Ethiopian TVET-System

# MEDICAL LABORATORY
# Level -III

**Based on Apr.2018G.C. Occupational Standard**

## Module Title: Perform Parasitological Examination

## TTLM Code: HLT MLT3 07 0919V1

## This module includes the following Learning Guides

**LG43: Identify concept of human parasitology**

**LG44: Process samples and associated request details**

**LG45: Set up and use microscope**

**LG46: Perform tests**

**LG47: Maintain a safe environment**

**LG48: Maintain laboratory records**

| Instruction Sheet 1 | LG43: Identify concept of human parasitology |
|---|---|

This learning guide is developed to provide you the necessary information regarding the following content coverage and topics –

1. **Basic Concepts of Medical Parasitology**
    - Definition of terminologies
    - Host parasite interactions
    - Techniques in Medical Parasitology
        - Wet mount
        - Concentration techniques
        - Staining
        - Immunodiagnostic techniques
    - Life cycle, morphological stage and classification of parasites
        - Medical Helminthological
            - ✓ Nematodes
            - ✓ Intestinal nematodes
            - ✓ Tissue and blood nematodes
        - Cestodes
        - Trematodes
        - Medical Protozoology
            - ✓ Intestinal protozoa
            - ✓ Protozoa of urogenital tract
            - ✓ Hemo-flagellates
            - ✓ Malaria Parasites

This guide will also assist you to attain the learning outcome stated in the cover page. Specifically, upon completion of this Learning Guide, you will be able to –

- Identify concepts of human parasitology.
- Identify principles of host and parasite interaction.
- Identify methods of parasitological examinations.
- Differentiate lifecycles and diagnostic stage of parasites.

**Learning Instructions:**

1. Read the specific objectives of this Learning Guide.

2. Follow the instructions described in number 3 to 16.
3. Read the information written in the “Information Sheets 1”. Try to understand what are being discussed. Ask your trainer for assistance if you have hard time understanding them.
4. Accomplish the “Self-check 1” in page 7.
5. Ask from your trainer the key to correction (key answers) or you can request your trainer to correct your work. (You are to get the key answer only after you finished answering the Self-check 1).
6. If you earned a satisfactory evaluation proceed to “Information Sheet 2”. However, if your rating is unsatisfactory, see your trainer for further instructions or go back to Information sheet 1.
7. Submit your accomplished Self-check. This will form part of your training portfolio.
8. Read the information written in the “Information Sheet 2”. Try to understand what are being discussed. Ask your trainer for assistance if you have hard time understanding them.
9. Accomplish the “Self-check 2” in page 11.
10. Ask from your trainer the key to correction (key answers) or you can request your trainer to correct your work. (You are to get the key answer only after you finished answering the Self-check 2).
11. Read the information written in the “Information Sheets 3”. Try to understand what are being discussed. Ask your trainer for assistance if you have hard time understanding them.
12. Accomplish the “Self-check 3” in page 19.
13. Ask your trainer the key to correction (key answers) or you can request your trainer to correct your work. (You are to get the key answer only after you finished answering the Self-check 3).
14. If you earned a satisfactory evaluation proceed to “Operation Sheet 1” in page 21; However, if your rating is unsatisfactory, see your trainer for further instructions or go back to Information sheet 3.
15. Read the “Operation Sheet 1 and try to understand the procedures discussed.

| Information Sheet-1 | Identify concepts of human parasitological |
|---|---|

**Identify concepts of human parasitological**

- Introduction to parasitology**:**

  Medical Parasitology: Parasitology is the most fascinating branch of biology that studies parasites and their relationship with their hosts. Medical parasitology, on the other hand, deals with parasites that cause diseases to human beings or Medical parasitology is the science that studies or deals with parasites infecting humans. The most accepted definition in medicine is, Medical parasitology is a subject that also deals with the biological features of medically important parasites, the relationship between the human being and the parasites, the diagnosis, treatment, prevention and control of the parasitic diseases. In general, while it is important to classify bacteria and fungi and viruses as parasites, parasitology has traditionally been limited to parasitic protozoa, helminthes, and arthropods. It follows, then, that parasitology encompasses elements of Protozology, helminthology, and medical arthropodology. Human parasitology, an important part of parasitology, studies the medical parasites including their morphology, life cycle, the relationship with host and environment.

- **Definition of terms**:

  - ✓ Parasitology: is a science that deals with parasites
  - ✓ Medical parasitology: is that study of parasites that causes disease in man
  - ✓ Parasite: is an organism living temporarily or permanently in or on another organisms (host) from which is physically or physiologically dependent up on other
  - ✓ A host: is an organism which harbors a parasite and provides food. A host is much bigger than a parasite and has lower reproductive capabilities compared to its parasite.
  - ✓ A vector: is an organism an arthropod which transfers infective forms of a parasite from one host to the other.
  - ✓ Infective Stage: it is a stage when a parasite can invade human body and continue to live there. The infective stage of ascaris is the embryonated egg.
  - ✓ Route of infection: is the specific entrance through which the parasite invades the human body. Hookworms invade human body by skin. Man gets infection with Ascaris by mouth.

- ✓ Modes of infection: means how the parasite invades human body, such as the cercariae of the blood fluke actively penetrate the skin of a swimming man and the infective Ascaris eggs are swallowed by human.
- ✓ Helminthes: are parasitic worms that feed on a living host to gain nourishment and protection, while causing poor nutrient absorption, weakness and disease in the host. The following groups of worms are classed as **helminths**: Nematodes or roundworms. Trematodes, which includes flukes or flatworms. Cestodes or tapeworms.
- ✓ Protozoa: are single celled organisms. They come in many different shapes and sizes ranging from an Amoeba which can change its shape to Paramecium with its fixed shape and complex structure. They live in a wide variety of moist habitats including fresh water, marine environments and the soil.
- ✓ Geohelminthes or Soil Transmitted Helminthes: refers to the helminthes which complete their life cycles not requiring the processes of the development in intermediate hosts. They have only one host and a simple life cycle, such as Ascaris, Hookworm, Pinworm and etc.
- ✓ Biohelminthes: refers to the helminths which have to undergo the development in intermediate hosts to complete their life cycles, such as filarial, liver fluke, pork tapeworm and so on.

| Self-check 1 | Written test |
|---|---|

**Write True if the statement is correct and False if it is incorrect**

1. Medical Parasitology deals with parasites that cause disease. (2 points)
2. A parasite is an organism that can survive without help of other organism.( 2 points )
3. A vector can transmit an infective stage of a parasite.( 2 points)

**Note: satisfactory rating is 4 points, unsatisfactory 2 points. You can ask your instructor for copy of correct answer.**

Score = _____

Rating: _____

**Answer Sheet**

Name: _____ Date: _____

| Information Sheet-2 | Host parasite interactions |
|---|---|

## ✓ Host parasite interactions

Any organism that spends a portion or all of its life closely associated with another living organism of a different species is known as a symbionts (or symbiotic), and the relationship is known as symbiosis (living together of organisms of two different species). There are four categories of symbiosis are commonly recognized: commensalism, phoresis, parasitism, and mutualism. The scope of this part is limited to relationships of medical importance, and, since parasitism is the major type of symbiosis meeting this criterion, definitions of the other forms is included for clarification only. **A. Commensalism:** Commensalism does not involve physiologic interaction or dependency between the two partners, the host and the commensal. Literally, the term means “eating at the same table.” In other words, commensalism is a type of symbiosis in which spatial proximity allows the commensal to feed on substances captured or ingested by the host. The two partners can survive independently. Although at times certain nonpathogenic organisms (e.g., protozoa) are referred to as commensals, this interpretation is incorrect since they are physiologically dependent on the host and are, therefore, parasites. An example of commensalism is the association of hermit crabs and the sea anemones they carry on their borrowed shells.

**B. Phoresis**: The term phoresis is derived from the Greek word meaning “to carry.” In this type of symbiotic relationship, the phoront, usually the smaller organism mechanically carried by the others usually larger organism or the host. Unlike commensalism, there is no dependency in the procurement of food by either partner. Phoresis is a form of symbiosis in which no physiologic interaction or dependency is involved. Both commensalism and phoresis can be considered spatial, rather than physiologic, relationships. Examples of phoresis are the numerous sedentary protozoans, helminthes eggs/larvae stages, algae, and fungi that attach to the bodies of aquatic arthropods, turtles, cockroaches (in case of human infections), etc.

**C. Parasitism**: Parasitism is another type of symbiotic relationship between two organisms: a parasite usually the smaller of the two, and a host upon which the parasite is physiologically dependent. The relationship may be:

**Permanent**, as in the case of tapeworms found in the vertebrate intestine,

**Temporary**, as with female mosquitoes, some leeches, and ticks, which feed intermittently on host blood. Such parasites are considered obligatory parasites because they are physiologically dependent upon their hosts and usually cannot survive if kept isolated from them.

**Facultative parasites**, on the other hand, are essentially free-living organisms that are capable of becoming parasitic if placed in a situation conducive to such a mode. An example of a facultative parasite is the amoeba Naegleria. Unlike commensals, parasites derive essential nutrients directly from the host, usually from such nutritive substances as blood, lymph, cytoplasm, tissue fluids, and host-digested food.

**D. Mutualism:** is an association in which the mutualistic and the host depend on each other physiologically. Simply the association is obligatory and living apart is impossible. That is, they must live together and one cannot survive in the absence of the other. A classic example of this type of relationship occurs between certain species of flagellated protozoans and the termites in whose gut they live.

**Note**: there is less overlap between phoresis/commensalism and parasitism as well as between phoresis/commensalism and mutualism than between parasitism and mutualism.

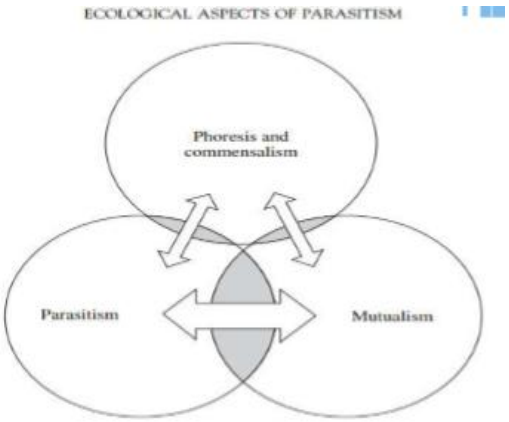



Figure 1.1Overlap between the major categories of symbiosis.

| Self-check 2 | Written test |
|---|---|

**Answer the following questions (2 points for each questions)**

1. Relationship between two different species is:
   A. Commensalism
   B. Phoresis
   C. Symbiosis
   D. Parasitism
2. Eating at the same table is;
   A. Commensalism
   B. Phoresis
   C. Symbiosis
   D. Parasitism
3. The dependence of one organism on other for food and shelter:
   A. Commensalism
   B. Phoresis
   C. Symbiosis
   D. Parasitism
4. An association of two different species for common benefit is:
   A. Mutualism
   B. Commensalism
   C. Parasitism
5. A form of symbiosis in which no physiologic interaction or dependency is involved is;
   A. Commensalism
   B. Phoresis
   C. Symbiosis
   D. Parasitism

**Note: satisfactory rating is 6 points, unsatisfactory 4 points. You can ask your instructor for copy of correct answer.**

**Answer Sheet**

Score = _____
Rating: _____

Name: _____ Date: _____

## ✓ Techniques in Medical Parasitology

**Basic diagnostic techniques for parasitic infection includes**

- Morphological diagnosis (Macroscopic /Microscopic diagnosis)

  By morphological diagnosis the stages of the parasites to be detected are eggs, larva and adult stage for **Helminths** and cyst, trophozoite stages for protozoans.

  - Macroscopic: using the naked eye for the presence of some adult worms. *E.g. Ascaris, E. vermicularis and gravid Taenia* spp.

- Microscopic: The majority of intestinal, blood, urinary and skin parasites are usually detected microscopically. Stained or unstained preparation of different specimens (such as blood, stool, urine, CSF, etc…) are examined either:
- **Directly**: Wet mount can be prepared directly from fecal material or from concentrated specimens.

  Basic types of wet mount are:

  - Saline: is initial microscopic examination of stool by direct stool smear. It primarily used to demonstrate adult worms, eggs, larvae of helminths and cysts and trophozoites of protozoan parasites. Can also reveal the presence of Red blood cells and White blood cells or pus cells.
  - Iodine: used mainly to stain glycogen and the nuclei of cysts of protozoa.
  - Materials and reagents used for wet mount preparation are the following.
    - Slides, cover slides, applicator sticks, and marker for labelling, microscope.
    - Reagents: normal saline 85%, Lugos iodine.

- **Concentration techniques:** The direct examination of faeces is essential to detect motile parasites and is usually adequate to detect significant helminth infections. Important exceptions are Schistosoma species because only a few eggs are usually produced even in moderate and severe infections, therefore a concentration technique should be performed when intestinal schistosomiasis is suspected and no eggs are found by direct examination.
  - Concentration techniques may also be required:
    - To detect Strongyloides larvae, the eggs of Taenia, cysts of G. lamblia, and to make it easier to detect small parasites, e.g. small fluke eggs, or the oocysts of intestinal coccidia prior to staining.

- To check whether treatment has been successful.
- To quantify intestinal parasites.

- The following techniques are commonly used to concentrate faecal parasites in district laboratories:
  - **Sedimentation techniques** in which parasites are sedimented by gravity or centrifugal force, e.g. formol ether concentration method which is the most frequently used technique because it concentrates a wide range of parasites with minimum damage to their morphology.
  - **Floatation techniques** in which parasites are concentrated by being floated in solutions of high specific gravity, i.e. solutions that are denser than the parasites being concentrated. Examples include the zinc sulphate method and saturated sodium chloride method. Unlike the formol ether sedimentation technique, a single floatation technique cannot be used to concentrate a wide range of parasites because of differences in the densities of parasites and the damage that can be caused by floatation fluids to some parasites
  - **Choice of concentration technique depends on** :
    - Why the technique is being performed, the species of parasite requiring concentration, and how well its morphology is retained by a particular technique.
    - The number of specimens to be examined and time available. –
    - The location, e.g. field or laboratory situation and equipment available.
    - Experience of staff performing the technique.
    - Health and safety considerations.

