Lab. : 1

LABORATORY DIAGNOSIS

Most of the parasitic infection cannot be conclusively diagnosed. On the basis of clinical features and physical examination laboratory diagnosis depends upon:

- Microscopy
- Culture
- Serological test
- Skin test
- Molecular method
- Animal inoculation
- Xenodiagnosis
- Imaging

Microscopy

An appropriate clinical samples should be collected for definitive diagnosis of parasitic infections.

Following specimens are usually examined to establish a diagnosis:

- Stool
- Blood
- Urine
- Sputum
- Cerebrospinal fluid (CSF)
- Tissue and aspirates
- Genital specimens.

❖ Stool Examination

Examination of stool is very important for the detection of intestinal infections like Giardia , Entamoeba , Ascaris , Ancylostoma , etc. Cysts and trophozoites of *E. histolytica*, *G. lamblia* can be demonstrated in feces. Eggs of roundworm and

tapeworm are also found in stool. The larvae are found in the feces in *S. stercoralis* infection .

❖ **Blood Examination**

Examination of blood is of vital importance for demonstrating parasites which circulate in blood vessels . Malarial parasite is confirmed by demonstration of its morphological stages in the blood.

❖ **Urine Examination**

The characteristic lateral-spined eggs of *S. haematobium* and trophozoites of *Tricomonas vaginalis* can be detected in urine . Microfilaria of *W. bancrofti* are often demonstrated in the chylous urine .

❖ **Sputum Examination**

The eggs of *P. westermani* are commonly demonstrated in the sputum specimen. Occasionally, larva stages of *Strongyloides stercoralis* and *Ascaris lumbricoides* may also be found in sputum.

❖ **Cerebrospinal Fluid Examination CSF**

Some protozoa like *Trypanosoma brucei*, *Naegleria* and *Acanthamoeba* can be demonstrated in the CSF.

❖ **Tissue and Aspirates Examination**

The larvae of *Trichinella* and eggs of *Schistosoma* can be demonstrated in the muscle biopsy specimens. By histopathological examination of brain, *Naegleria* and *Acanthamoeba* can be detected. In kala-azar , *Leishman Donovani* bodies can be demonstrated in spleen and bone marrow aspirate. Trophozoites of *Giardia* can be demonstrated in intestinal aspirates. Trophozoites of *E. histolytica* can be detected in liver pus in cases of amebic liver abscess.

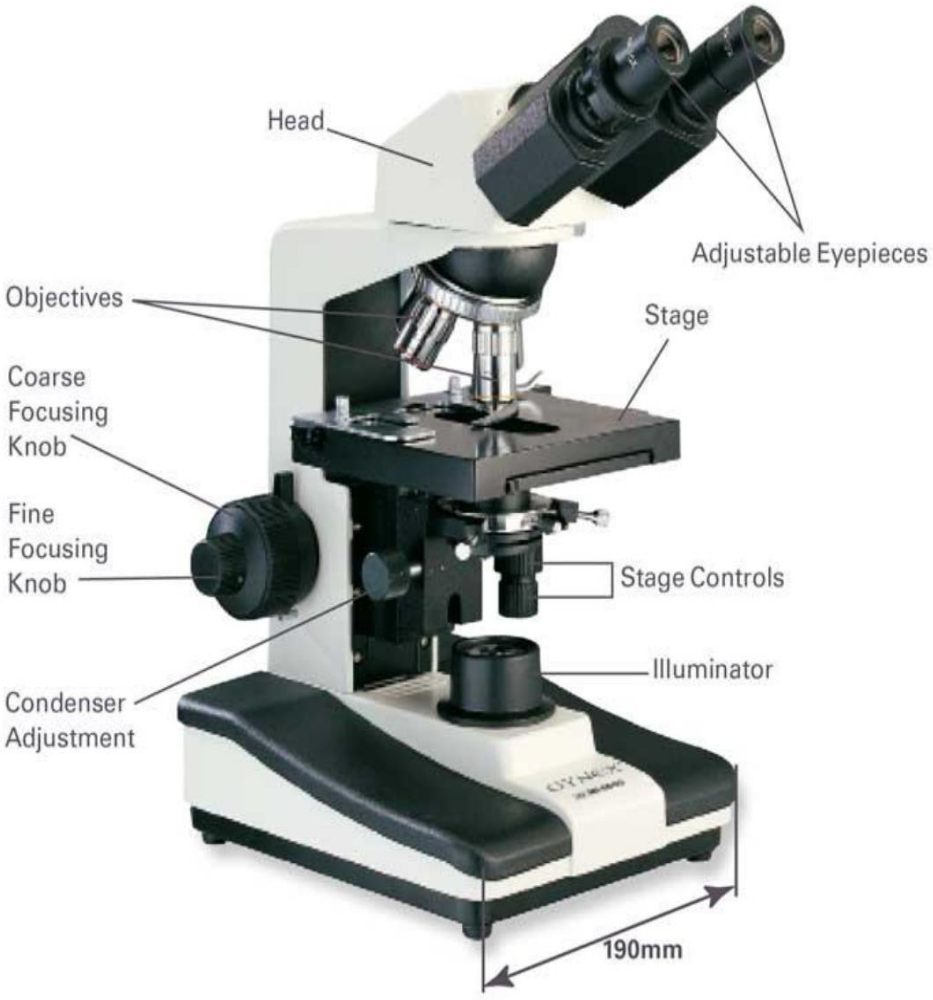
❖ **Genital Specimen Examination**

Trophozoites of *Trichomonas vaginalis* are found in the vaginal and urethral discharge. Eggs of *Enterobius vermicularis* are found in anal swabs.

Lab. : 2

Sample : a small part of something intended as representative of the whole . **or** A specimen of a whole entity small enough to involve no threat or damage to the whole , e.g. blood , stool , urine , CSF ….

Microscope : is an instrument used to see objects that are too small to be seen by the naked eye.



