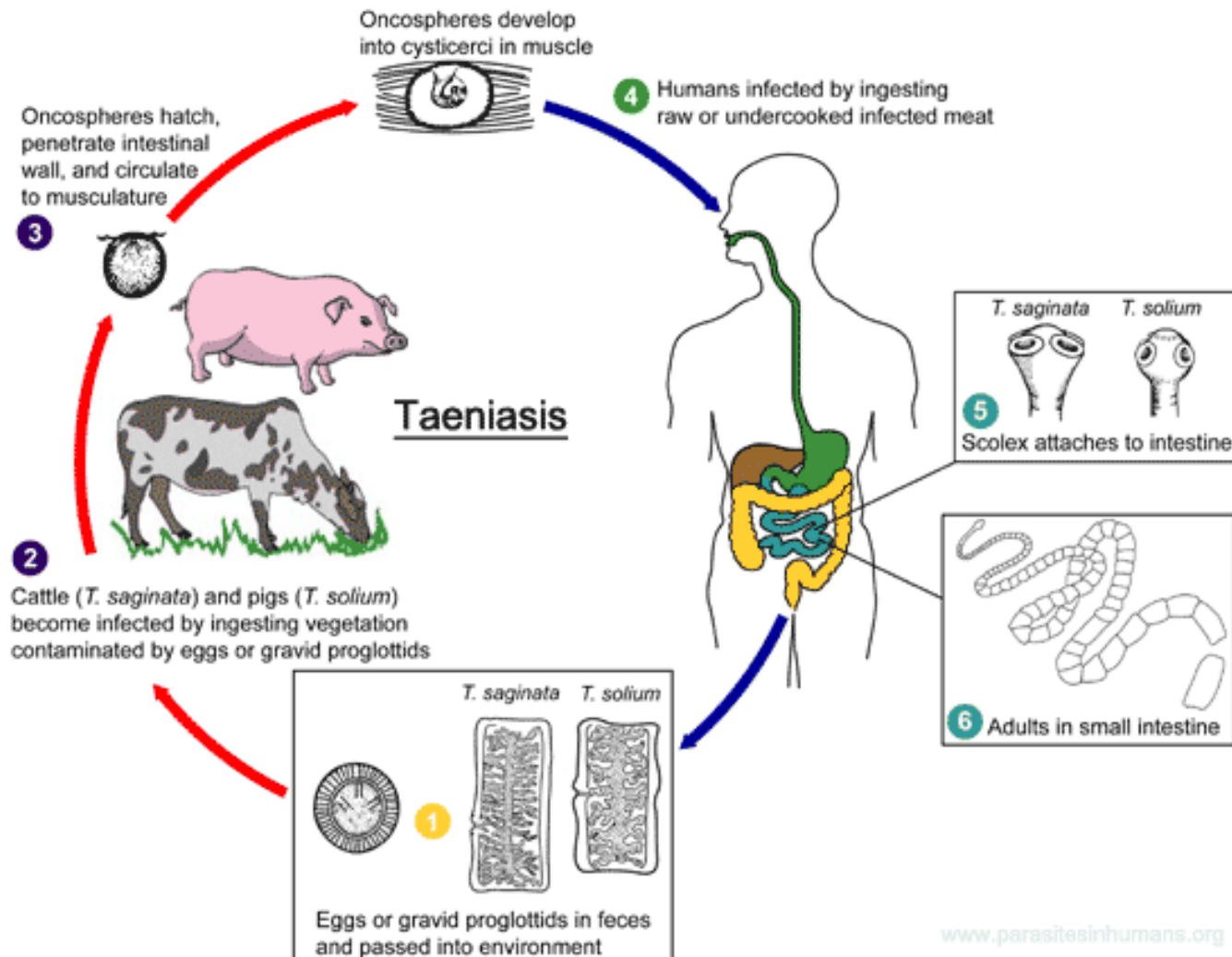
# *Taenia sp.*

Lab diagnosis of Taeniasis infection is by finding:

- **Gravid segments** from clothing or stool.
- **Eggs** in concentrated stool smear.
- *T. saginata* has longer segments than *T. solium*. But there eggs cannot be differentiated.