A. **Formol ether concentration technique**: This is recommended for use in laboratories because it is rapid and can be used to concentrate a wide range of faecal parasites from fresh or preserved faeces. Risk of laboratory acquired infection from faecal pathogens is minimized because organisms are killed by the formalin solution. The technique, however, requires the use of highly flammable ether or less flammable ethyl acetate.

**Principle:** In the Ridley modified method, faeces are emulsified in formol water, the suspension is strained to remove large faecal particles, ether or ethyl acetate is added, and the mixed suspension is centrifuged. Cysts, oocysts, eggs, and larvae are fixed and sedimented and the faecal debris is separated

in a layer between the ether and the formol water. Faecal fat is dissolved in the ether. The require reagent is formol water 10%V/V.

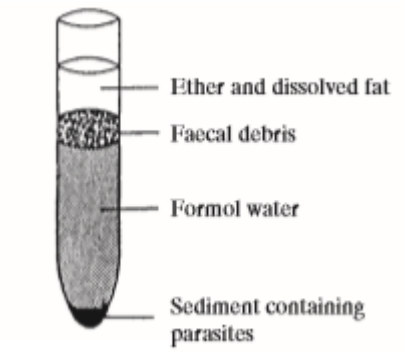



*Fig. 1.2 Formal ether sedimentation concentration technique, after centrifugation*

B. **Zinc sulphate floatation technique:** zinc sulphate technique is recommended for concentrating the cysts of G. lamblia and E. histolytica/E. dispar, and the eggs of T. trichiura species. Other nematode eggs are concentrated less well. The technique is not suitable for concentrating eggs or cysts in fatty faeces. Adequate safety precautions should be taken because faecal pathogens are not killed by zinc sulphate.

**Principle:** A zinc sulphate solution is used which has a specific gravity (relative density) of 1.180–1.200. Faeces are emulsified in the solution and the suspension is left undisturbed for the eggs and cysts to float to the surface. They are collected on a cover glass. The require reagent is Zinc sulphate solution, 33% w/v.

C. **Saturated sodium chloride floatation technique**:The saturated sodium chloride technique is a useful and inexpensive method of concentrating hookworm or Ascaris eggs, e.g. in field surveys. The technique is the same as that described for the zinc sulphate floatation technique.

♣ **Staining:** as many parasitic organisms cannot be cultured, microscopic examination is the mainstay of diagnostic parasitology. Examination after proficient staining of fresh and unconcentrated specimens, as well as preserved and or concentrated specimens with permanent stained preparations, most often provides for rapid and accurate diagnosis. A variety of reagents and stains are available for these purpose, and each laboratory must decide which ones to use to best serve its patient population. In addition most specimens are transported in fixatives and preservatives.

There are three categories of chemicals used to preserve stool, prepare slides for staining and stain the preparation:

1. Fixatives

2. Preparatory reagents
3. Stains

- **Temporary stains**
    1. Eosin
    2. Saline
    3. Acridine Oragne
    4. Lugol's Iodine
    5. Thomson's stain
    6. Sargeant's stain
    7. Burrow's stain
- **Permanent stains**
    1. Auramine Phenol
    2. Field's stain (A/B)
    3. Giemsa stain
    4. Iron Hematoxylene (A/B)
    5. Trichrome
    6. Modified Trichrome
    7. Modified Ziehl – Neelsen

- **Sodium Acetate -** Acetic Acid - Formalin Fixative (SAF)

  SAF fixed material is suitable for:
    - ☐ Direct examination
    - ☐ Concentration and
    - ☐ Permanent staining
- **Field Stain A & Field Stain B**
    - ☐ Enables rapid staining of fixed thin films.
    - ☐ This particular method is very useful for staining films of unformed feces, fecal exudates, duodenal aspirates etc.
- **Lugol's Iodine (Aqueous)**
    - ☐ Temporary Stain for Protozoa.
- **Iron Haematoxylen Solution A/B**

  **Method: p**reparation of Working Iron Haematoxylin Solution
    - ☐ Mix equal volumes of the two solutions and filter.
    - ☐ Allow to stand at least two hours.
    - ☐ Parasite stain blue if used immediately after preparation
    - ☐ Mature stains stain light blue with grey back ground
    - ☐ If a slide appears cloudy, then dehydration has been inadequate.

- ☐ Agitation in the final alcohols can improve the clarity of the smear.

- **Trichrome For Protozoa**
  - ☐ May be used to stain fresh faeces, prefixed faeces or cultured organisms.
  - ☐ The method varies slightly depending on the sample preparation used.

♣ **Immunological diagnosis (Antibody and antigen detection**

Pertaining to diagnosis by immune reactions. Is based on the detection of:

A. **Antibody (Ab) from person's serum**

Ab is produced in response to a particular parasitic infection

B. **Antigen (Ag) detection**

- ☐ Ag. Is excreted by parasites and can be found in the serum, urine, CSF, feces or other specimens.
- ☐ Antigen tests provide evidence of present infection

Why Immunodiagnostic techniques are required?

- Parasites live in the tissue of internal organ and cannot easily obtained for examination.
- Parasites can be found in specimens only in certain stages of infection,
  - ☐ E.g. in the acute stage not in the chronic stage.
- Parasites are present intermittently or in too few numbers to be easily detected in the specimens.
- The techniques used to detect parasites are complex or time consuming.

Immunodiagnosis is particularly used for the following types of parasites

- ■ South American trypanosomiasis , Chronic stage
- ■ African trypanosomiasis, when parasitaemia is low
- ■ Leishmaniasis
- ■ Filariasis
- ■ Amoebic liver abscess
- ■ Trichinosis
- ■ Toxoplasmosis
- ■ Toxocarisis
- ■ Hydatid disease
- ■ Schistosomiasis
- ■ Malaria

| Self-Check 3 | Written Test |
|---|---|

***Directions:*** Answer all the questions listed below. Use the Answer sheet provided in the next page:

1. Macroscopic diagnosis technique involves detection_____ stage of parasite. (2 points)
2. List the chemicals used for temporary staining in parasitology lab. (5 points)
3. List the chemicals used for permanent staining in parasitology lab. (5 points)
4. Describe wet mount technique. (5 points)
5. Immunological diagnosis technique detects _____and _____? (2 points)
6. List at least five parasites for which we can use immunological diagnostic technique? (5 points)
7. Briefly explain why the immunological diagnosis method is required. (4 points)

**Answer Sheet**

Score = _____
Rating: _____

Name: _____ Date: _____

| Information sheet 4 | Life cycle, morphological stage and classification of parasites |
|---|---|

## 4. Life cycle, morphological stage and classification of parasites

### 4.1. Introduction:

**Parasitism:** A parasite is an organism that is entirely dependent on another organism, referred to as its host, for all or part of its life cycle and metabolic requirements. Parasitism is therefore a relationship in which a parasite benefits and the host provides the benefit. The degree of dependence of a parasite on its host varies. An obligatory parasite is one that must always live in contact with its host. The term free-living describes the non-parasitic stages of existence which are lived independently of a host, e.g. hookworms have active free-living stages in the soil.

### 4.2. Terms used to describe parasite hosts

- Definitive host: This is the host in which sexual reproduction takes place or in which the most highly developed form of a parasite occurs. When the most mature form is not obvious, the definitive host is the mammalian host.
- Intermediate host: This is the host which alternates with the definitive host and in which the larval or asexual stages of a parasite are found. Some parasites require two intermediate hosts in which to complete their life cycle.
- Reservoir host: This is an animal host serving as a source from which other animals can become infected. Epidemiologically, reservoir hosts are important in the control of parasitic diseases. They can maintain a nucleus of infection in an area.

### 4.3. Features of parasites

- Smaller than their host
- Outnumber of the host
- Have short life span than their host
- Have greater reproductive potential than their host

### 4.4. Classification of parasites

#### 4.4.1. According to their habitat

- **Ecto-parasites: parasites** living on or affecting the skin surface of the host e.g. Lice, tick, flea, bed-bugs, etc---
- **Endo -parasites**: parasites living with in the body of the host. E.g. leishmonia species, Ascaris lumbricoides etc.

### 4.4.2. According to their dependent on the host

- **Permanent** (Obligate) parasites: the parasite depends completely up on its host for metabolites, shelter, and transportation e.g. malaria.
- **Temporarily** (facultative) parasite: the parasite is capable of independent existence in addition to parasitic life e.g. stronglyloids sterecolaris, Naegleria fowlery etc. …

### 4.4.3. According to their pathogencity

- **Pathogenic parasites**: It causes disease in the host e.g. E. histolytica
- **Non-pathogenic** (Commensally) **parasite**: the parasite derives food and protection from the host but not harms the host. E.g. Entamoeba coli.
- **Opportunistic parasites**: parasites, which cause mild disease in immunologically healthy individuals but they cause, sever disease in immuno – deficient hosts. e.g. pneumocystis carnii, Toxoplasma gondii, Isospora belli ,etc

## 4.5. Source of parasitic infections

- **Contaminated soil**: polluted soil with human excreta is commonly responsible for exposure to infection with Ascaris lumbricodes, Trichuris trichuria and hookworms.
- **Contaminated water**: water may contain
  a) Viable cysts of amoeba, flagellates and T.Solium eggs
  b) Cercarial stage of human blood flukes
  c) Cyclopes containing larvae of dracunculus medinesis,
  d) Fresh water fishes, which are sources of fish tapeworm and intestinal flukes infection.
  e) Crab or cray fishes that are sources for lung fluke and
  f) Water plants which are sources of Fasciolopsis buski
  g) Insufficiently cooked meat of pork and beef which contains infective stage of the parasite e.g. Trichenilla spirals. Taenia species
  h) Blood sucking arthropods: - these are responsible for transmission of e.g.
     * Malaria parasites
     * Leishmania
     * Trypanosoma
     * Wuchreria (Filariasis)

2. Animals (domestic or wild animals harboring the parasites)
   * Dog – hydatid cyst, cutaneous larva migrans
   * Herbivores animal – Trychostrongylus species.

3. Human beings – Another person's his clothing, bedding or the immediate environment that he contaminated e.g. Amoeba, E.vermicularis, H.nana
4. Sexual intercourse e.g. Trichomonas vaginalis
5. Auto infection e.g. S.stercoralis, E.vermicularis, and taemia solium.

## 4.6. Mode of transmission

### 4.6.1. Direct- mode of transmission

The parasite does not require biological vectors and or intermediate hosts and requires only a single host to complete its life cycle. It may require mechanical vectors direct mode of transmission can be classified as.

4.6.2. **Horizontal direct mode of transmission**:

Mainly transmitted through

- Feco-oral route
- Sexual intercourse
- Blood transmission
- Direct skin penetration
- Air born

4.6.3. **Vertical direct mode of transmission occurs from mother to child through**

- Congenital /transplacental
- Transmammary (breast milk)

## 4.7. Indirect- mode of transmission

The parasite has complex life cycle and requires biological victor and /or one or more intermediate hosts for transmission.

## 4.8. Host parasite relation ship

### 4.8.1. Effects of parasites on their hosts

- Consumption of the nutritive e.g. hook worm-sucks blood, D.latum remove $B_{12}$
- Obstruction of passages – e.g. Ascaris lumbricoides
- Bleeding e.g. schitosome eggs.
- Destruction of tissues e.g. Trophooites of E. histolytica, causes necrosis of liver, leishmania donorani – bone marrow destruction
- Compression of organs e.g. Hydatid cysts in liver, brain cause presure

- Release of toxic substances e.g. rupture of E. granulosus result anaphylactic shock
- Opening pathway to secondary infections e.g. ulcer. Eg D. medenesis for bacteria
- Allergy development e.g. Bite of arthropod
- Transmission of pathogens to man e.g. lice transmitte rickettsia
- Predisposition to malignancy. Eg Bilharziasis
- Chronic immune stimulation leading to unresponsiveness to infections.

### 4.8.2. Host susceptibility factors

#### 4.8.2.1. Host factors

- Genetic constitution
    - Age
    - Sex
    - Level of immunity: - natural and acquired immunity
    - Nutrition (malnutrition or under nutrition)
    - Intensity and frequency of infections
    - Presence of co-existing disease or conditions which reduced immune response e.g. pregnancy, HIV,
    - Life style and occupation

### 4.8.3. Parasite factors

- Strain of the parasite and adaptation to human host
- Parasite load (number of parasite)
- Site (occupied) in the body
- Metabolic process of the parasite, nature of the waste products or toxins

## 4.9. Escape mechanism of parasite from the immune system.

- Site e.g. intracellular parasites e.g. T. curzi, leishmania and plasmodia are to some extent protected from the action of antibodies and forming cysts as T.gondii and larva of T.solium, E.granulous and Trichinella spiralis,
- Avoidance of recognition e.g. Recognition of some parasites as self-cell. Masking their selsves, eg. Schistosomes. Variation of antigens e.g. African trypanosomes. This types of relationship is known as molecula mimicry

- Suppression of immune response e.g. plasmodium, Toxoplasm, Trypanosome and Trichinella are able to suppress the ability of the host to response immunologically and increasing other infections results
    - Combine with the antibody and preventing it from attaching to the parasite
    - Induce B or T-cell tolerance either by blocking antibody or by depleting specific antigens.
    - Activating specific suppressor cells

## 4.10. Nomenclature of Human parasites

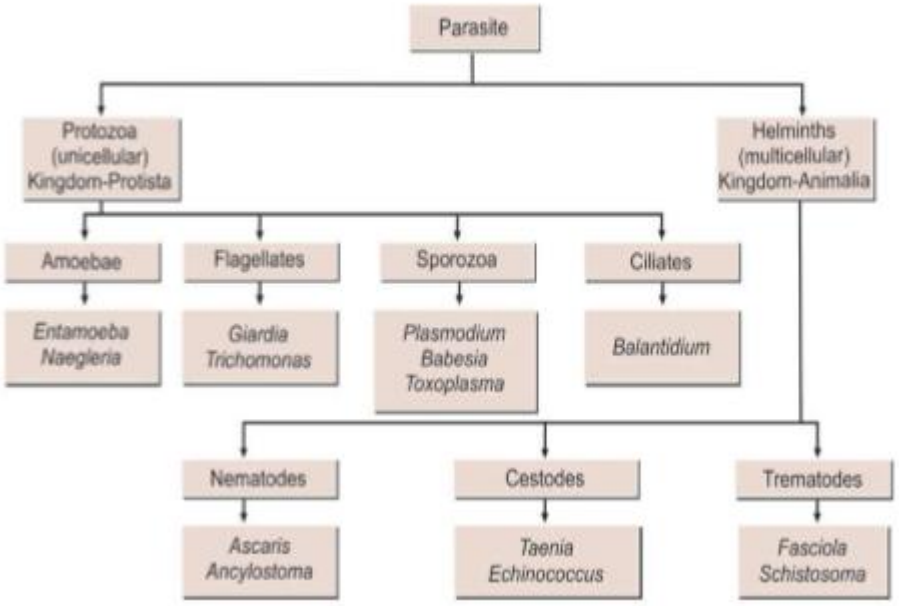
All animals and plants must have names by which they can be distinguished. Although common names are frequently, used for this purpose. These are not universally understood, partly because of language barriers and partly because of a common name not necessarily applied to the same organism in different countries. To overcome this difficulty, a binomial scientific name is used. Based on the international code of zoological nomenclature.