❖ STOOL EXAMINATION :

Conditions & notes of collection & transport of stool sample :

- Stool samples should be collected in a clean , watertight container with a tight-fitting lid. The acceptable amount of stool required for parasite study is 2 to 5 g .
- Urine should not be allowed to contaminate the stool specimen .
- Stool should not be retrieved from toilet bowl water .
- The typical stool collection protocol consists of three specimens , one specimen collected every other day or a total of three collected in 10 days . One exception is in the diagnosis of Amebiasis in which up to six specimens in 14 days is acceptable.
- It is recommended that :

- Liquid specimens be examined within 30 minutes of passage, contain trophozoite.
- Semiformed specimens may yield a mixture of protozoan cysts and trophozoites and should be evaluated within 1 hour of passage .
- Formed stool specimens are not likely to contain trophozoites; therefore, they can be held for 24 hours following collection.

- The sample container should be labeled with the **patient's name** and **identification number** and the **date** and **time** of sample collection .

- If it is not possible to obtain feces, collect a specimen by inserting a cotton wool swab into rectum for about 10 sec.
- Certain medications and substances may interfere with the detection of parasites.

- If these guidelines cannot be done , the specimen should be placed into a preservative. The specimen can be preserved by placing it directly into a fixative at the time it is collected or on receipt in the laboratory.

Stool examination include the following processing :

Once a stool specimen has been received in the laboratory , In this phase samples are examined from two perspectives : macroscopic and microscopic .

- **Macroscopic Examination**

Determine the : **Consistency** , **Color** , **Gross abnormalities**

- **Consistency or degree of moisture** in a stool specimen may serve as an indication of the types of potential parasites present . For example :
  - Soft or liquid stools may suggest the presence of protozoan trophozoites
  - Semi formed specimens have trophozoite & cyst .
  - Protozoan cysts are more likely to be found in fully formed stools .
  - Helminthes eggs and larvae may be found in liquid ,semi or formed stools .
- **Color** of a stool is important because it may indicate the condition of the patient such as (the patient is on antibiotic therapy) . The range of colors varies including black to green to clay and colors in between . The color of normal stool is brown .
- **Gross abnormalities** possibly found in stool include adult worms , segments ,blood , pus and mucus . The sample should then be broken up—a wooden applicator stick works nicely for this task—and examined once more for macroscopic parasites, especially adult helminths .

- blood and/or mucus in loose or liquid stool may suggest the presence of amebic ulcerations in the large intestine . Bright red blood on the surface of a formed stool is usually associated with irritation and bleeding .

- **Microscopic Examination**

To detect the presence of parasites in a stool sample , microscopic examinations are performed .

Cysts/Trophozoites	Eggs		Larvae	Adult worms
• Entamoeba histolytica	Cestodes	• Gastrodiscoides hominis	Strongyloides stercoralis	• Taenia solium
• Giardia lamblia	• Taenia spp.	• Heterophyes heterophyes		• Taenia saginata
• Balantidium coli	• Hymenolepis nana	• Metagonimus yokogawai		• Diphyllobothrium latum
• Sarcocystis spp.	• Hymenolepis diminuta	• Opisthorchis spp.		• Ascaris lumbricoides
• Isospora belli	• Dipylidium caninum	Nematodes		• Enterobius vermicularis
• Cyclospora cayetanensis	• Diphyllobothrium latum	• Trichuris trichiura		• Trichinella spiralis
• Cryptosporidium parvum	Trematodes	• Enterobius vermicularis		
	• Schistosoma spp.	• Ascaris lumbricoides		
	• Fasciolopsis buski	• Ancylostoma duodenale		
	• Fasciola hepatica	• Necator americanus		
	• Fasciola gigantica	• Trichostrongylus orientalis		
	• Clonorchis sinensis			

Parasites and their developmental stages found in stool

Lab. : 3

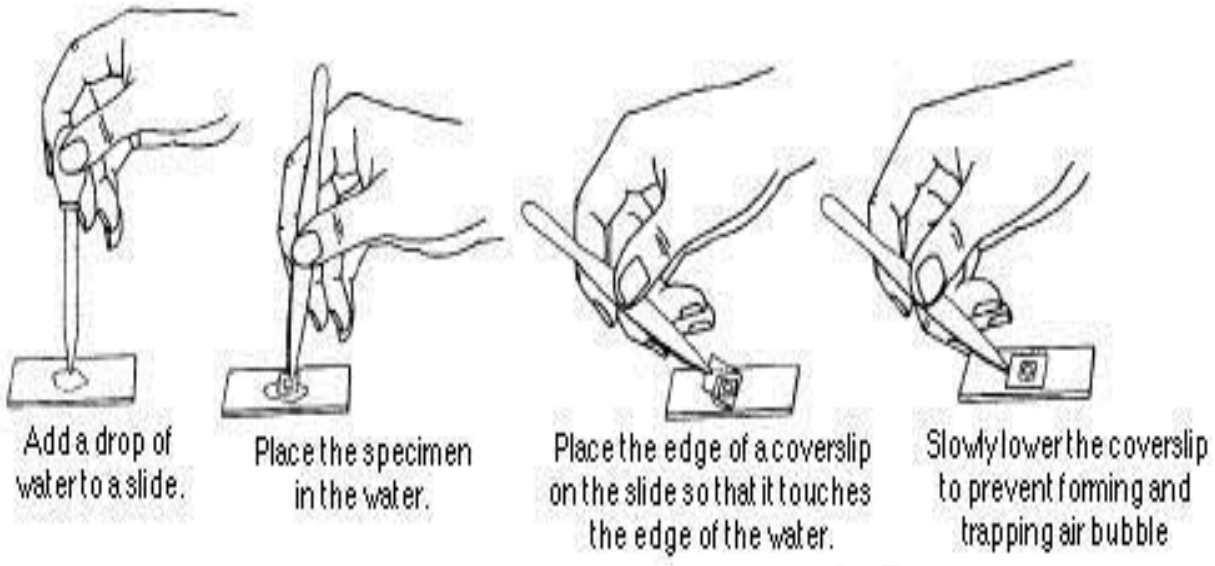
General Stool Examination (G.S.E.)

- **Microscopic Examination**

To detect the presence of parasites in a stool sample , microscopic examinations are performed .