# *T.saginata* vs. *T.solium*

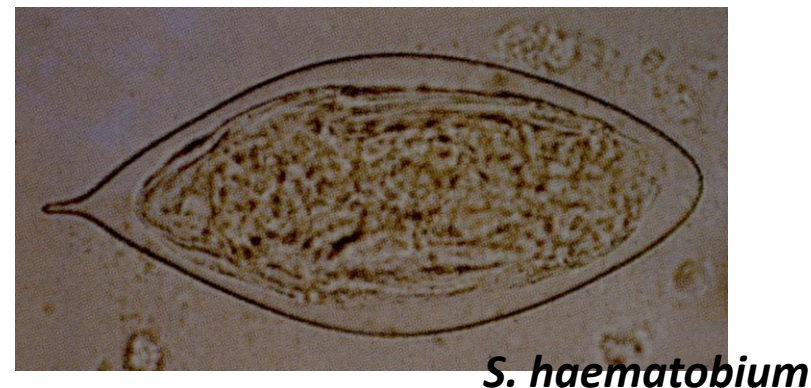


## *T.saginata* vs. *T.solium*



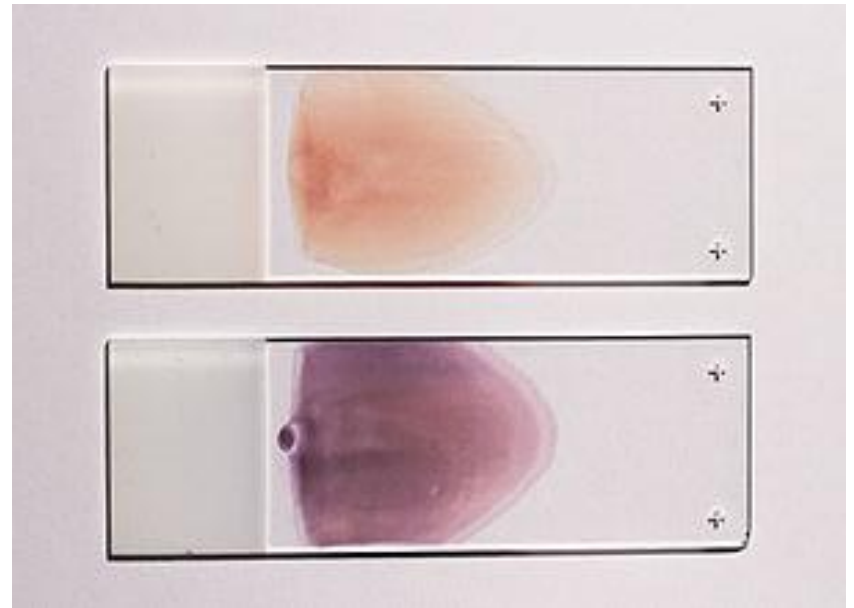
# *Schistosomes sp.*

- Lab diagnosis of intestinal Bilharzia (*S. mansoni*) is by finding eggs in stool sample (direct wet preparation, or concentrated smear), or rectal biopsy.
- Lab diagnosis of urinary Bilharzia (*S. haematobium*) is by finding eggs in urine, rectal biopsy, or bladder biopsy. Count eggs/10ml urine.



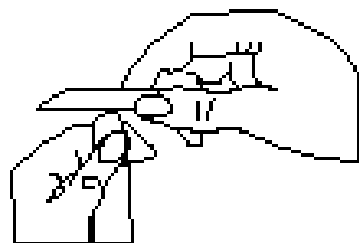
# Blood Film for Malaria

- Collect EDTA peripheral blood, make blood film.
- The thick film provides the greatest sensitivity and should be performed on all malaria requests.
- Thin films have a lower sensitivity and are primarily used to make the species identification (fixed smear).
- Stain with Giemsa stain or Field's stain and examine using 40x and 100x obj.
- *P. falciparum* is the most severe form of infection.



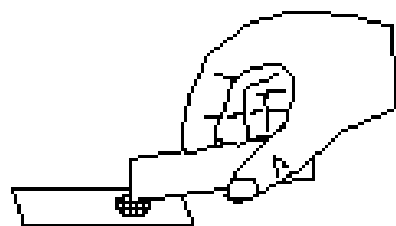
**FIGURE A-2. Preparation of a thin and thick blood film on the same slide**

**1**



Touch the blood drop with a clean slide.

**2**



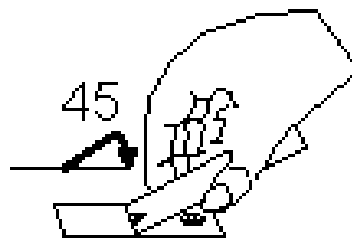
Using the corner of another slide, spread the blood drop into the shape of a circle or square of  $\sim 1 \text{ cm}^2$ .

**3**



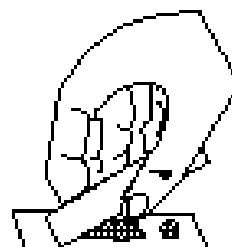
Gently squeeze the patient's finger again, and touch the edge of a clean slide to the newly formed blood drop.