The first name in the binomial is that the genus to which the organism belongs, and the second is that of the species this combination of in designating an animal or plant species is termed binomial nomenclature.

Taxonomic classification of medically important parasites of men belongs to the kingdom of Animalia and most of parasites are members of three phyla.

1. Phylum Nemathelminths
2. Phylum Platyhelminths
3. Phylum protozoa

The following flow chart shows the general classification medically important parasites.




***Flow chart 1.1. Nomenclature of parasites***

4.11. **The life cycles of parasites**:

The life cycles of parasites describes the cycle of development of the parasites that Involves passing through a number of developmental stages either in the host or the environment.

The life cycle a parasite has three phases (components) that are very important for the successful survival of a parasite:

- Growth and maturation
- Reproduction
- Transmission.

The life cycle can be **simple (direct)** or **complex (indirect**) depending on how many hosts it required to complete its cycle.

- **Simple life cycle or direct:** only one host is required to complete the life cycle. Transmission is through contaminated food (grass), water, air, contaminated material or directly from person to person through sexual intercourse.

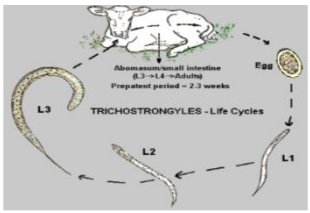



Fig. 1.3. The direct life cycle of Trichstrogyles.

- **Complex (indirect**): two or more hosts are required to complete the life cycle.

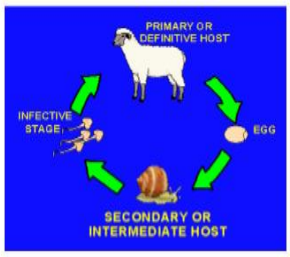



Fig. 1.4. Indirect life cycle involving more than one host.

## 4.12. Medically important helminthes

### 4.12.1. General features of helminthes:

- The word ‘helminths’ is a general term meaning ‘worm’. All helminths are multicellular eukaryotic invertebrates with tube-like or flattened bodies. Many helminths are free-living organisms in aquatic and terrestrial environments whereas others occur as parasites in most animals and some plants. Parasitic helminths are an almost universal feature of vertebrate animals; most species have worms in them somewhere. They are parasitic metazoans from the phyla Platyhelmintha (flatworms), Nematoda (roundworms). That means they are multicellular parasites belong to the kingdom Metazoa.The term 'helminths' (Greek helmins’worm’) originally referred to intestinal worms, but now comprises many other worms, including tissue parasites as well as many free - living species. The size of the adult worms varies in size (6mm->30m). Their life cycles may be simple or complex. Pathology, clinical sign and symptoms depend on the location of the organisms. This may be caused by adults, larva, or egg (in case schistosomiasis).Laboratory diagnosis mainly depends on detection and identification of egg, larva or embryo and rarely adults in case of Ascariasis).

**4.12.1. Classification of helminthes:** Helminths, which occur as parasite in humans, belong to two phyla:

- Phylum Nemathelminths: It includes Nematoda
- Phylum Plathyhelmiths (flatworms). It includes two classes:
    - Class Cestoda (tapeworms)
    - Class Trematoda (flukes or digeneans)

### 4.12.2. Phylum Nemathelminths

**Class: Nematoda**

**Introduction**

Nematodes are the most common helminthes parasiting humans, and include intestinal nematodes as well as blood and tissue nematodes. The most common nematodes of medical importance are those inhabiting the intestinal tract. Most of

these have a direct life cycle and their presence may be confirmed by detecting eggs in stool.

The filarial are among the most important of blood and tissue nematodes. They are long, slender, round worms that parasitize the blood, lymph, subcutaneous and connective tissue of humans. They are transmitted by insect vectors and most produce larvae called microfilariae.

### 4.12.3. General characteristics of nematodes

- Non segmented cylindrical or round worms
- Possess a shiny cuticle which may be smooth, spine or ridged.
- Mouth is surrounded by lips or papillae
- Sexes are separated with the male & female
- They live in the tissues or intestinal tract
- Intestinal nematodes are feco – oral route and soil.

### 4.12.4. Intestinal round worms (Nematodes)

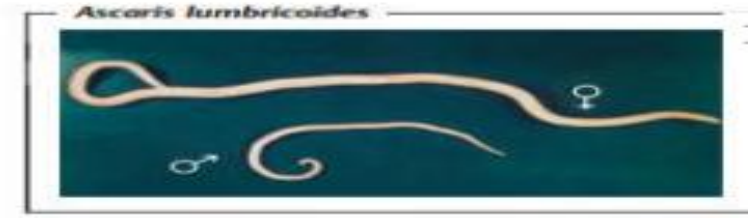
**General characteristics**

- Adult worms live in the intestinal tract
- Female worms are oviparous (lay eggs)
- Humans are the only or the most significant hosts.
- Most species are soil transmitted
- Before becoming adults in their human host, the larvae of A.lumbricoides, S.stercoralis, and hookworms have heart lung migration.

## 4.13. *Ascaris Lumbricoides* (round worm)

*A. lumbricoides* has a worldwide distribution. It is particularly common in the tropics and subtropics in places where environmental sanitation is inadequate and untreated human faeces are used as fertilizer (night-soil). Habitat: Adult in the small intestine. Egg in the faeces.

- Morphology: Adult pinkish color
- Male about 15cm in size curved tail and two copulatory spi cules of equal size.
- Female – 2 – 25cm long with straight tail eggs.



1.5. Male and female worms

There are five types of Ascaris eggs.

**a. Fertilized egg with double shell**

- Size – 70µm, shape oval or sometimes round.
- Shell – the two layers are distnict, rough, brown color, covered with little lumps external shell.
- Color brown external shell, and the contents are colorless or pale yellow.
- Content: a single rounded granular central mass.

**b. Unfertilized egg with double shell.**

- Size: 80 - 90µm
- Shape: more elongated (elliptical)
- Shell: brown, puffy external & thin internal shell.
- Content: full to large round very refractile granules.

**c. Semi – decorticated fertilized egg**

- Similar to **type (a) above** , but without the external shell
- Content: a single rounded colourless granular central mass
- Color: colorless or very pale yellow

**d. Semi – Decorticated unfertilized egg**

- Shell: a single smooth thin colorless (double line).
- Content: large rounded colorless refractile granules.

**e. Embryonated egg.**

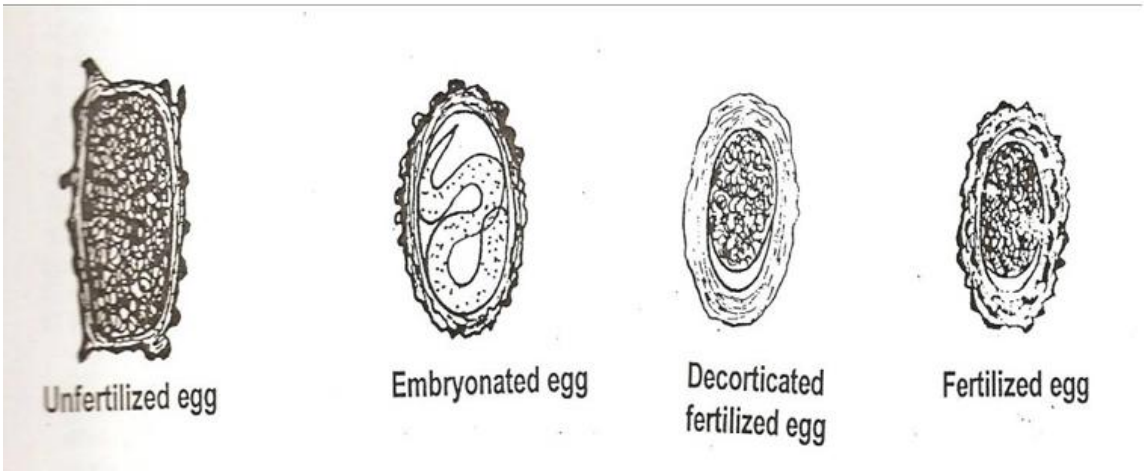



***Fig. 1.6. types of eggs of A. lumbricoides***

### 4.13.1. Mode of transmission

The infective stages is the egg containing second stage, rhabditiform larvae by ingestion in contaminated food or drink, from contaminated hand.

**Life cycle:***Ascaris lumbricoides* is spread by faecal pollution of soil.

**Embryonated egg:** is an infective stage(egg containing 2nd stage larva) Infection may occur through: Ingestion of food or water contaminated with embryonated eggs, eating soil (geophage) frequently seen in children, putting contaminated finger or toys with infective egg in to mouth, rarely by inhalation of eggs carried in air.

**Larva**: After human ingests an embryonated egg, it would hatch larvae. The larvae invade the mucosa of the intestine and are then carried through lymphatic to the portal vein and finally to the lungs. The larvae mature in the lungs, penetrate the alveolar walls, and move up the bronchial tree to the throat, where they are swallowed.

**Adult worms:** the larva matured to Adult in the small intestine, where the female reportedly can lay up to 200,000 eggs per day after mating or without mating. The eggs are passed from the host with the feces. After eggs are passed with the feces, they require a period of 2 weeks' incubation in the soil before they are viable and are capable of causing an infection. After eating the eggs that have contaminated vegetables harvested from the soil, the eggs hatch in the intestine and then enter the circulatory system by means of the hepatic portal circulatory system. This circulatory route takes

the blood containing the newly hatched larvae directly through the heart and into the lungs (Figure below).

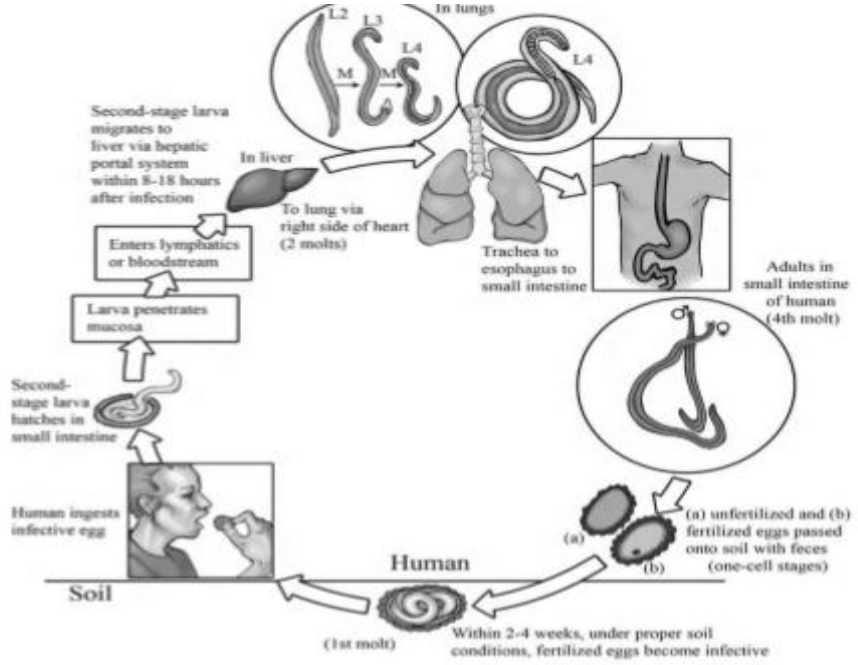



*Fig.1.7. Life cycle of Ascaris lumbricodes*

### 4.13.2. Pathogenesis:

1. by larval stage: offensive pneumonia results from lung tissue damage happens with migratory larvae during heart-lung migration
2. Pathogenesis adult worm stage: Bowel obstruction (intestinal obstruction) due to wandering many adult worms.
3. During feeding by adult worms: Parasite secretes trypsin inhibitor, prevents host from digesting proteins.
4. Migration of adult worms in the human body: Aberrant migration of “irritated” adult worms to; common duct, liver, Pharynx, peritoneum

**4.13.3. Clinical Features:** disease caused by A. lumbricoides is called as Ascariasis. Ascaris often causes mild or asymptomatic illness. Clinical manifestation is causes by both the migrating larvae and the adult worms.

The clinical manifestation is divided in to **three phases:**

**The lung phase:** occurs 5-6 days after infection

**Pneumonitis (Loffler’s pneumonia)**: due to penetration of lung capillaries by larval stage causing hemorrhage.

**Intestinal phase:** occurs 2-3 months after infection.

The most important clinical findings are:

- Abdominal pain due to migratory worms
- Diarrheal diseases
- Mal absorption of nutrients by adult worms
- stunted growth of children due to depletion of essential elements
- Cognitive impairment
- Intestinal obstruction by large number of adult worms. Wandering worms causes serious diseases such as, Intestinal perforation (see fig 7) by migratory worms and blacking bile ducts by adult worms.
- Liver abscesses due worms in the liver.

***Fig. 1.8****. Intestinal obstruction and perforation by adult worms of A. lumbricoides*

**4.13.4. Laboratory Diagnosis:** Most often is made by the identification of either or both fertilized and unfertilized eggs of *A. lumbricoides*. Adult worms may also be identified, as they may pass from anybody opening (orifices) including the anus, nose, or mouth. Larvae may also be found in sputum or gastric washings from infected individuals.

Thus, laboratory finding is often finding and identification of eggs in the stool through:

- **Direct wet mount**: adequate for detecting moderate to heavy infections
- **Concentration technique:** may be used in light infection, Sodium chloride floatation technique & Formol-ether concentration techniques are used.
- Larvae can be identified in sputum or gastric aspirate during the pulmonary migration phase.
- Finding adult worms macroscopically passed in stool, or through mouth, nose or anus.