❖ Direct wet preparations

The primary purpose of a direct wet preparation (also known as a **Direct wet mount**) , defined as a slide made by mixing a small portion of unfixed stool (stool with no added preservatives) with saline or iodine and subsequent examination of the resultant mixture under the microscope, is to detect the presence of motile **Protozoan Trophozoites** . **Trophozoite motility can only be demonstrated in fresh specimens, especially those of a liquid or soft consistency**. If the specimen is received in the laboratory in a fixative, this procedure can be eliminated from the ova and parasites (O&P) assay. Other parasite stages that might be observed in a direct wet preparation include **Protozoan Cysts** , **Oocysts** , **Helminth Eggs (Ova)** , and **Larvae** . Because the diagnostic yield of this procedure is low , most experts agree that technical time is better spent on the concentration procedure and permanent stained smear and recommend only performing the direct wet preparation on fresh specimens .



Kingdom : 1. Protista (Unicellular)

Subkingdom : Protozoa

Phylum : 1. Sarcomastigophora 2.Sporozoa 3.Ciliate

Apicomplexa Ciliophora

Subphylum : 1.Amoeba 2. Flagellate

Sarcodina Mastigophora

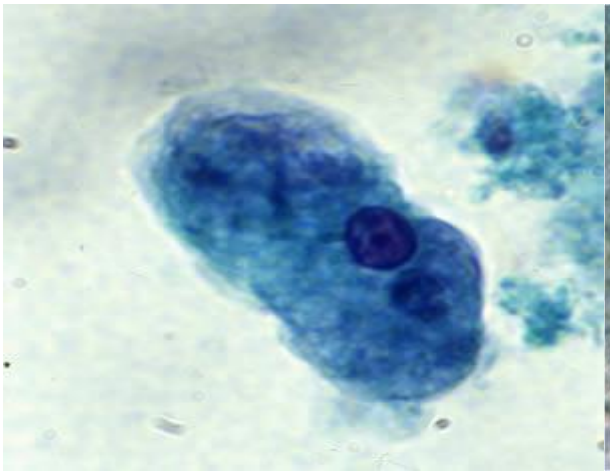
- **Amoebae (Sarcodina)**

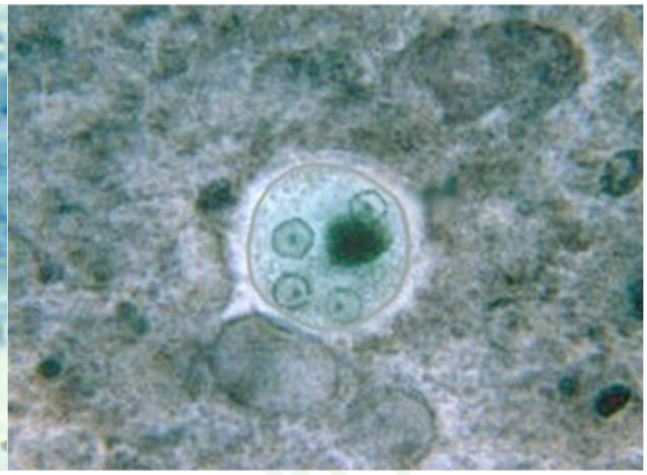
Amoeba is a single-celled life form characterized by its irregular shape . move by pseudopodia .

A. Trophozoite : is motile, feed , pathogenic and reproductive stage

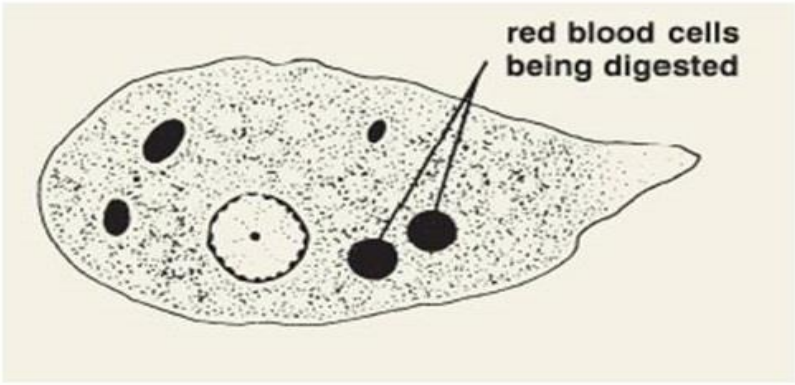
B. Cyst : is non motile , non-feeding , infective and non-reproductive stage .