**4**



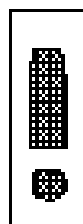
Take this slide and hold the edge that has the blood drop at an  $\sim 45^\circ$  angle against the surface of the first slide. Wait until the blood completely spreads along the edge of the second slide.

**5**



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

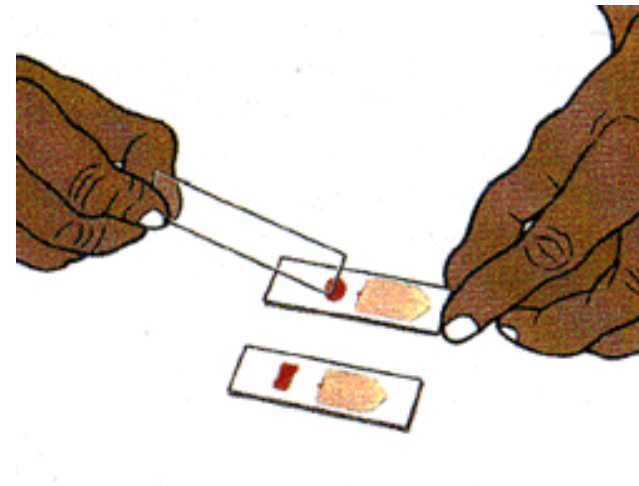
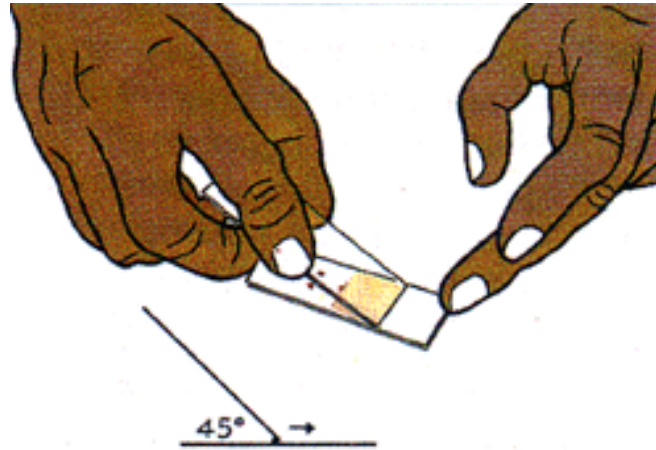
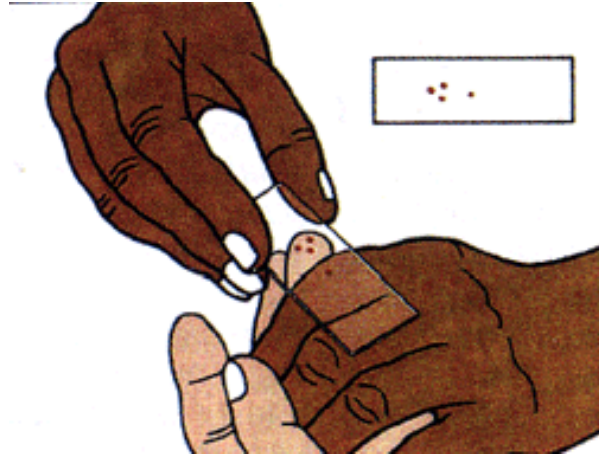
**6**