**4.13.5. Treatment:** Mebendazole, 200 mg, for adults and 100 mg for children, for 3 days is effective.

**4.13.6. Prevention and control:** prevention of infection by washing hands before eating, trimming finger nails and avoiding eating uncooked foods such as vegetables. In addition, preventing soil become faecally polluted and avoiding the use of night soil as a fertilizer. Treatment of infected individuals and health education and mass de-worming programmes for elementary school children, repeated at 3-6 month intervals, have been advocated in areas of high prevalence.

### *4.14. Trichuris trichuria*

*Trichuris trichiura* is an intestinal nematode affecting an estimated 795 million persons worldwide. Also known as whipworm due to its characteristic shape, Trichuris can be classified as soil-transmitted helminths because its life cycle mandates embryonic development of its eggs or larvae in the soil. It is the second most common nematode found in humans, behind Ascaris. Morphology of *T. trichuria*: Adults: whip-like shape, anterior 3/5th of the worm resembles a whip & the posterior 2/5th is thick. Males are 30-45 mm in size and coiled tail. Females are 35 - 50mm with straight thick tail. Eggs 50-54 µm (micrometer) in Size. The shape resembles a "tea tray" or barrel- shaped with a colorless protruding mucoid plug at each end (look arrow) with eggs Shell thick and smooth having two layers & bile stained. It is yellow brown and the content is a central granular mass which is unsegmented ovum inside

**Fig. 1.9. Egg of *T. trichuria***

**Life cycle:**

Following ingestion of infective eggs the larvae in the small intestine and penetrates villi. After about a week, the larvae leave the small intestine and migrate to large intestine (caecum) where they develop in to mature worms.

- After mating, the female worms lay eggs which are passed in the faeces.
- In damp warm soil, the larvae develop and after 2-3 weeks each egg contains an infective larva.
- The eggs can remain infective for several months in moist warm soil but they are unable to withstand desiccation. ( see fig. 1.10 below)

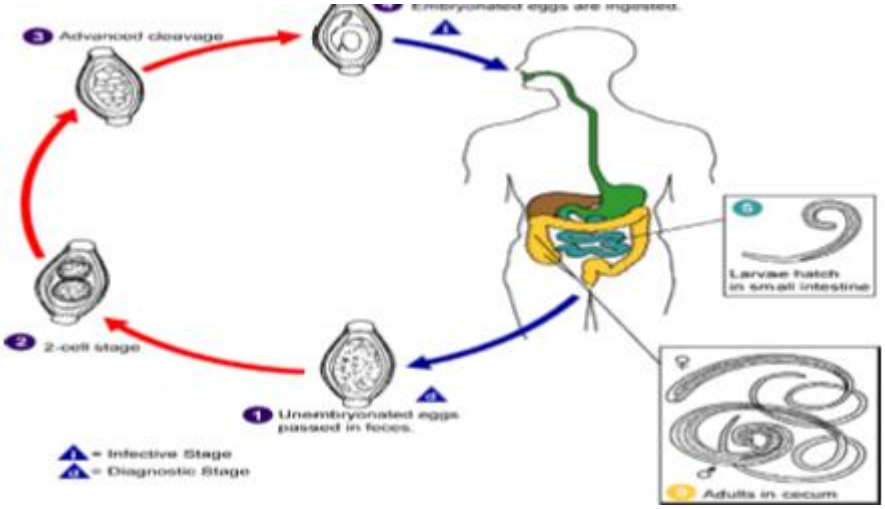


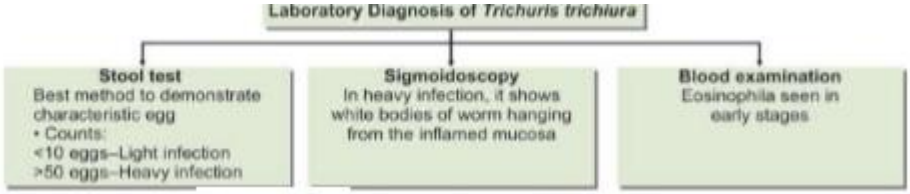

***Fig. 1.10 lifecycle of T. trichuria***

**Clinical features:** Clinical features are largely determined by dose of infection and worm burden. Often, less than 10 worms are asymptomatic (99% asymptomatic) while heavy worm burden results in mechanical damage to the intestinal mucosa. Infection with adult worms results in chronic profuse mucoid and bloody diarrhea with abdominal pain and edematous prolapsed rectum. Anemia from blood loss and iron deficiency, malnutrition, weight loss, and sometimes death. Each adult worm sucks about 0.005 ml per day.


***Fig. 1.11. Prolapsed rectum in trichuriasis***.

**Laboratory diagnosis**

Both infective stages: embryonated eggs while the diagnostic stage is unembryonated egg. Stool Examination: the characteristic barrel-shaped eggs are found in stools. The degree of infection can be assessed by egg counts. Less than 10 eggs per smear in direct stool preparation is considered light infection and More than 50 per smear as heavy infection (Flowchart below).




***Flow chart 1.2: Laboratory diagnosis for T. trichuria***

**Treatment**: Mebendazole (100 mg 12 hourly for 3–5 days)  Albendalzole (single dose of 400 mg) are effective with cure rates of 70–90%.

**Prevention and control:**

- Hand wash before and after toilet.
- Sanitary disposal of faeces in latrine.
- Treatment of infected individuals

### 4.15. *Entrobius vermicularis*

*Entrobius vermicularis* is a tiny worm living in the intestine. The common name is Pinworm, Seat worm, thread worm. E. vermicularis is considered to be world's most common parasite, which specially affects the children.

**Habitat:** Adult: in caecum, appendix and adjacent portions of the ascending colon while gravid female will be found around rectum (anus) to lay eggs.

**Transmission:** person to person through handling & sharing of contaminated clothes or bed linens and through surfaces in the environment that are contaminated with pinworm eggs.

- Self (autoinfection): Ingestion of eggs due to scratching of perianal area with fingers leading to deposition of eggs under the nails. This type of infection is mostly common in children. This mode of infection occurs from anus to mouth. Children who suck their fingers are more likely to be infected. Eggs are infectious upon leaving the host.

**Life cycle: see below**

*Enterobius vermicularis* (Pin Worm" or "threadworm" or seat worm)

| | |
|---|---|
| **Pathogenic** | Yes |
| **Disease** | Enterobiasis |
| **Acquired** | Fecal-oral transmission of infective eggs via contaminated food or water or on fingers and hands |
| **Body site** | Intestine (Large intestine) |
| **Symptoms** | Anal itching may be the only symptom; nervousness, insomnia, nightmares; occasional vaginal discharge |
| **Clinical specimen** | Scotch tape anal swab |
| **Epidemiology** | Worldwide, primarily human-to-human transmission |
| **Control** | Improved hygiene, adequate disposal of fecal waste, adequate washing of hands |

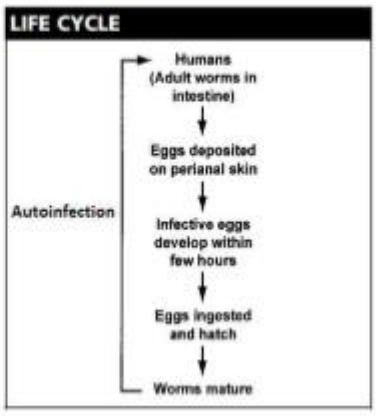


***Fig 1.12. Life cycle of Entrobius vermicularis***

**Morphology:**

**Adults**: are short, white, tapering at each end worms with pointed ends, looking like bits of white thread. Adults are yellow white in color. Males are 25 mm in length &Coiled tailed while Females are 8-13mm with thin pointed tails.

***Fig. 1.13. Adult Entrobius vermicularis***

**Eggs:** are deposited on perianal skin & occasionally in faeces. They are 50-60µm in size and oval with flattened in one side.

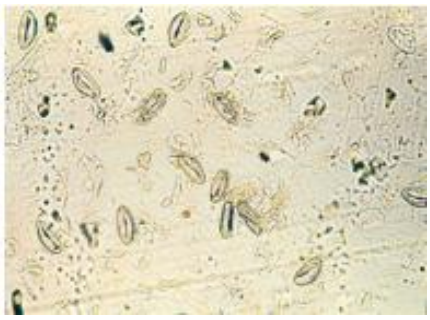
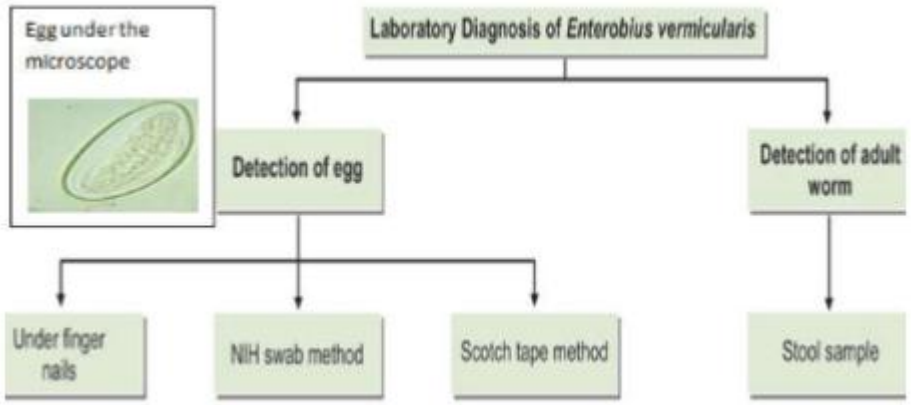


***Fig. 1.14. Eggs of Entrobius vermicularis***

**Laboratory Diagnosis:** Diagnosis depends on finding eggs or adult worms. This is normally done by sampling the perianal and perianal skin with cellulose tape (Scotch tape), which is applied sticky side down to the skin. The tape is transferred to a glass slide and examined under the microscope for eggs or adult worm.




***Flow chart 1. 3. Laboratory diagnosis of E. vermucularis***

**Treatment:** Pyrantel pamoate (11 mg/kg once, maximum 1 g), Albendalzole (400 mg once) or Mebendazole (100 mg once) can be used for single dose therapy, while piperazine has to be given daily for one week.

**Prevention and control:** Keeping personal and family hygiene. Frequent hand washing. Finger nail trimming and regular bathing. And frequent washing of night clothes and bed linen.

4.16. *Strongyloides stercoralis*:

*Strongyloides stercoralis* is the smallest nematode known to cause human infection and it is called dwarf thread worm.

**Transmission**:

- The main modes of transmission are through penetration of skin by filariform larva.
- Ingestion of food or water contaminated with filariform larva( oral route-causes Wakana syndrome) and autoinfection with rhabiditform larva.

**Unique Characteristics of Strongyloides stercoralis:**

- Parasitic males are absent
- Parasitic females are present in the sub-mucosa of small intestine which produces eggs parthenogenically (laying eggs without mating with male worm).
- Can develop in to free living generation in the soil outside the human host
- Has internal autoinfection (re-infection before larvae exit from the host)

**Habitat:**

The adult worm is found in the small intestine (duodenum and jejunum) of human.This parasite is a facultative parasite and has both free living and parasitic generations are present. Infective stage is Filariform larvae and diagnostic stage is Rhabditiform larvae. **Life cycle:** Adult: only females are parasite to human and they buried in the mucosal epithelium of the small intestine. Free living male and female worms mate on external environment.

- Eggs: are laid in the sub mucosa of small intestine. They are not found in feces/stool.
- Larvae: Rhabditiformlarvaeare passed in the faeces to the external environments and these motile larvae will be observed in stool. Filariformlarvae are infective stages which are found in soil & water.

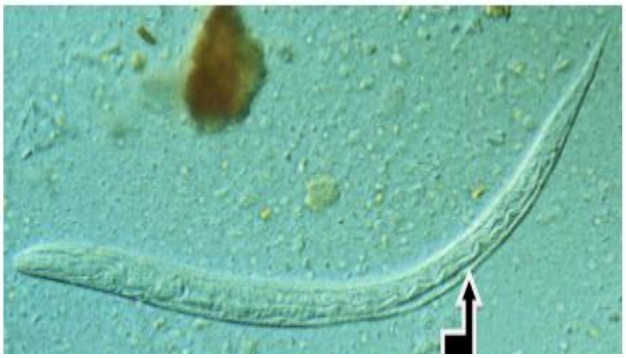


***Fig.1.15. Rhabditiform larvae of Strongyloides stercoralis in stool***

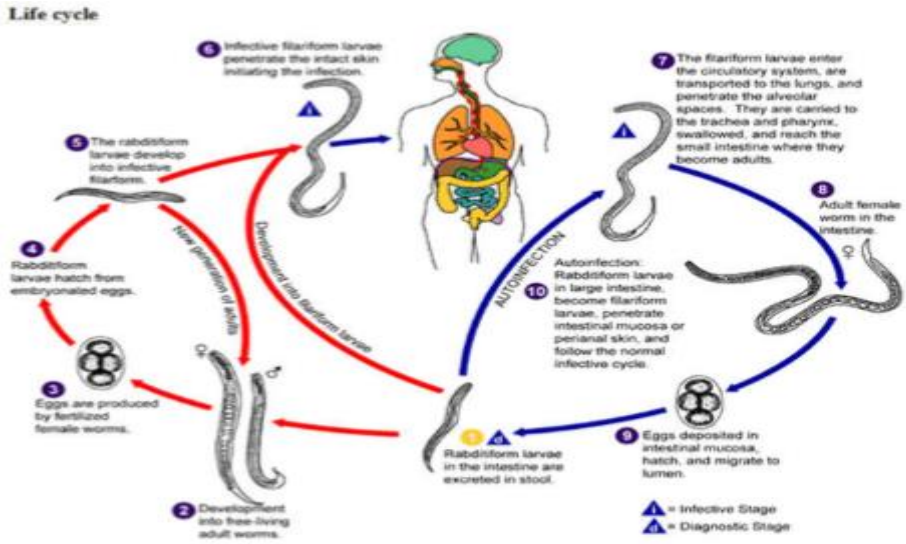



***Fig. 1. 16. Life cycle of S. stercolaries***

**Clinical feature:** It is usually asymptomatic. People with weaker immune systems such as elderly people & children are more susceptible.