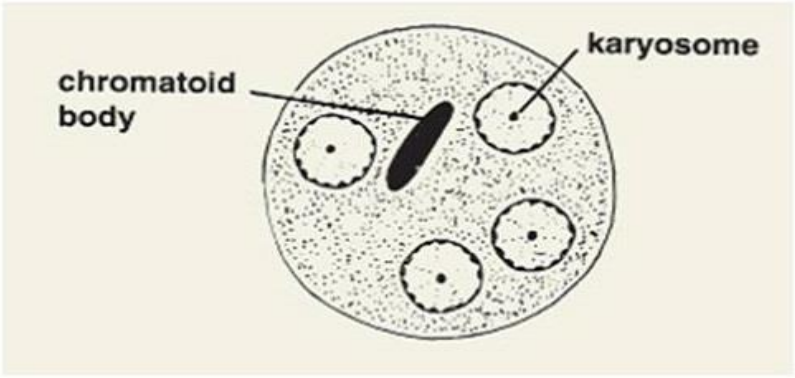
❖ ***Entamoeba histolytica***



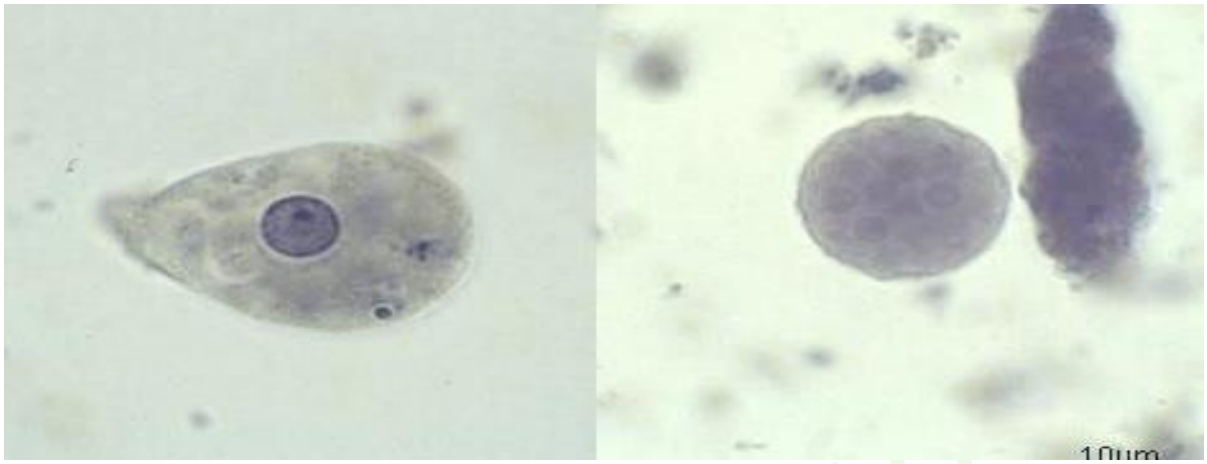


Trophozoite Cyst

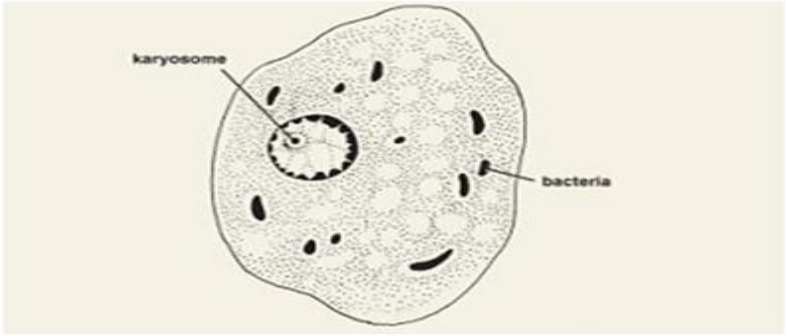


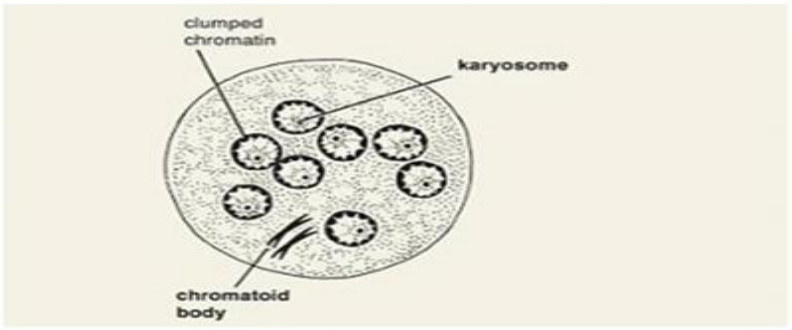


❖ ***E. coli***



Trophozoite Cyst





Diagnosis :

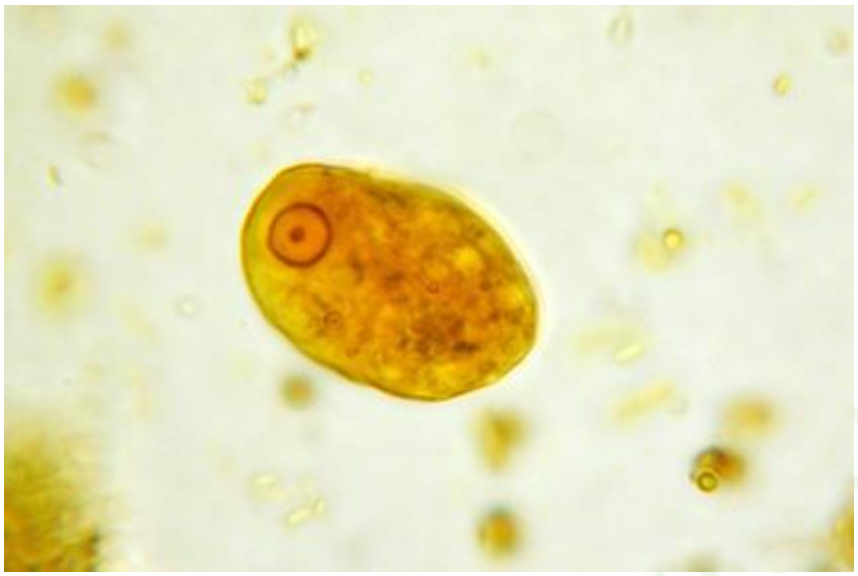
1. Microscopic examination , wet preparation of stool sample with staining , found trophozoites & cyst
2. Microscopic examination of hepatic abscess material .found trophozoites .
3. Serological tests as ELISA
4. Molecular tests as PCR

Lab. : 4

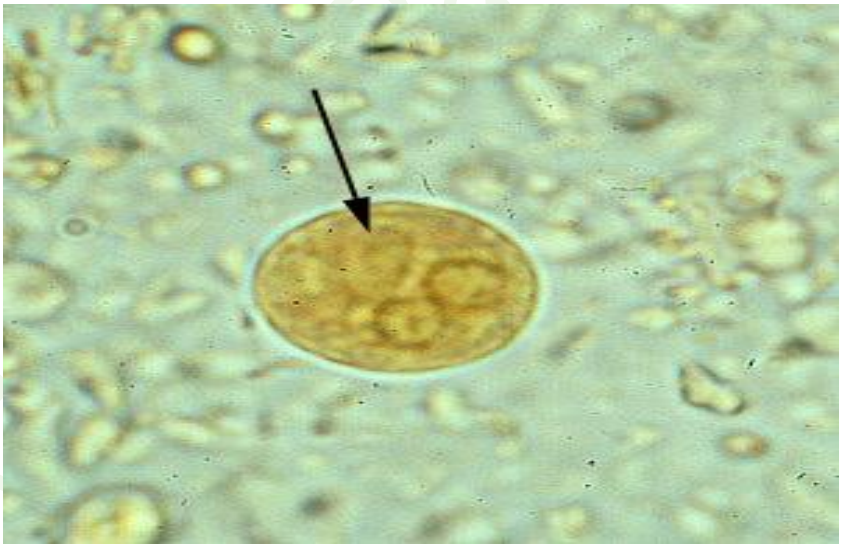
➢ Macroscopy & Microscopy

- **Lugol's iodine solution :** also known as aqueous iodine and strong iodine solution, is a solution of potassium iodide with iodine in water . It is a medication and disinfectant used for a number of purposes
- **The stool of Amoebiasis is characterized by :** Foul-smelling , copious, semiliquid, brownish -black in color, intermingled with blood and mucus, and it does not adhere to the container.
- The cellular exudate is scanty and consists of only the nuclear masses **(pyknotic bodies)** of a few pus cells, epithelial cells and macrophages.
- ****Pyknotic bodies** are the nuclear remains of tissue cells and leukocytes , they may present in the stools of persons suffering from Amoebiasis.
- ****Charcot-Leyden crystals** are often present. These are diamond-shaped, clear and refractile crystals.