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

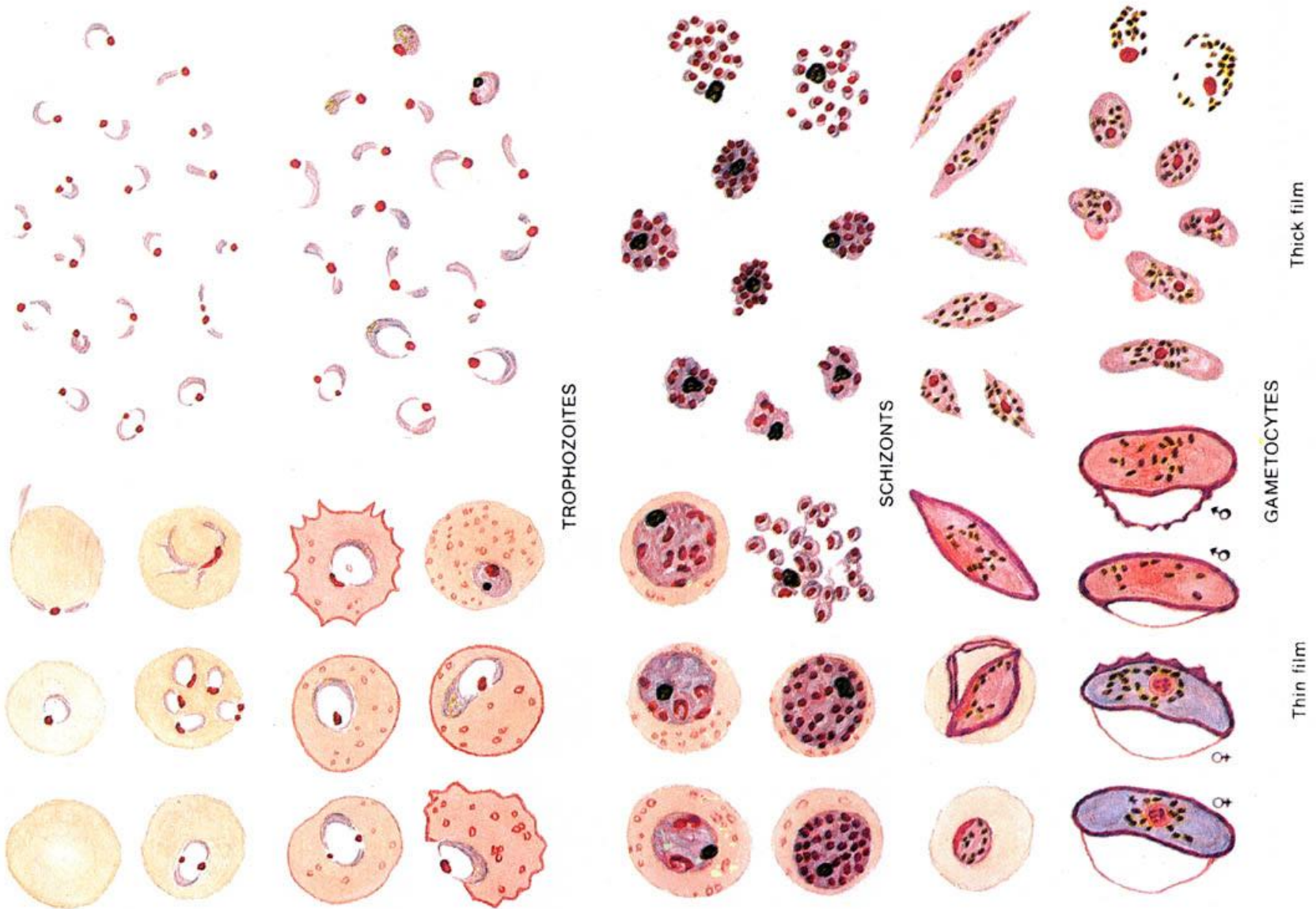


# Preparation of thin & thick BF

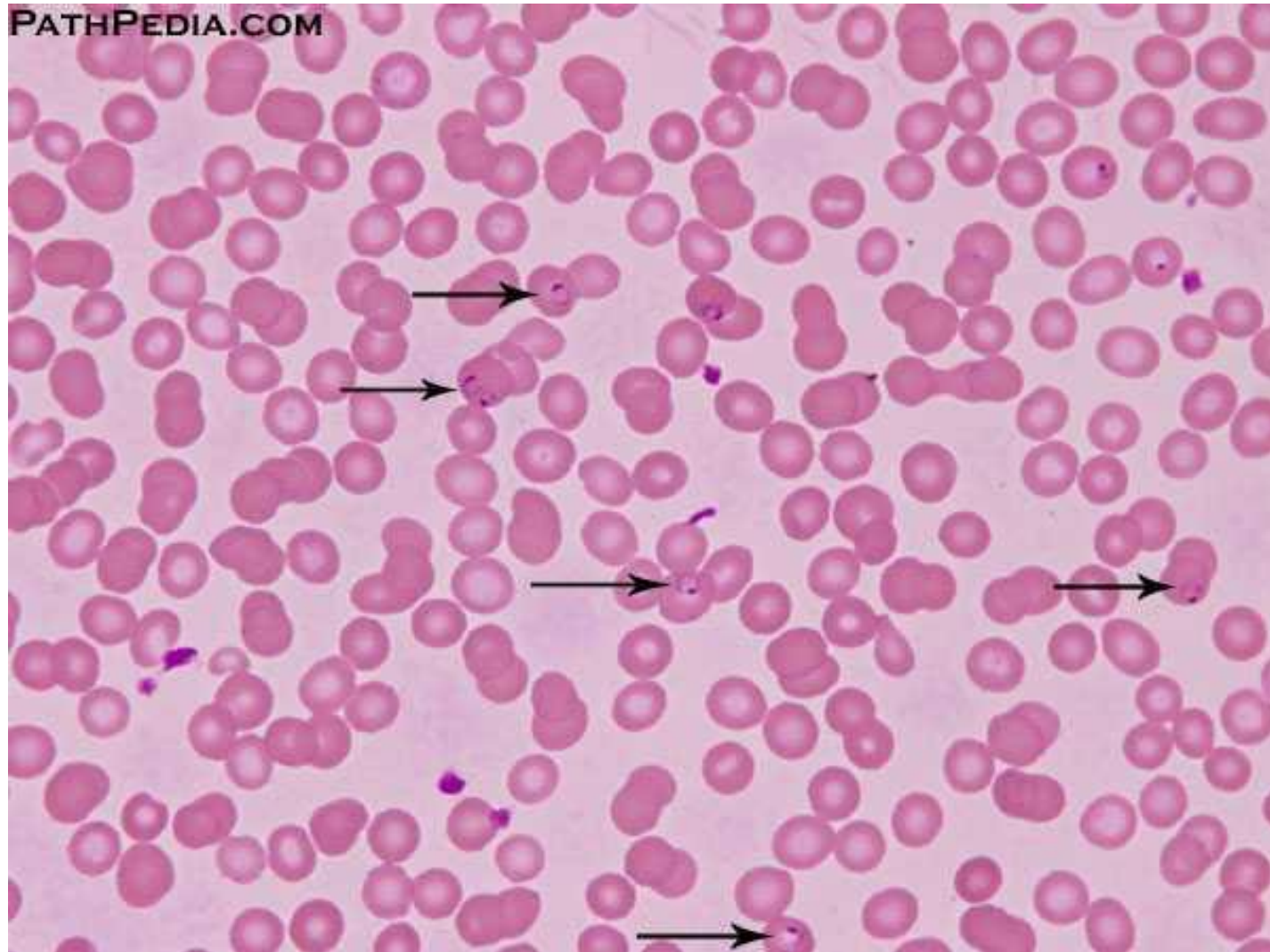