There are three clinical phases:

- Cutaneous Manifestations: Large numbers of larva produce itching erythematic &allergic response at the site of infection within 24 hours of invasion by filariform larvae.
- Pulmonary Manifestations: when the larva escape from the pulmonary capillaries into the alveoli, hemorrhages in the alveoli and bronchioles may occur. Bronchopneumonia may be present, which may progress to chronic bronchitis and asthmatic symptoms in some patients. Larva of Strongyloides may be found in the sputum of these patients.
- Intestinal Manifestations: The symptoms may resemble those of peptic ulcer or mal-absorption syndrome. Mucus diarrhea is often present. In heavy infection, the mucosa may be honeycombed with the worm and there may be extensive sloughing and causing dysenteric stools.

- Other manifestations are protein losing enteropathy and paralytic illus.

**Laboratory Diagnosis:**

- Microscopy Direct wet mount of stool: Demonstration of the rhabditiform larvae in freshly passed stools is the most important method of specific diagnosis.
- Concentration methods of stool examination: Stool may be concentrated by Formol ether concentration examined for larvae more efficiently. Larvae may sometimes be present in sputum or duodenal aspirates and jejunal biopsies. Peripheral eosinophilia (>500/µmL of blood) is a constant finding. However, in severe hyper infection, eosinophilia may sometimes be absent.

**Treatment:** For all cases either symptomatic or not, should be treated to prevent severe invasive disease. Ivermectin (200 mg/kg/2 days) is more effective than albendazole (400 mg daily/3 days). For disseminated strongyloidosis, treatment with ivermectin should be extended for at least 57 days.

**Prevention and control:** Wearing protective foot wear and contact with infective soil and contaminated surface waters and treatment of infected individuals.

4.17. **Hook worms**:

Hook worms are hematophagous (blood feeders) nematodes. Common name: hookworms. Scientific name: Two species. Ancylomstoma duodelnale and Necator americanus.

**Infective form**: Filariform larva.

**Habitat: -** Adults are found in Jejunum and less often in the duodenum of man. - Eggs are found in the faeces; not infective to human (no infective stage).

- Rhabditiform & filariform larvae: free in soil and water.

- Morphology: Eggs: shape is oval. The egg shell is very thin & appears as black line. Eggs contains an ovum which appears segmented usually 4-8 blastomeres (undifferentiated immature cells witch finally develop to larvae stage in the eggs)

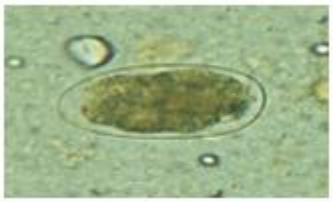


***Fig. 1.17. Ova of Hook worm***

**Life cycle of hookworms:**

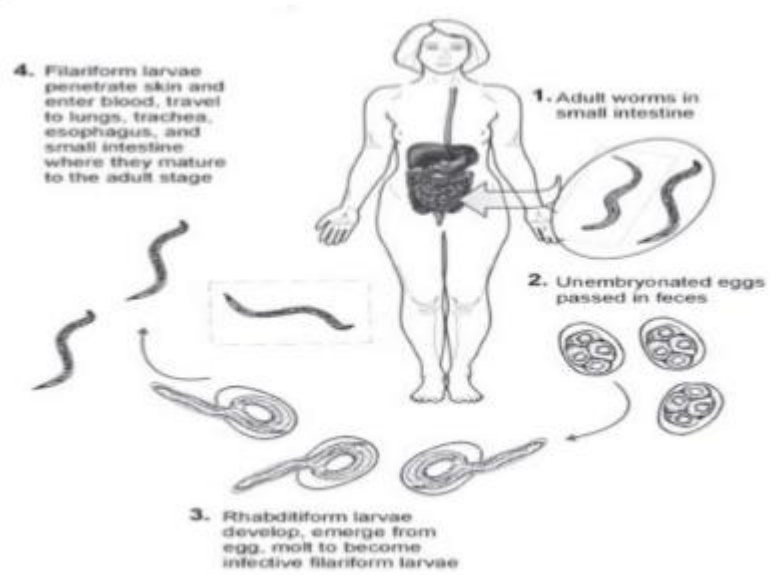


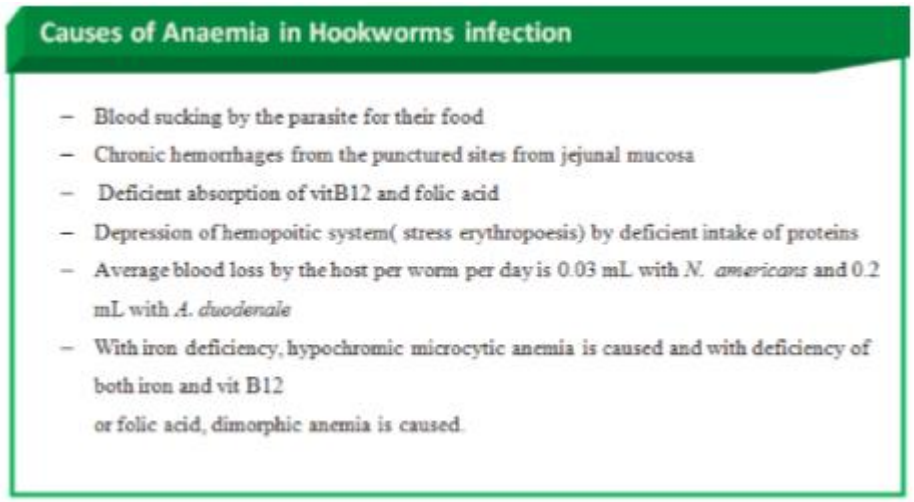

***Fig. 1.18. Life cycle of Hook worm***

An infective stage is filariform larva and diagnostic is egg. This is different from S.stercoralis. Transmission is same as that of S. stercoralis.

**Clinical features:** The name of disease is called hookworm infection. Intestinal inflammation & progressive iron/protein-deficiency anemia are the typical disease. About 90% of individuals with hookworm infection are asymptomatic. High loads of the parasite(20 – 100 worms) coupled with poor nutrition (inadequate intake of protein & iron) eventually lead to anemia which is a major clinical outcome of infection.

In general, hook worm causes:

- malnutrition, stunt growth & poor mental development in children
- Anemia leads to weakness & fatigue in adults.
- Other clinical features are same as S. stercoralis.




**Laboratory diagnosis:** Finding eggs in faeces by microscopic identification of eggs in the stool is the most common method. A.duodenale & N.americanus eggs morphologically indistinguishable by microscope.

- Freshly passed faeces should be examined.
- If the stool is more than 12 hours old, larva maybe seen inside the egg. –
- If the stool is more than 24 hours old, the larva will hatched and mislead with Strongyloides larva.
- The indirect method is blood examination reveals microcytic, hypochromic anemia and eosinophilia.

**Treatment:** A single 500 mg dose of Mebendazole often achieves a low cure rate, with a higher efficacy with a single, 400 mg dose of Albendalzole. Cutaneous larva migrants should be treated empirically with either albendazole 400 mg daily for three days, or ivermectin 200 mcg/kg/d for 1-2 days.

### 4.18. Blood and Tissue Nematodes (Filarial worms)

- General features: Filarial worms are threadlike (filum: thread) nematodes. The nematode genera of the super family filarioidea (order Spirurida) are included under the collective term filariae, and the diseases they cause are designated as flariasis. In the life cycle of filariae infecting humans, insects (mosquitoes, black flies, horse flies etc.) function as intermediate hosts and vectors, whereas human are definitive hosts. Hence, these parasites show complex life cycles involving more than one host. This indicates that it is difficult to control parasites involving more than one host. Generally, it is easy to control parasites which have direct life cycles than those having complex life cycles. The length of the adult stages of the species varies between 2–50 cm and the females are larger than the males. The females give birth to larvae stage called microfilariae.

These are about 0.2 – 0.3 mm long and can be sheathed microfilariae (covered by external protective cover) or unsheathed microfilariae(uncovered). They can be detected mainly in the skin or in blood. Based on the periodic appearance of microfilariae in peripheral blood, they can be periodic filarial species or non-periodic ones showing continuous presence. The periodic species produce maximum microfilaria densities either at night (nocturnal periodic) or during the day (diurnal periodic). The insect species that actively bite during the day or night function as intermediate hosts accordingly to match these changing levels of microfilaremia.

**Life Cycle of Filariae**

**Insect:** → Ingestion of microfilaria with a blood meal → development in thoracic musculature with two moltings to become infective larva → migration to mouth parts and tranmission into skin of a new host through puncture wound during the next blood meal.

**Human:** → Migration to definitive localizations and further development with two more moltings to reach sexual maturity.

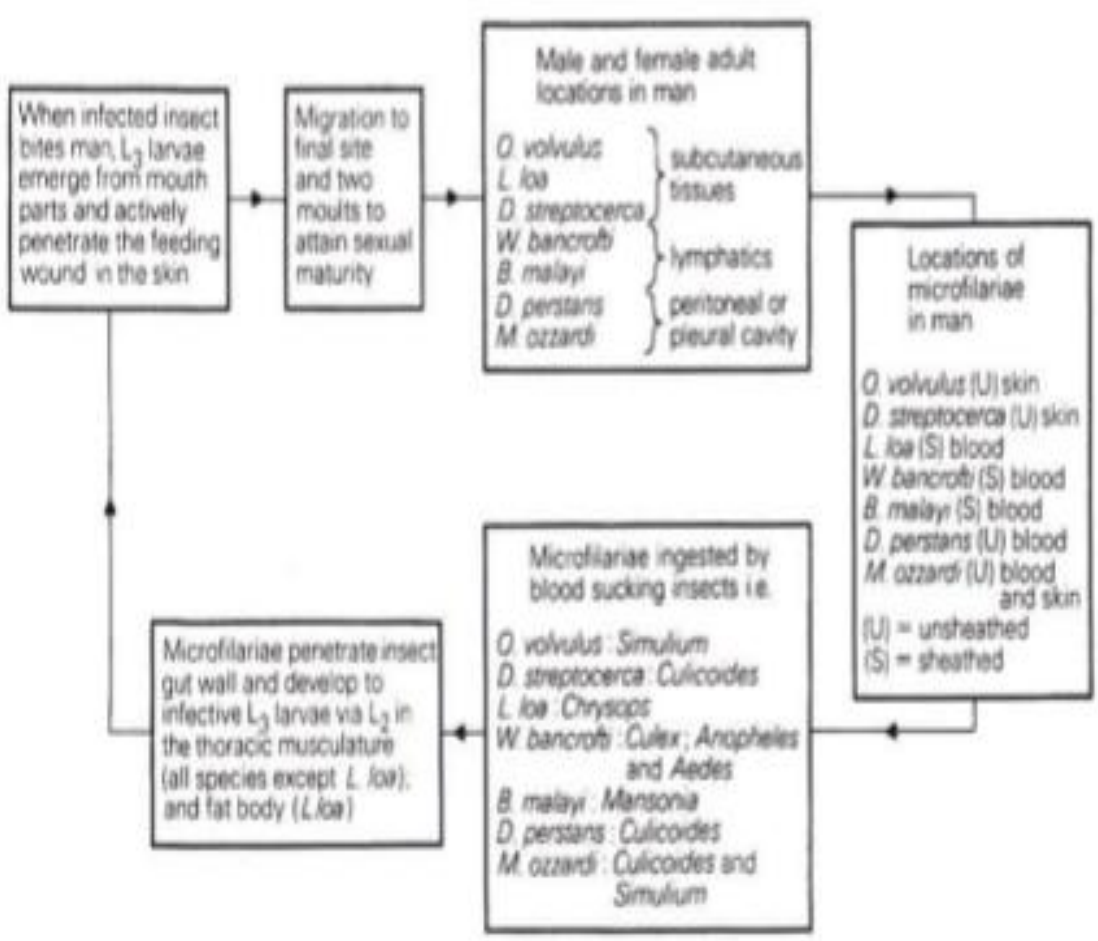



***Fig. 1.19. Life cycles of all filarial worms***

**4.18.1. Wuchireria banchrofti and Brugia species**: They are causative agents of lymphatic flariasis or elephantiasis. The intermediate hosts of W. banchrofti and B.malayi are various diurnal or nocturnal mosquito genera. The development of infective larvae in the insects is only possible at high environmental temperatures and humidity levels; in W. banchrofti the process takes about 12 days at 280C. After penetration to human from the mosquito, the filariae migrate into lymphatic vessels where they develop to sexually mature. Microfilariae (Mf) will appear in the blood after three months at the earliest (B. malayi, B. timori) or after seven to eight months (W. banchrofti) of infection. The adult parasites survive for several years in the infected individuals.

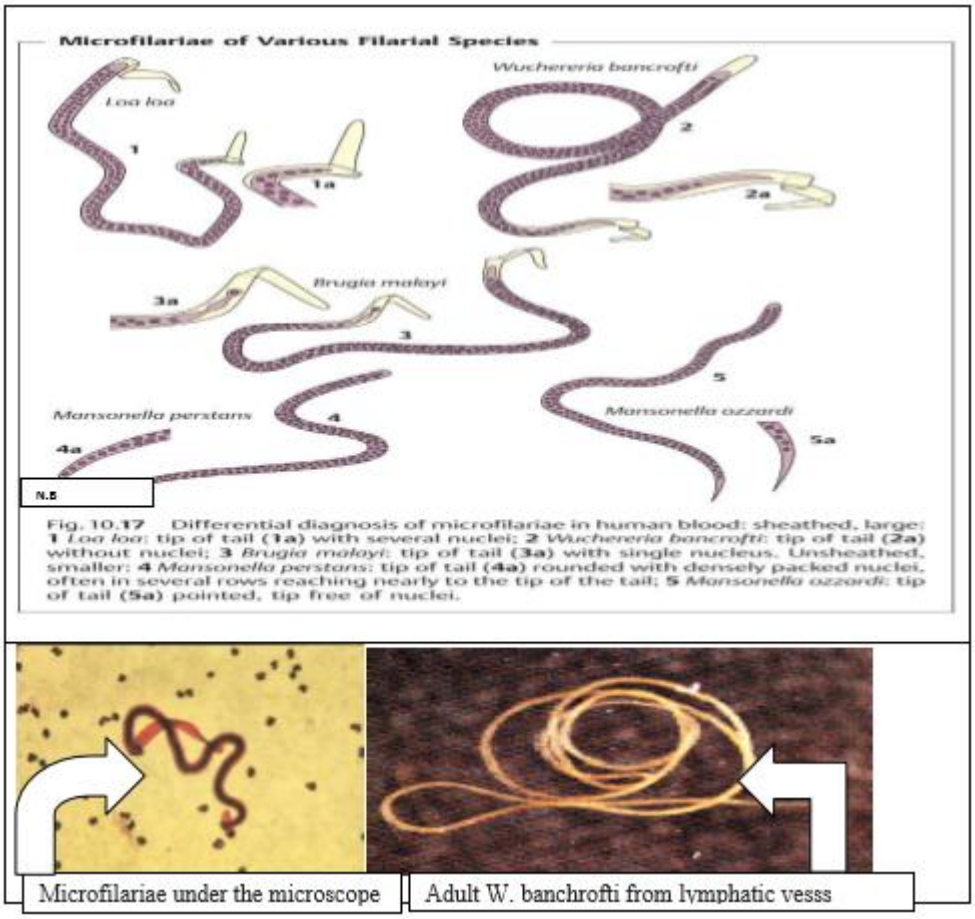



***Fig 1.20. Morphology of filarials***

**Life cycle:** Generally, the life cycle of W. banchrofti filarial worms are shown below. The infective stages are L1 larvae while the diagnostic stages are L3 larvae.