- Iodine preparation : For the demonstration of cysts or dead trophozoites, stained preparations may be required for the study of the nuclear character
- The trophozoite of *E. histolytica* stains yellow to light brown.
- The RBCs are in clumps and yellow or brown -red in color.
- Nucleus is not visible but a faint outline may be detected (without iodine) . The nucleus is clearly visible with a central karyosome (with iodine) .



- The cytoplasm of the cystic stage shows a smooth and hyaline appearance.
- Nuclear chromatin and karyosome appear bright yellow.
- Glycogen masses stain golden brown and chromatoid bars are not stained.



➢ **Mucosal scrapings :** Scraping obtained by sigmoidoscopy is often contributory . The examination method includes a direct wet mount and iron hematoxylin , immunofluorescent staining with anti-*E.hislolytica* antibodies.

➢ **Stool culture :** Stool culture is a more sensitive method in diagnosing chronic and asymptomatic intestinal amebiasis. The culture of stools gives higher positivity for *E. histolytica* as compared to direct examination.

Media used for stool culture include to diagnosis the *E. histolytica* :

- Boeck and Drbohlav's biphasic medium.
- NIH polygenic medium
- Craig's medium, Nelson's medium
- Robinson's medium
- Balamuth's medium.

- **Serodiagnosis (Serological tests) :** become positive only in invasive amebiasis.
  Serological tests include :
- Enzyme-Linked Immunosorbent Assay (ELISA)
- Indirect Hemagglutination Assay (IHA)
- Indirect Fluorescent Antibody (IFA)
- Counter-Current Immune Electrophoresis (CIEP)
- Latex Agglutination Tests.

  - **Antibody detection :** Amebic antibodies appear in serum only in the late stages of intestinal amebiasis . Tests for antibodies in serum help in the diagnosis of mainly extraintestinal infections .

  - **Antigen detection :** Amebic antigen in serum are detected only in patients with active infections and disappears after clinical cure . Antigen like Lipophosphoglycan (LPG), amebic lectin , Serine-Rich *E. histolytica* Protein (SREHP) are detected using monoclonal antibodies by ELISA.

- **Molecular diagnosis :** Recently, Deoxyribonucleic Acid (DNA) probes and radioimmunoassay have been used to detect *E. histolytica* in the stool. It is a rapid and specific method .

Lab. : 5

Flagellates

Parasitic protozoa, which possess whip-like flagella as their organs of locomotion are called as flagellates and classified as:

- SubPhylum: Sarcomastigophora
- Class : Zoomastigophora

Depending on their habitat, they can be considered under :

- Lumen-dwelling flagellates: Flagellates found in the alimentary tract and urogenital tract .
- Hemoflagellates : Flagellates found in blood and tissues .

Giardia Lamblia

- found in the alimentary tract

Laboratory Diagnosis

➢ ***Stool Examination***

Giardiasis can be diagnosed by identification of cysts of *Giardia lamblia* in the formed stools and the trophozoites and cysts of the parasite in diarrheal stool **.**

- On macroscopic examination , fecal specimens containing G. *lamblia* may have an offensive odor, are pale colored and fatty, and float in water.
- On microscopic examination, cysts and trophozoites can be found in diarrheal stools by saline and iodine wet preparations.

- Often multiple specimens need to be examined and concentration techniques like formal ether or zinc acetate are used. In asymptomatic carriers, only the cysts are seen.

➢ ***Enterotest (String Test)***

A useful method for obtaining duodenal specimen is *enterotest*. A coiled thread inside a small weighted gelatin capsule is swallowed by the patient, after attaching the free end of the thread in the cheek. The capsule passes through the stomach to the duodenum. After 2 hours, the thread is withdrawn, placed in saline, and is mechanically shaken. The centrifuged deposit of the saline is examined for *Giardia*. The use of enterotest is not recommended because of the very high cost of the test.

➢ **Serodiagnosis**

- **Antigen detection:** Enzyme-linked immunosorbent assay (ELISA), immunochromatographic strip tests and indirect immunofluorescence (IIF) tests

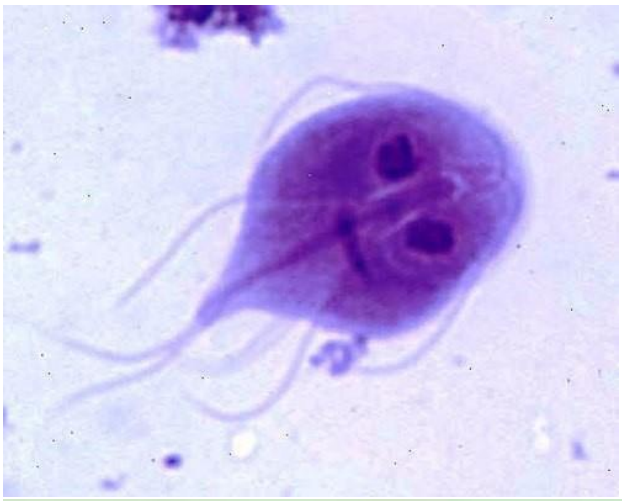
using monoclonal antibodies have been developed for detection of *Giardia* antigens in feces **.**

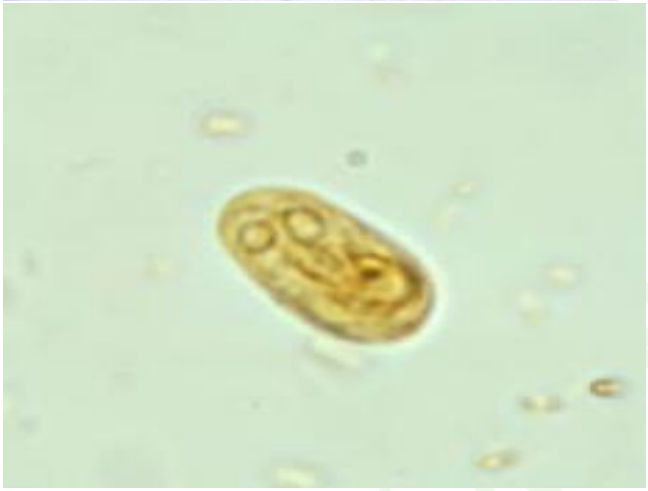
- The presence of antigen indicates active infection.
- Commercially available ELISA kits (ProSpecT/ Giardia kit) detects Giardia specific antigen 65 (GSA 65).
- the sensitivity of the test is 95% and specificity is 100% when compared to conventional microscopy.
- The test may be used for quantification of cysts and in epidemiological and control studies, but not for routine use.