# *Plasmodium sp.*

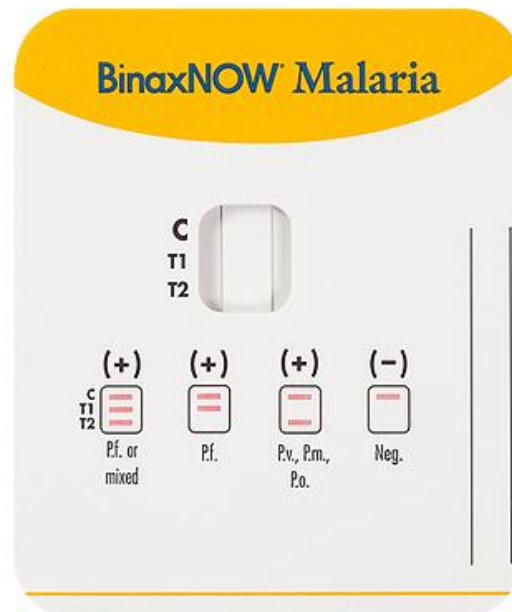


# ***BF Stained with Giemsa***



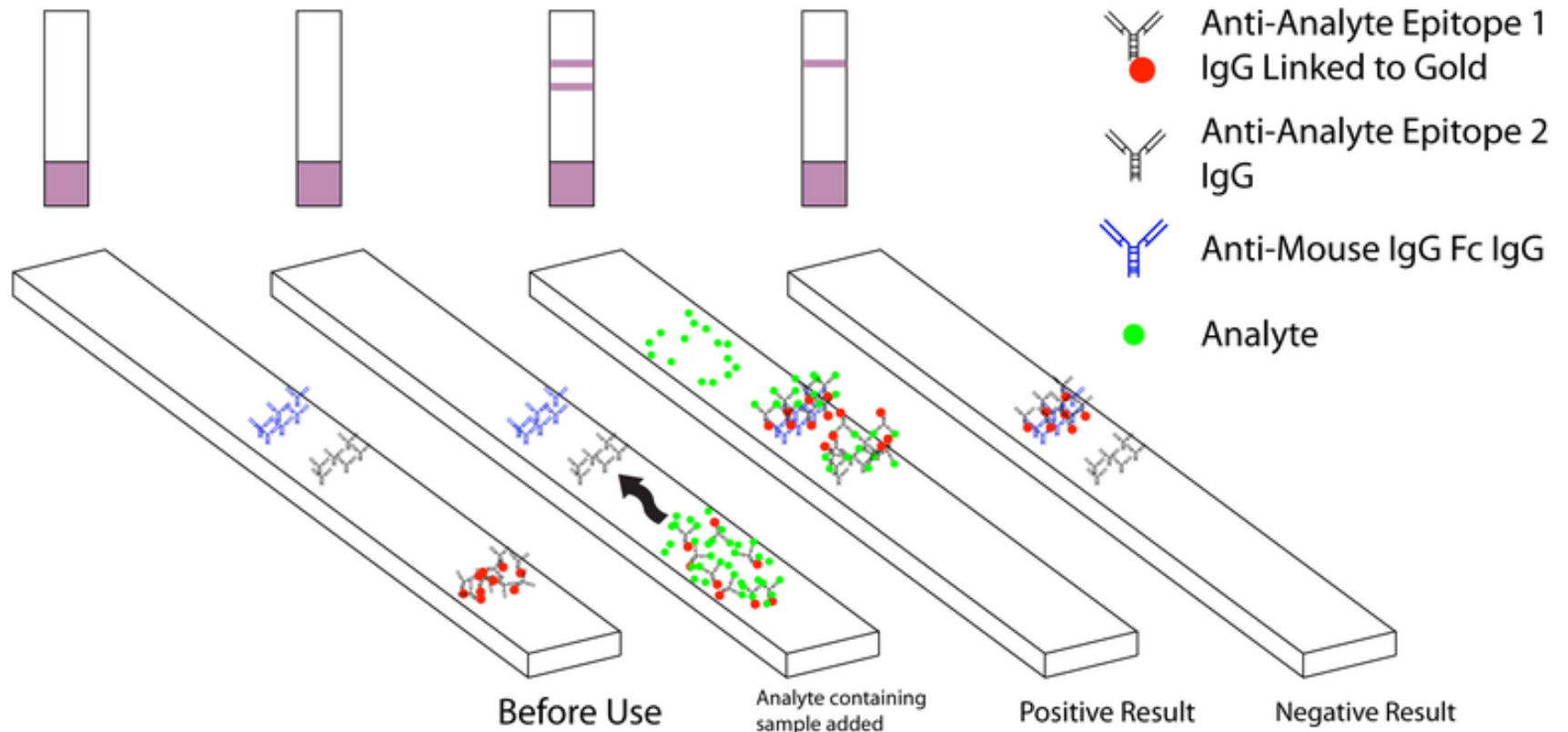
# Malaria RDT

- **Malaria Rapid Detection Tests-** are rapid qualitative immunochromatographic antigen detection methods. There are currently over 20 such tests commercially available in the shape of: dipstick, cassette, or card form.