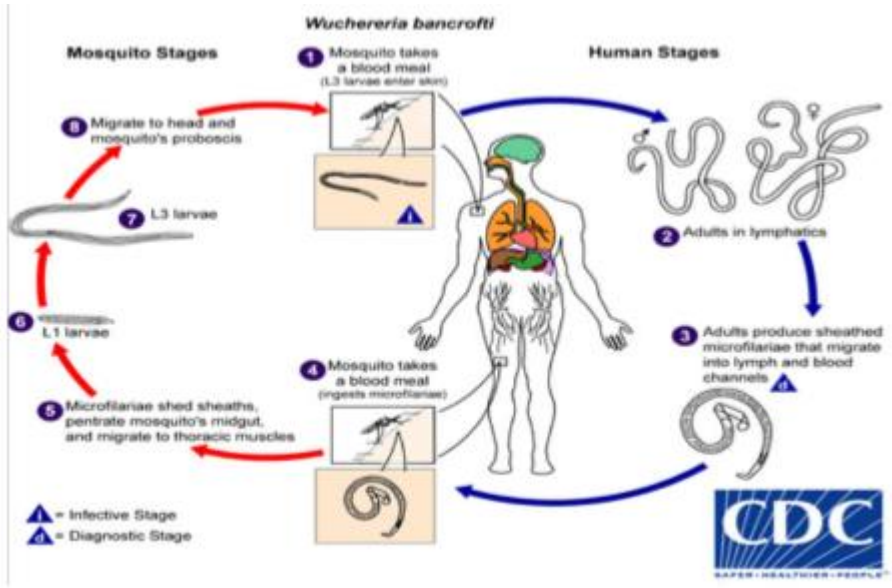



***Fig 1.21. Life cycle of W. bancrofti***

**Pathogenesis and clinical manifestations:** The pathologies caused by W. banchrofti and Brugia species are very similar. The initial symptoms can appear as early as incubation period although in most cases the incubation period is five to 12 months or much longer. The different courses taken by such infections can be showed as follows. Lymphatic flariasis most often consists of asymptomatic microfilaremia. Some patients develop lymphatic dysfunction causing lymph edema and elephantiasis (frequently in the lower extremities) and additionally with W. banchrofti, hydrocele and scrotal elephantiasis. Episodes of febrile lymphangitis and lymphadenitis may occur. Persons who have newly arrived in disease-endemic areas can develop afebrile (having no fever) episodes of lymphangitis and lymphadenitis. An additional manifestation of filarial infection is pulmonary tropical eosinophilia syndrome, with nocturnal cough and wheezing, fever, and eosinophilia. Laboratory Diagnosis: The standard method for diagnosing active infection is the identification of microfilariae in a blood smear by microscopic examination. The microfilariae that cause lymphatic filariasis circulate in the blood at night (called nocturnal periodicity). Blood collection should be done at night to coincide with the appearance of the microfilariae, and a thick smear should be made and stained with Giemsa or hematoxylin and eosin. Concentration techniques can be used to increase the sensitivity of the diagnosis.

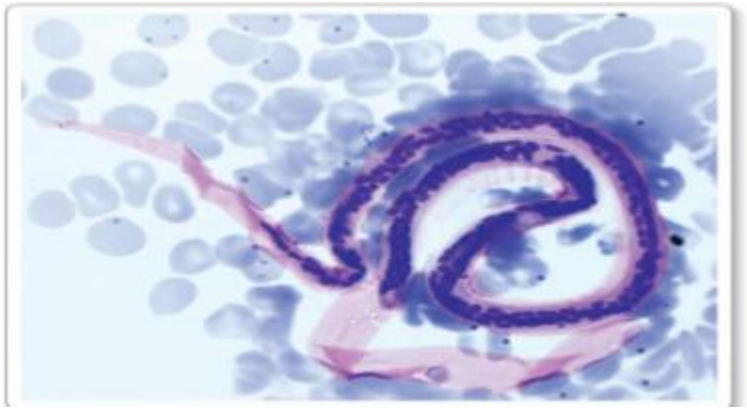


***Fig. 1.22. Microfilaria in blood***

Serologic diagnosis provide an alternative to microscopic detection of microfilarie for the diagnosis of lymphatic filarialsis. Patients with active filarial infection typically have elevated levels of anti filarial antibody in the blood and these can be detected by using serologic tests.

**TreatmentW. Banchrofti:** The main aim of treatment is to kill the adult worms by diethyl carbamazine (DEC). General measures: include rest, antibiotics, antihistamines, bandaging and surgical measures for elephantiasis.

**Prevention and control**: Mosquitoes control, avoid mosquito bite, treating patients and health information for the community.

**4.18.2.** Onchoreca vovulus :

Onchoreca vovulus causes Onchocerciasis(common name: river blindness). The adult worms live (habitat) in nodules in subcutaneous connective tissue of infected persons. This filarial species causes onchocerciasis; a disease that manifests mainly in the form of skin alterations, lymphadenopathy, and eye damage, which latter is the reason for the special importance of the disease. Life cycle Humans are the only definitive host whereas day-biting female black flies of the genus Simulium(black flies) are intermediate hosts. The vector Simulium species breed in ‘fast flowing rivers; and therefore, the disease is most common along the course of rivers hence, the disease named as ‘river blindness. The female black flies are ‘pool feeders’ and suck in blood and tissue fluids after accumulated at cutting site. Microflariae from the skin and lymphatics are ingested and develop within the vector and become infective third-stage larvae, which migrate to its mouth parts. The normal incubation period is about 6 days in vector. Infection is transmitted when an infected Simulium bites a person.

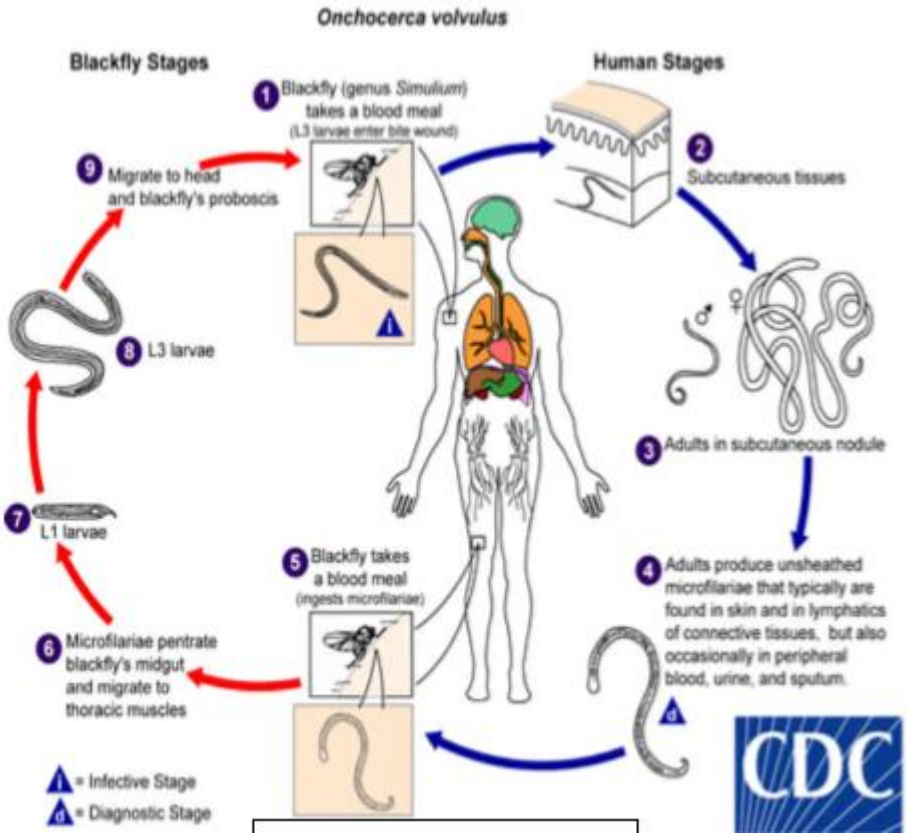



***Fig. 1. 23. Life cycle of O. volvulus***

**Pathogenesis and clinical manifestations:**

Pathogenesis depends on the host's allergic and inflammatory reactions to the adult worm and microflariae. Pathological reactions are produced by adult and microfilariae parasites. These reactions are influenced by the immune status of infected individuals. Reactions to adult parasites: enclosure of adult filariae in fibrous nodules (onchocercomas), usually 0.5–2 cm (sometimes up to 6 cm) in diameter in the subcutis along the iliac crest, ribs, scalp, etc., more rarely in deeper tissues. Nodulation occurs about one to two years after infection and is either asymptomatic or causes only mild symptoms. Reactions to microfilariae: microfilariae appear in the skin about 12–15 (seven to 24 months of incubation period). Initial symptoms occur after about 15–18 months: the clinical features are pruritus, loss of skin elasticity with drooping skin folds, papules, depigmentation, and swelling of lymph nodes; blood eosinophilia may also be present.

Eye changes: "snowflake" corneal opacities, in later stage sclerosing keratitis, the main cause of blindness, chorioretinitis (inflammation of the choroid layer behind the retina) and ocular nerve atrophy; tendency toward bilateral damage and finally results in eye blindness.

**Laboratory Diagnosis:** The microflariae may be demonstrated by examination of skin snip from the area of maximal microflarial density such as iliac crest or trapezius region, which is placed on a slide in water or saline. The specimen is best collected around midday. This

method is specific and most accurate. Microflariae may also be shown in aspirated material from subcutaneous nodules. In patients with ocular manifestations, microflariae may be found in conjunctival biopsies. Adult worms can be detected in the biopsy material of the subcutaneous nodule.


***Fig. 1.24. Skin sample***

**Treatment:** Chemotherapy with Ivermectin is the main stay of treatment. Ivermectin is given orally in a single dose of 150µg/kg either yearly or semiannually. A 6 week course of doxycycline is microfilaria static, rendering the female worm sterile as it targets the wolbachia endosymbiont (which help the parasite for further existence) of filarial parasites. Surgical excision is recommended when nodules are located on the head due to the proximity of the worm to the eyes.

**Prevention and control:** Protective clothing and application of repellents tothe skin can provide some degree of protection from black fly bites. WHO involving repeated applications of insecticides to streams and rivers with the aim of selective eradication of the developmental stages of Simuliidae in western Africa have produced impressive regional results,

### *4.18.3. Dracunculus medinensis*

Most common in areas of limited water supply where individuals acquire water by physically entering water sources. The disease is known as Dracunculus medinensis and Common name: Medina worm or Guinea worm Medina worm is found Nile valley, India and areas where wells are used for water in supply Africa but currently it is in the verge of eradication Habitat: Adults in subcutaneous tissues of man/reservoir animals Adult: thread like, cylindrical esophagus.

- Male: About 3 cm in length posterior end coiled and 2 unequal spicules.
- Female: 30 to 100 cm in length, swollen anterior end, hooked posterior end and inconspicuous vulva near anterior

***Fig.1.25. Adult stage of Dracunculus medinensis***

Larva (or embryo): Rhabditiform esophagus tapering and long tail (1/3 bod

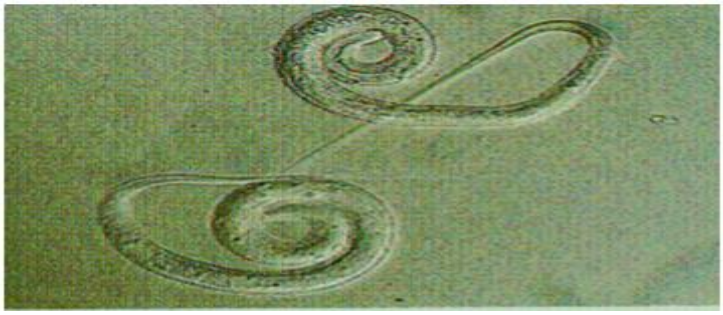


***Fig. 1.26. Larvae of Dracunculus medinensis***

**Life cycle:** A blister is formed from the female worm's production of embryos released beneath the skin, due to a burning pain that comes with this, the victims often immerse their legs in water for relief.

- With the sudden drop in temperature that follows, the blisters usually rupture, releasing the worms.
- These worms may release thousands of infective juveniles at this time, which enter the water
- Once within the copepod, the infective juvenile larvae moves into the hemocoel where they develop into 3rd stage juveniles.
- These get consumed when humans drink water with infected copepods.