- **Antibody detection:** Indirect immunofluorescence test and ELISA are used to detect antibodies against *Giardia*.
  - Demonstration of antibodies is useful in the epidemiological and pathophysiological studies.
  - These tests cannot differentiate between recent and past infection and lack sensitivity and specificity.

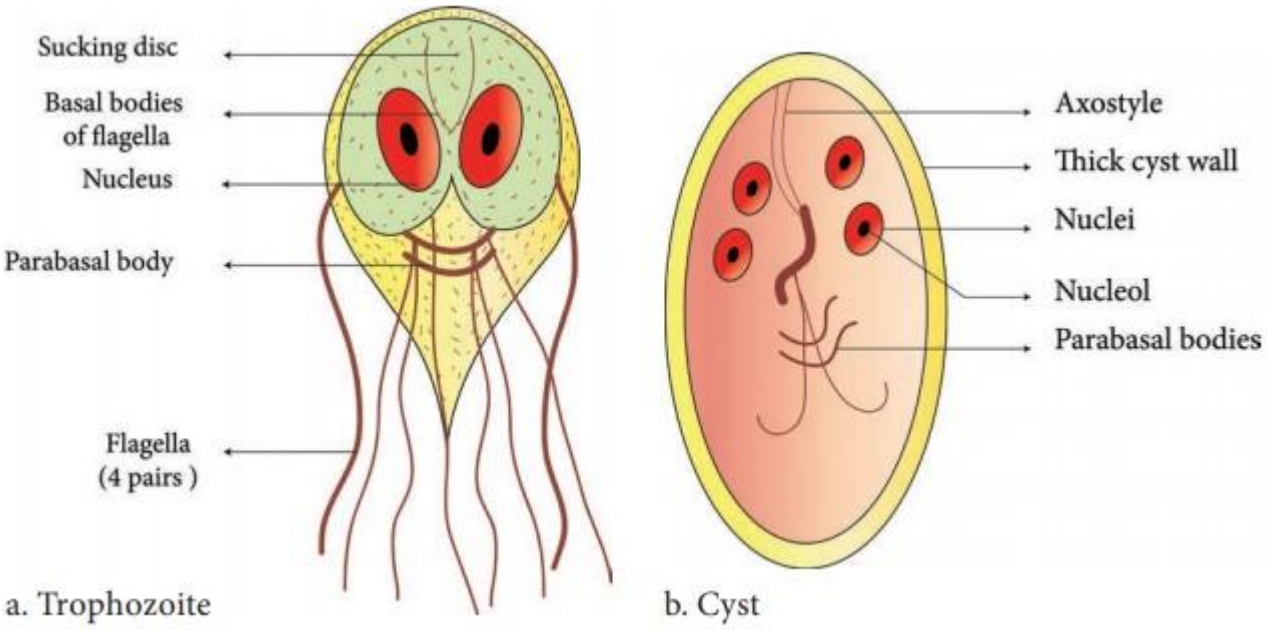
➢ **Molecular Method**

- Deoxyribonucleic acid (DNA) probes and polymerase chain .
- Reaction (PCR) have been used to demonstrate parasitic





Trophozoite **Cyst**



a. Trophozoite b. Cyst

Lab. : 6

- ***Trichomonas vaginalis***

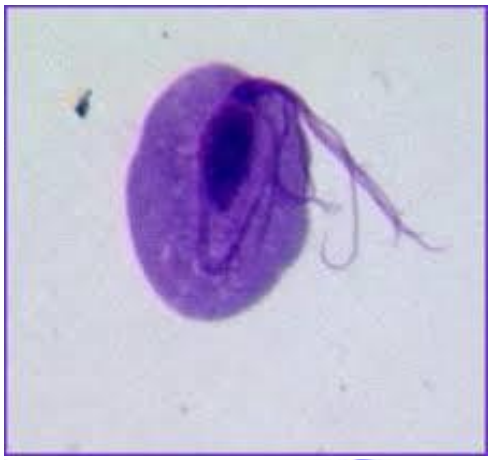
  They exist only in trophozoite stage . Cystic stage is not seen

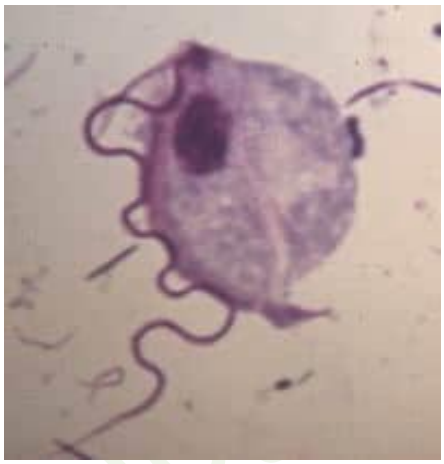
  Laboratory diagnosis

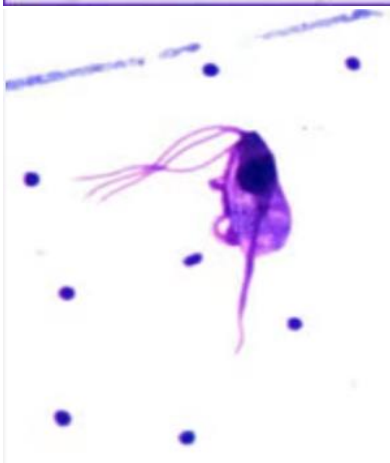
  - In female patient, *Trichomonas vaginalis* maybe demonstrated in sedimented urine, vaginal secretion, or from vaginal scraping.
  - In male patient *Trichomonas vaginalis* maybe found in the centrifuged urine and prostatic secretions following massage of the prostatic gland.