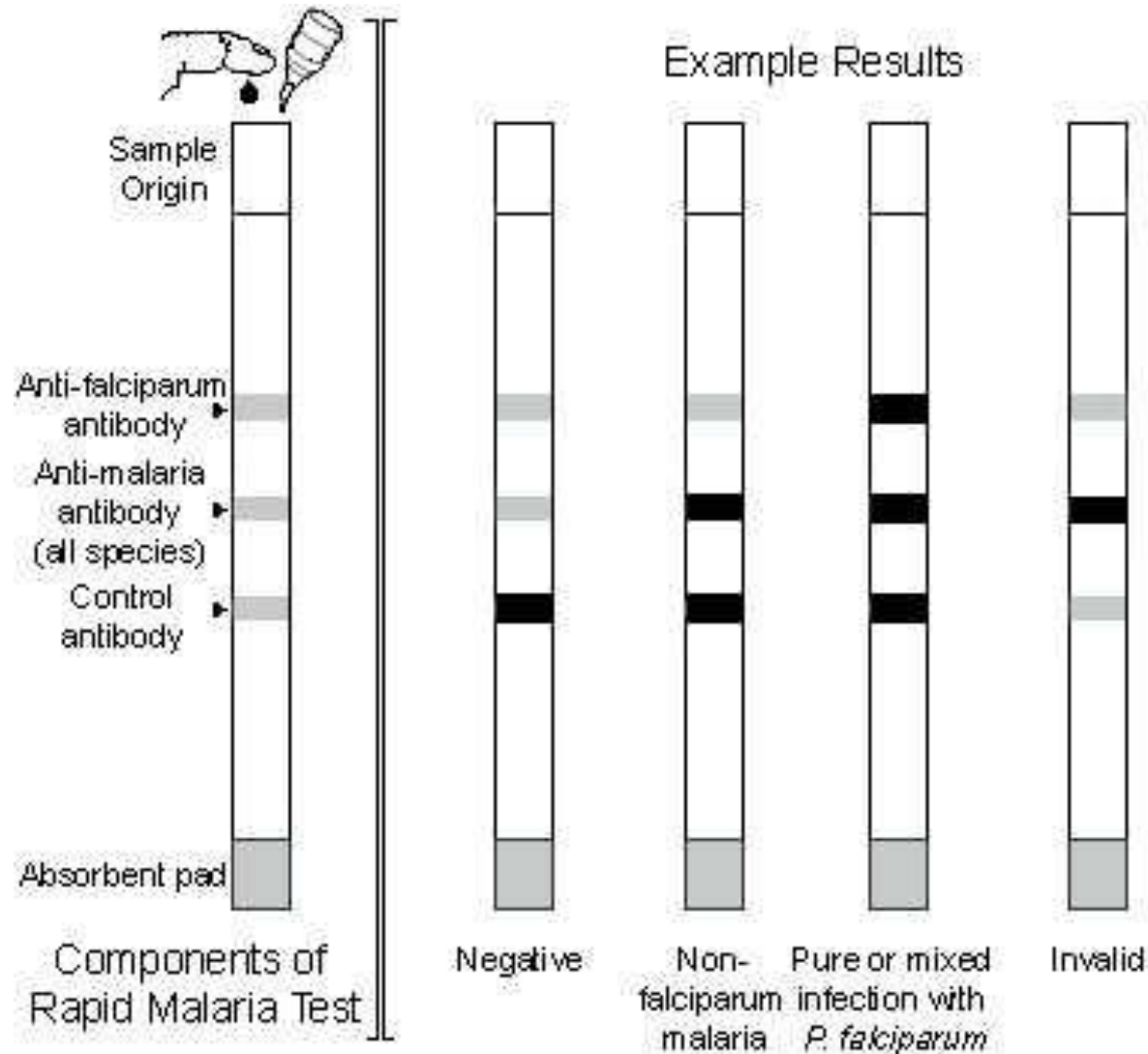


**Card Form**

# Dipstick Malaria Method



# Results of Dipstick Malaria Method

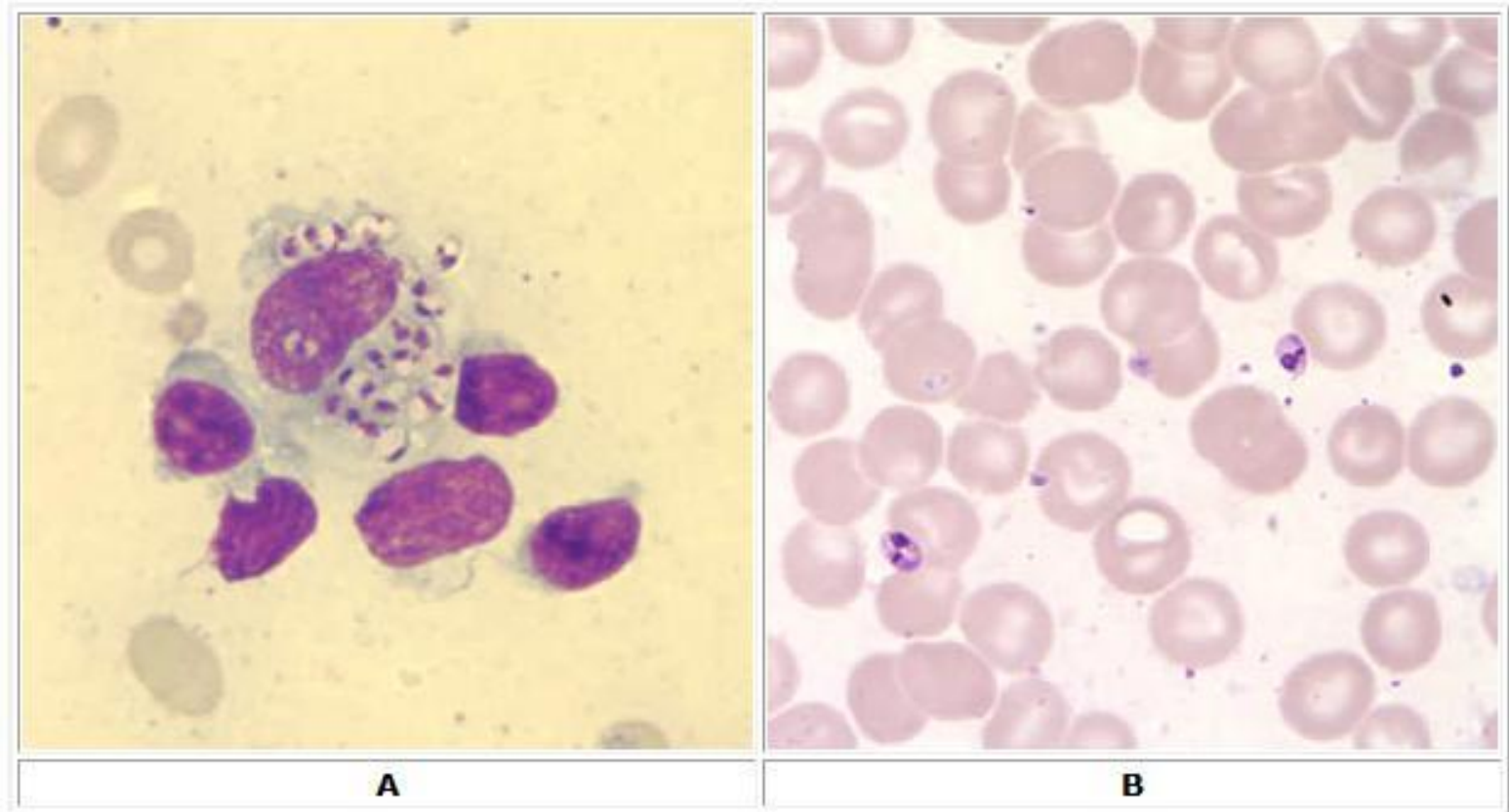




# ***Smear for Leishmania sp.***

- *Leishmania* is found in macrophages around the point of infection.
- **Visceral, cutaneous, & mucocutaneous Leishmaniasis.**
- **Visceral:** fluid from spleen, lymph node, bone marrow, nasal secretions, or peripheral blood.
- **Cutaneous & mucocutaneous:** scrapings from infected areas.
- Make a smear, fix with methanol, stain in Giemsa, examine under oil immersion (X1000) for the presence of **amastigotes**.

# *Leishmania*



**A**

**B**

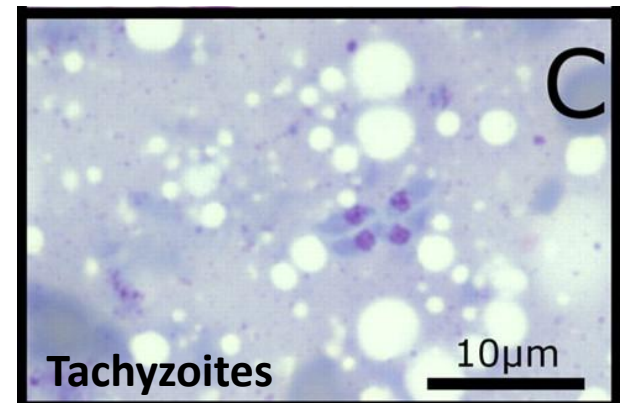
**A:** *Leishmania* spp. amastigotes in a Giemsa-stained tissue scraping.

**B:** *Leishmania* (*Viannia*) *panamensis* amastigotes in a Giemsa-stained tissue scraping. Identification to the species level is not possible based on morphology and other diagnostic techniques such as isoenzyme assay or PCR are needed.



# *Toxoplasma gondii*

- **Specimen:** CSF
  - *This is considered to be a non-routine procedure therefore it should only be performed by experienced personnel.*
1. Prepare smears from spun sediment.
  2. Fix in absolute methanol and stain with Giemsa for 50 minutes.