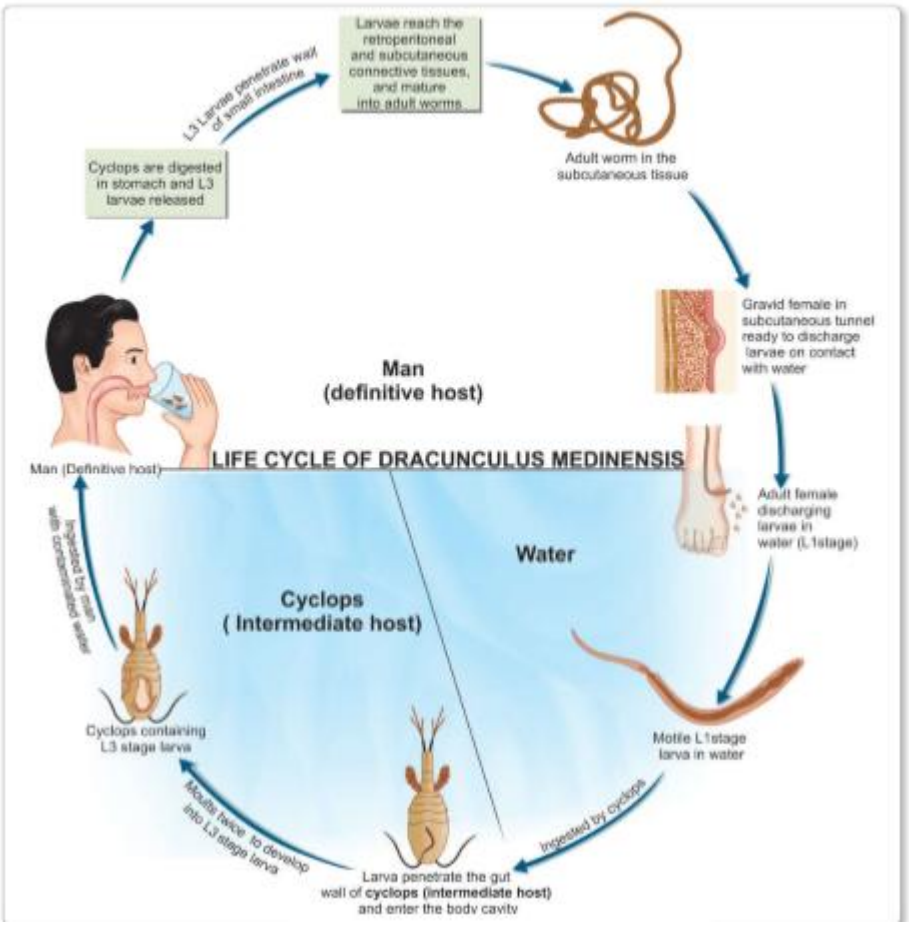
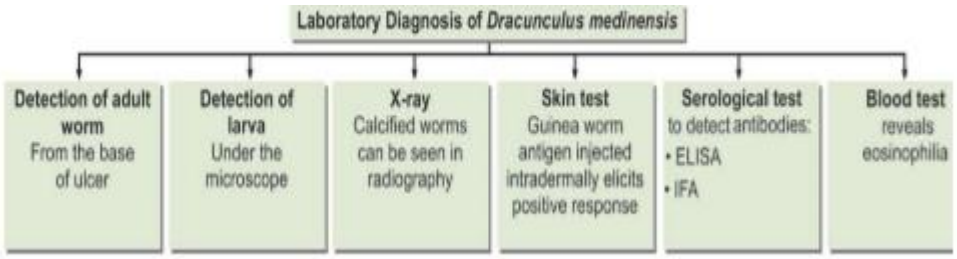



***Fig. 1.27. Life cycle of Dracunculus medinensis***




***Flow chart: 1. 4. Laboratory diagnosis for Dracunculus medinensis***

**Prevention and control:** preventing people with an open Guinea worm wound should not enter ponds or wells used for drinking water, boiling drinking water, filtered through tightly woven nylon cloth, or treated with a larvae-killing chemical

### 4.19. Phylum Plathyhelmiths (flat worms):

#### 4.19.1. Class Cestoda (tapeworms)

**General features**: Various tapeworm species can parasitize in the small intestine of humans. Cestoda species are hermaphrodites and consist of the head (scolex or “holdfast”), followed by an unsegmented germinating section (neck) and a posterior chain of segments (proglottids). There are no digestive (alimentary) system, so nutrients are taken up through the absorptive integument. The life cycle of Cestoda require one or two intermediate hosts. Humans can also be infected by larval stages of

various tapeworm species of cysticerci or metacestodes. These stages develop in body tissues and generally cause considerably greater pathological damage than the intestinal cestodes stages. Cestodes have tape - like, dorsoventrally flattened and segmented bodies. The scolex (head) carries suckers and some also have hooks. They are monoecious (no separate male and female organisms) and body cavity is absent. They are oviparous and reproduction via parthenogenesis (self-fertilization), thus the egg outputs rates are higher in this case than sexually reproducing parasites of separate male and female organisms.

**4.19.2.** Taenia species: The Taenia species cause a disease known as Taeniasis (intestinal manifestation) and cysticercosis (tissue manifestation). The two known species are *Taenia saginata* and *T. solium.T. solium* is 3–4 m long and is and smaller than *T. saginata*. The scolex of *T. solium* has a rostellum armed with two rows of hooks in addition to the four suckers which similar to T. saginata.

> Taeniosis is a small intestine infection of humans caused by *Taenia* species. In the case of *T. saginata*, the intermediate hosts are cattle, in the musculature of which metacestodes (cysticerci) develop and can be ingested by humans who eat raw beef. The infection runs an inapparent course or is associated with mild intestinal symptoms. The metacestodes of *T. solium* develop in the musculature of pigs, or through accidental infection in humans as well (CNS, eyes, musculature, skin), causing cysticercosis. *T. saginata asiatica* is closely related to *T. saginata*, but its metacestodes parasitize mainly in the livers of pigs and ruminants.

**Morphology:** Egg: 33-40µm in size while the shape is round. It has dark yellowish-brown shell and light yellowish gray in content. The shell is thick, Smooth, radially striated (embryophore).The content is round granular mass enclosed by a fine membrane with six hooklets (this is the diagnostic features of this parasite. Gravid proglotides: are those detached when fully develop & pass through the anus independently. It attains an opaque white color with 20mmX6m in size. Inside the gravid segments, the number of lateral uterus branches is usually 7–13 in T. solium which is less than that of T. saginata (usually >15). Pathogenesis and clinical manifestations of *T. saginata*. Taeniasis which is a morphological changes in intestine (villus deformation, enterocyte proliferation, cellular mucosal infiltration, etc.) and functional disturbances. Blood eosinophilia may occur sometimes. The infection takes an asymptomatic course in about 25% of cases. Symptoms of infection include nausea, vomiting, upper abdominal pains, diarrhea or constipation and increased or decreased appetite. Infection does not confer levels of immunity sufficient to prevent rein. Pathogenesis and clinical manifestations of *T. solium*: causetaeniasis in the intestine which has no or only mild symptoms, similar to infections with T. saginata. Cysticercosis is caused by T. solium cesticerci larvae encysted in brain or tissues of the central nervous system

(neurocysticercosis) or of the eye (ocular cysticercosis) are among the more severe forms of the infection. In the CNS, the metacestodes are usually localized in the cerebrum (ventricle, sub-arachnoidal space), more rarely in the spinal cord; they can cause epilepti form convulsions, raised intracranial pressure, and other neurological symptoms. The cysticerci can also develop in subcutaneous tissues, in the heart, and in the skeletal muscle Causative agent of *T. solium* taeniasis and cysticercosis.

**Life cycle:** The life cycle is similar, except that T. saginata uses cattle as intermediate host and *T. solium* uses the pig as intermediate host, in which the metacestode (Cysticercus cellulosae) develops to infectivity within two to three months.

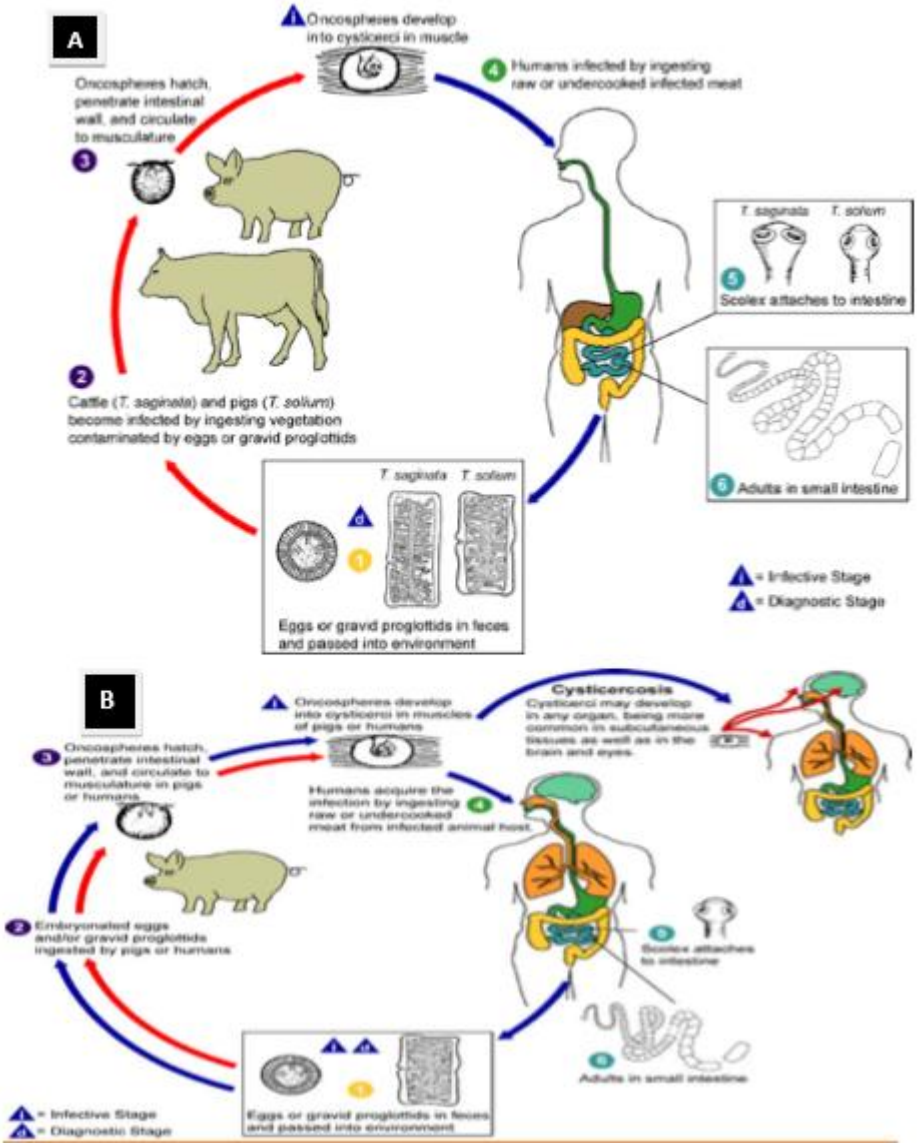



***Fig. 1.27. Life cycle of intestinal Taenia species. A. intestinal case, B. cesticercosis to brain***

**Laboratory diagnosis:** Detecting eggs in faeces is the first step. Macroscopic Identification of gravid segments/ proglotides in underclothing/ bedding/ in faeces. Scolex can be

recovered from clothing or passed in faeces; T.saginata ova on perianal skin (cellotape slide is the best methode) T. solium (cysticercosis): Finding calcified larvae in histological or X-rays examination (by X-ray technicians).

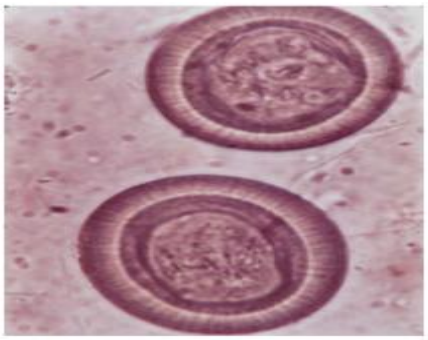


***Fig. 1.28. Eggs of T.saginata and T.solium***

**Treatment:** Praziquantel in adults 2.5-10mg/kg given in a single dose. Albendalzole for adults 6.6mg/kg or two doses each of 200mg/day on 3consecutive days (this drug should not be usedin children less than 2years old or during pregnancy.

**4.19.3.** Hymenolepis nana (Dwarf Tapeworm):

Hymenolepis nana (common name: dwarf tapeworm),1–4 cm long (rarely 9 cm) and 1mm wide, is a small intestinal parasite that occurs worldwide. The highest prevalence being found in warm countries and in children. The final hosts are rodents and humans. Infection results from ingestion of eggs, from which oncospheres hatch in the small intestine, penetrate into the villi, and develop into larvae (cysticercoids). The larvae then return to the intestinal lumen, where they develop into adult tapeworms within two to three weeks. Alternatively, H. nana develops in a cycle with an intermediate host (insects: fleas, grain beetles, etc.). The closely related species Hymenolepis diminuta (10–60 mm) is not as frequent in humans. The developmental cycle of this species always involves intermediate hosts (fleas, beetles, cockroaches).

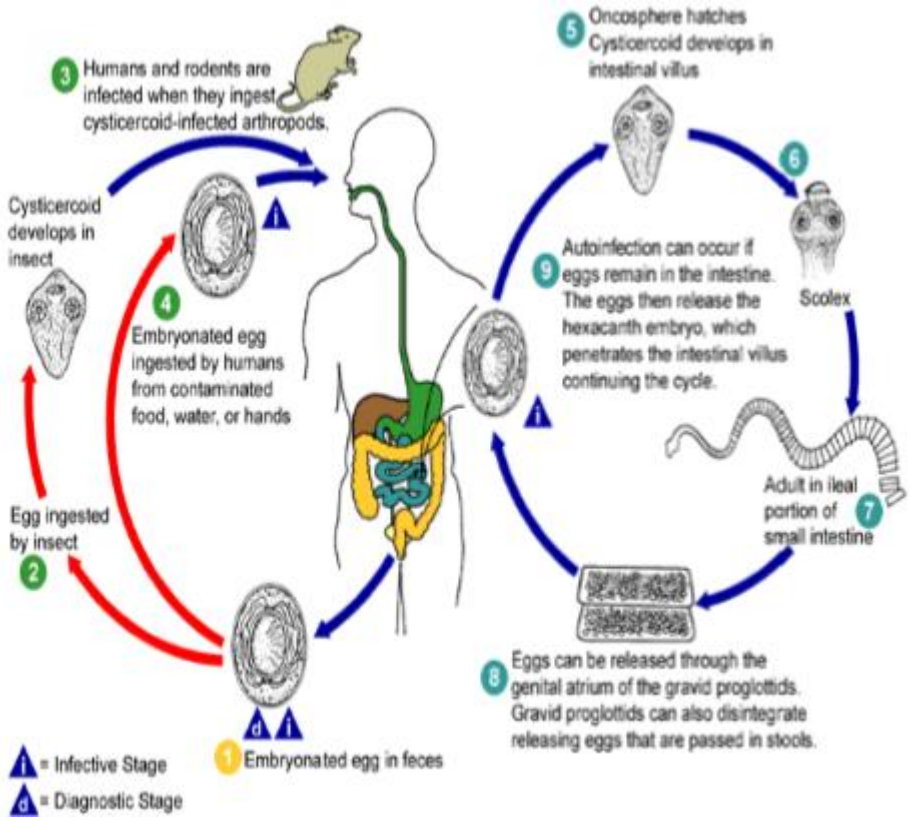



***Fig. 1. 29. Life cycle of H.nana***

**Clinical manifestations:** Infections gastrointestinal distress.

**Laboratory diagnosis:** Finding eggs in feces Size: 35-50µm.

- Shape: oval, almost round.
- Shell: double; thin external membrane and internal membrane often thicker at the poles.
- Thread like polar filaments coming from both poles.
- Color: color less or very pale gray
- Content: Rounded mass (embryo) with six retractile booklets arranged in fan shaped.