However, care should be taken to prevent contamination of the specimen with feces, since *Trichomonas hominis* maybe has seen and thus misdiagnosed as *Trichomonas vaginalis*. The smear is stained using Giemsa , PAS stain , Papanicolaou stain , Leishman stain , Diff Quick stain (Romanowsky stain) & acridine orange stain .

Trichomonas vaginalis ***Trichomonas homonis*** ***Trichomonas tenax***







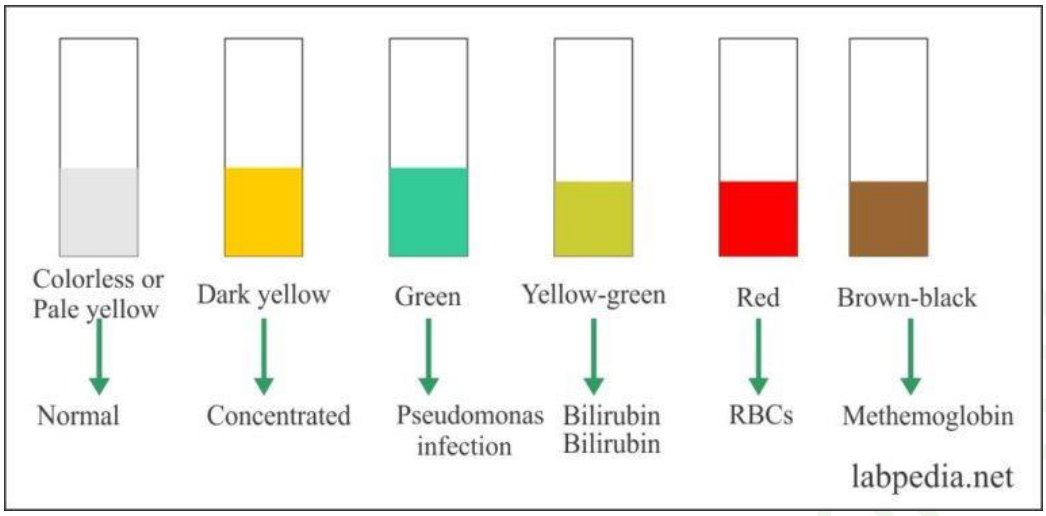
Trophozoite

General urine examination

It includes two basic tests:

A. Macroscopic or physical examination :

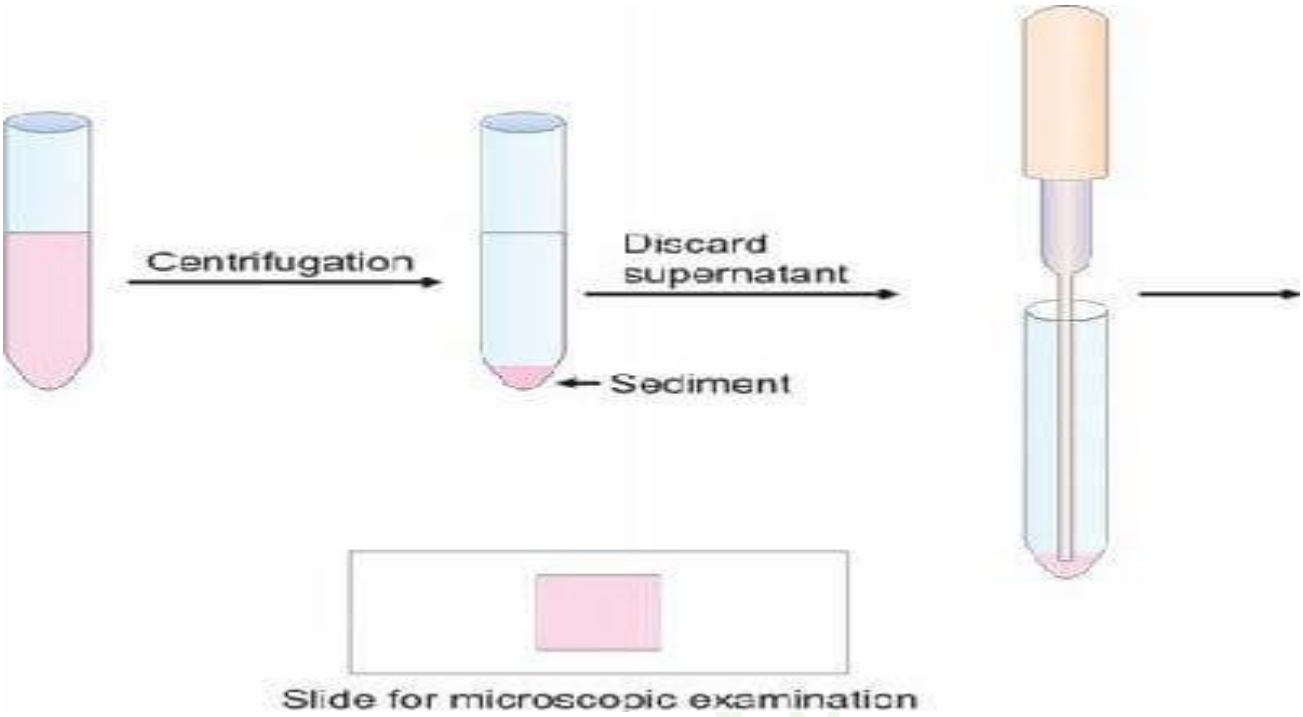
Include : Volume , Specific gravity , Color , pH , Odor .