# Other Methods for Detecting Parasitic Infections

## Serological Assays

- Antigen Detecting Assay
- Antibody Detecting Assay
- **E.g. Enzyme Linked Immunosorbant Assay (ELISA), Hemagglutination (HA), Immunofluorescent (IFA), Compliment Fixation (CF), Rapid Diagnostic tests (RDTs).**

## Molecular Assays

- **PCR, Real-Time PCR, Loop-Mediated Isothermal Amplification (LAMP), Flow-cytometry, Proteomics.**

# FOBT

- Faecal Occult Blood Tests are to detect hidden blood in stool samples.

- **Guaiaac faecal occult blood Test (gFOBT):**

Smear some feces on to some absorbent paper that has been treated with a chemical. Hydrogen peroxide is then dropped on to the paper; if trace amounts of blood are present, the paper will change color in one or two seconds. This method works as the heme component in hemoglobin has a peroxidase-like effect, rapidly breaking down hydrogen peroxide. Advise the patient not to eat food rich in iron e.g. spinach, ..

# gFOBT



**Example of a fecal occult blood test.** Using an applicator stick, you apply a stool sample to two test windows on a supplied card. You then mail the card to to your doctor's office or lab.



Once the card reaches a doctor's office or a lab, a chemical is applied to the back of the card over the test windows. If blood is present, the chemical will react and appear as a different color.