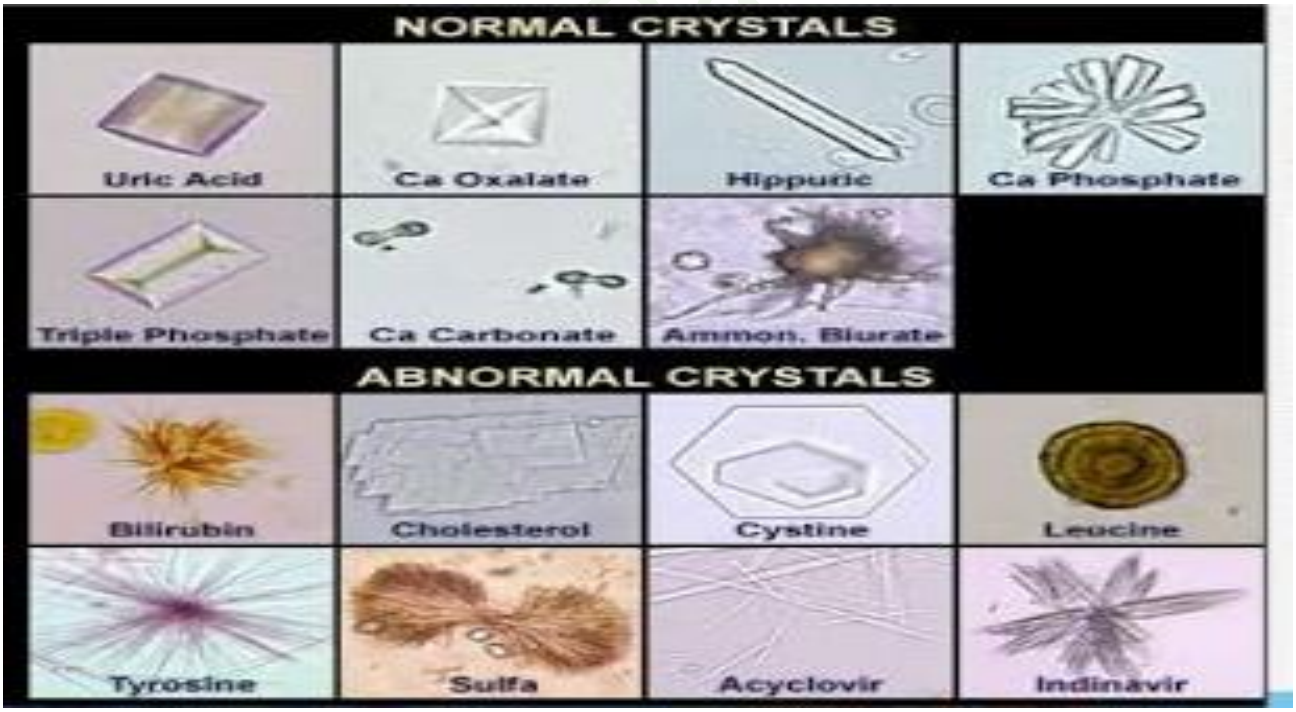


B. Chemical examination :

Protein , Glucose , Bilirubin , Ketone Bodies , Blood , …….

C. Microscopic examination


Centrifugation
Discard supernatant
Sediment
Slide for microscopic examination


NORMAL CRYSTALS
Uric Acid
Ca Oxalate
Hippuric
Ca Phosphate
Triple Phosphate
Ca Carbonate
Ammon. Biurate
ABNORMAL CRYSTALS
Bilirubin
Cholesterol
Cystine
Leucine
Tyrosine
Sulfa
Acyclovir
Indinavir

Lab. : 7

Hemoflagellates

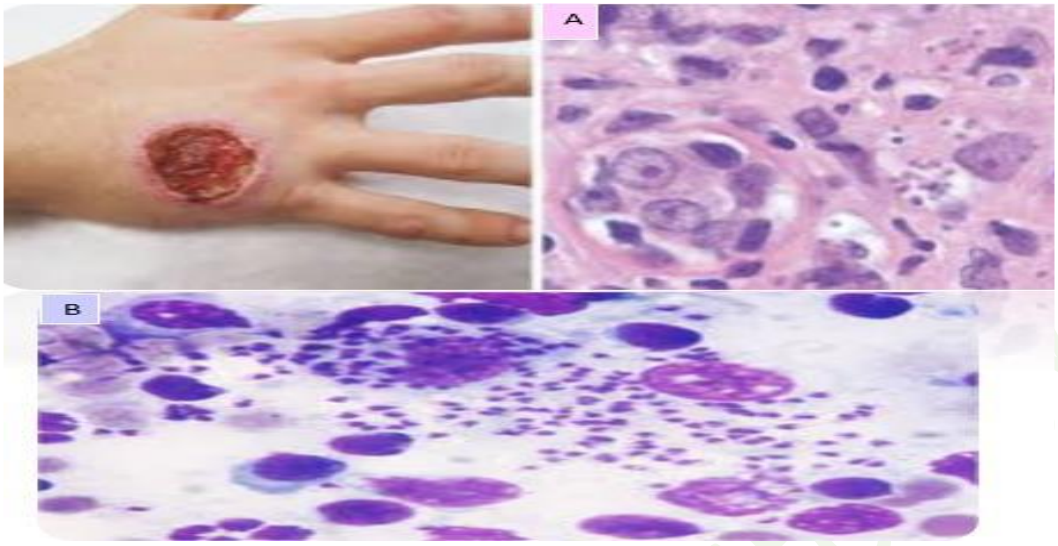
Laboratory Diagnosis

1. *Leishmania donovani* cause Visceral Leishmaniasis

- Giemsa-stained slides of blood , bone marrow , lymph node aspirates and biopsies of the infected areas for the diagnosis of amastigote forms.
- Culture of blood, bone marrow and other tissues these samples show the promastigote forms
- Serological tests.

2. *Leishmania tropica* cause Cutaneous Leishmaniasis

- The specimen of choice for identify the amastigotes of *L. tropica* is a biopsy of the infected ulcer .
- Microscopic examination of the Giemsa-stained preparations should reveal the typical amastigotes . Promastigotes may be present when the sample is collected immediately after introduction into the patient.
- Culturing the infected material, which often demonstrated the promastigote stage.
- Serological tests.



L. tropica amastigote : A-intracellular , B-intercellular

3. *L. brasiliensis* cause Mucocutaneous Leishmaniasis

- Microscopic examination of giemsa-stained slides of aspiration of fluid underneath the ulcer bed for typical amastigotes.
- Culture of the ulcer tissue may also reveal the promastigote forms.
- Serological tests such as Indirect Fluorescent Antibody (IFA) also available.