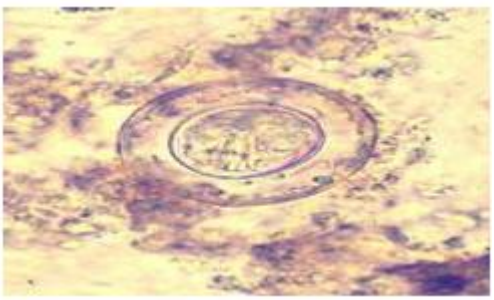


***Fig. 1. 30. Egg of H.nana***

**Treatment**: Praziquantel or albendazole are the drugs of choice.

**Prevention and control**: Preventive measures include general hygiene and treatment of infected persons void eating raw or insufficiently cooked meat. Inspecting meat for larvae and provide latrine for proper waste disposal. Protection of cattle from grazing on faeces or sewage polluted grass. Avoiding open field defecation and treating infected persons & providing health education may break the life cycle of these parasites.

**4.19.4.** Echinococcus

## Echinococcus

Causative agent of echinococcosis

■ The most important species of the genus *Echinococcus* are *Echinococcus granulosus* (intestinal parasite of Canidae) and *E. multilocularis* (intestinal parasite of fox species, dogs, cats, and other carnivores). Both species occur in Europe. Their metacestodes can cause cystic echinococcosis (CE, hydatid disease) or alveolar echinococcosis (AE) in humans. Humans are infected by peroral ingestion of *Echinococcus* eggs, from which in CE, liquid-filled cystic metacestodes (the hydatids) develop, particularly in the liver and lungs. In AE the metacestodes primarily parasitize the liver, where the metacestodes proliferate like a tumor and form conglomerates of small cysts; secondary metastatic spread to other organs is possible. Clinical imaging and immunodiagnostic methods are used for diagnosis. Treatment involves surgery and/or chemotherapy. ■

**Parasite species.** *Echinococcus* species are small tapeworms that parasitize the small intestine of carnivores and produce eggs that are shed to the environment by the host. Pathogenic larval stages (metacestodes) develop following peroral ingestion of such eggs by the natural intermediate hosts (various mammalian species), as well as in humans and other accidental hosts (which do not play a role in the life cycle). Four *Echinococcus* species are currently known, all of them pathogenic for humans (*Echinococcus granulosus*, *E. multilocularis*, *E. vogeli*, and *E. oligarthrus*).

**Life cycle : see below**

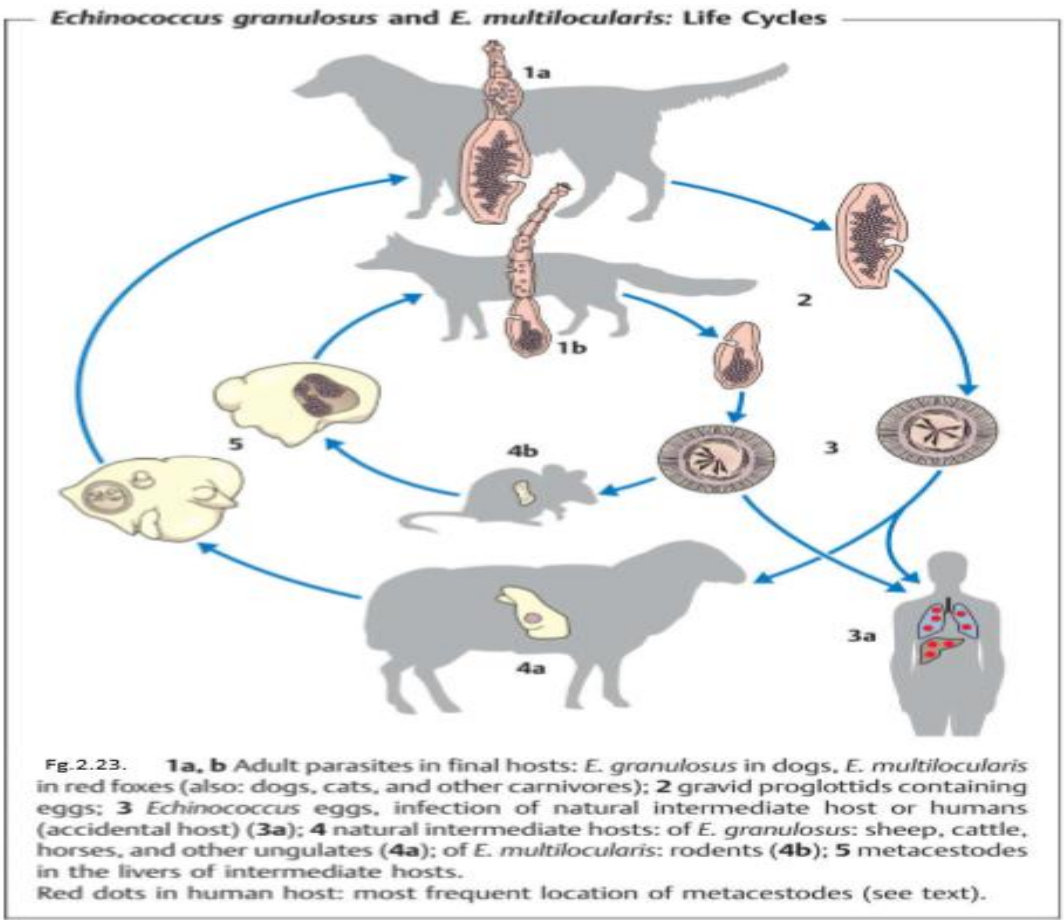



***Fig 1. 31. Life cycle of E. granulosus and E. multiiocularis***

**Laboratory diagnosis:** Microscopic diagnosis is impossible. But diagnosis is based on detection of cysts using imaging techniques (ultrasonography, computer tomography, thoracic radiography, etc.) in connection with serological antibody detection. Specific antibodies occur in about 90–100% of patients with cystic hepatic echinococcosis, but in only about 60–80% of cases with pulmonary echinococcosis. Diagnostic cyst puncture is generally not advisable due to the risks described above (secondary echinococcosis, anaphylactic reactions).

### 4.19.5. Trematodes (Flukes) or Platyhelminthes

**General Characteristics:** They vary in size from the species just visible to the naked eye, like Heterophyes to the large fleshy flukes, like Fasciola and Fasciolopsis. Most of the trematode species that parasitize humans are dorsoventrally flattened with an oval to lancet shape, although others have different shapes such as the threadlike schisotosomes. Suckers (trema: hole, opening) serve as attachment organs: an oral sucker around the mouth connected to the esophagus and the blind-ending intestine, and a ventral sucker. The body surface of adult trematodes is covered by a cellular tegument (composed of an outer annucleate, syncytial layer of cytoplasm connected

by cytoplasmic strands to inner nucleated portions) through which substances can be absorbed from the environment. Most species are hermaphroditic, only the schisotosomes have separate sexes. Snails are the first intermediate hosts; some species require arthropods or fish as a second intermediate hosts.

**Schistosoma species**

Schistosomosis (bilharziosis) is one of the most frequent tropical diseases with about 200 million infected persons. The occurrence of schistosomosis depends on the presence of suitable intermediate hosts (freshwater snails). Human infections result from contact with standing or slow-moving bodies of water (freshwater) when *Schistosoma* cercariae penetrate the skin. *Schistosoma hematobium* causes urinary schistosomosis; *S. mansoni*, *S. japonicum*, *S. intercalatum*, and *S. mekongi* are the causative agents of intestinal schistosomosis and other forms of the disease. Diagnosis can be made by detection of either *Schistosoma* eggs in stool or urine or of specific antibodies in serum.

- They are unisexual (diecious)
- They lack a muscular pharynx
- Their intestinal caeca re-unite after bifurcation to form a single canal
- They produce non-operculated eggs
- They have no redia stage in larval development
- The cercariae have forked tails and infect by penetrating the unbroken skin of definitive host

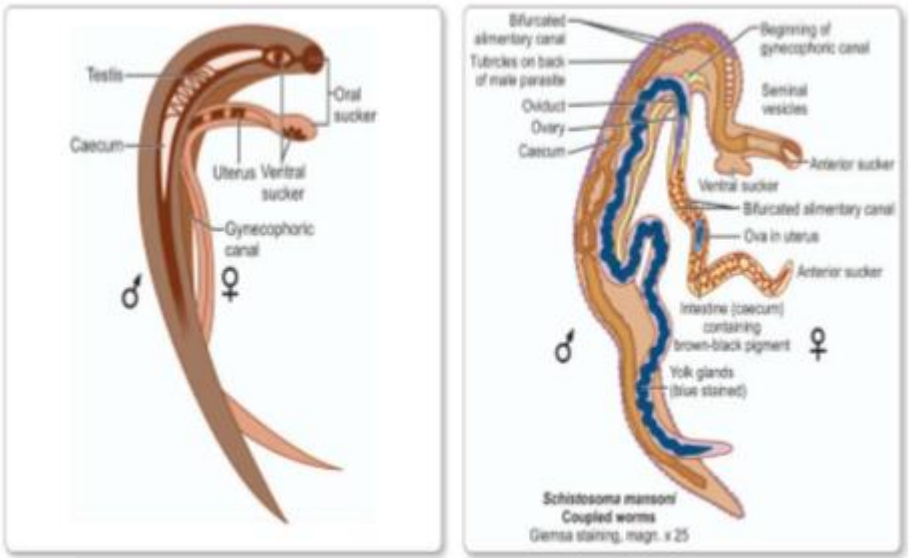



***Fig. 1.32. Adult worms of schistosoma species.***

**History and Distribution:** In 1902, Manson discovered eggs with lateral spines in the feces of a West Indian patient that led to the recognition of this second species of human schisotosomes. It was, therefore named S. mansoni. It is widely distributed in Africa including Ethiopia. **Habitat:** Adult worm lives in the inferior mesenteric vein.

**Morphology:** S. mansoni resembles S. haematobium in morphology and life cycle, except the adult worms are smaller and their integuments studded with prominent coarse tubercles. In the gravid female, the uterus contains very few eggs, usually 1–3 only. The prepatent

period (the interval between cercarial penetration and beginning of egg laying) is 4–5 weeks. The egg has a characteristic lateral or apical spine and are non operculated with yellowish brown.

**Life cycle:** Definitivehost**:** humans are the only natural definitive hosts.

**Intermediate host:** snails of the genus Biomphalaria.

**Infective form:** Fork-tailed Cercaria.

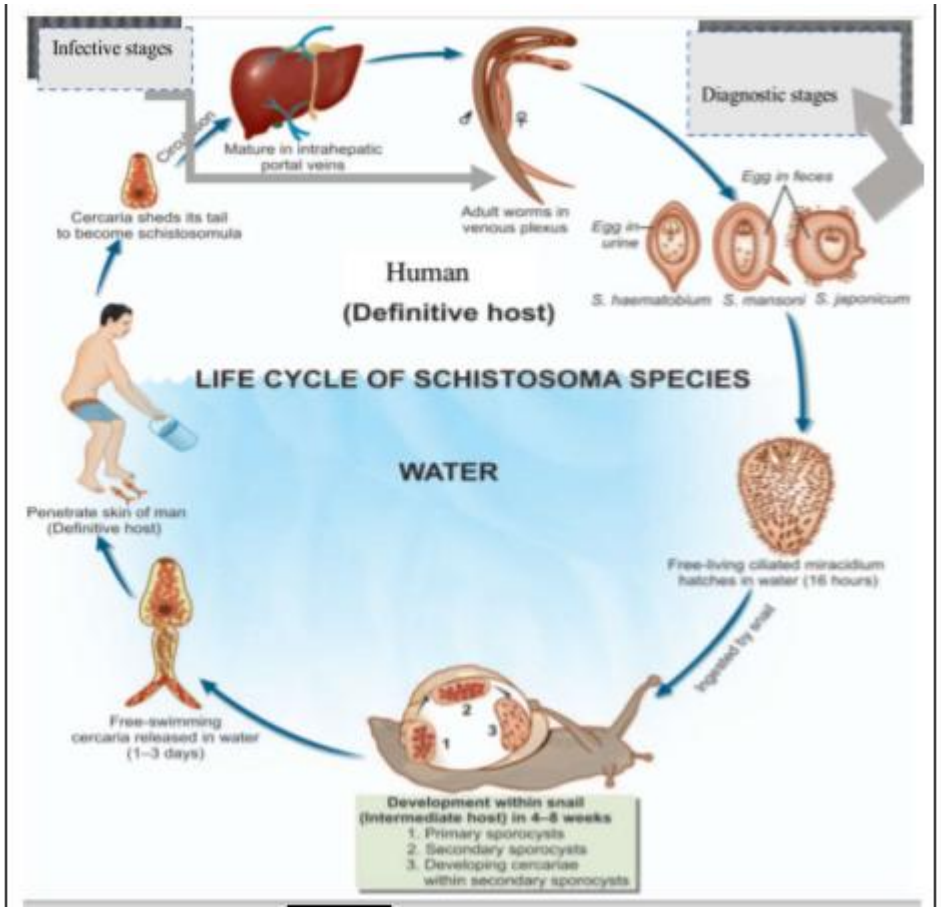



***Fig 1.33. Life cycle of schistosoma spp.***

**Description of the life cycle in brief:**

**Migration of Schistosomes in the Human Body**

Infection → schistosomula penetrate subcutaneous tissues → find venous capillaries or lymph vessels → migrate through the venous circulatory system into the right ventricle of the heart and the lungs → travel hematogenously into the intrahepatic portal vein branches where development into adult worms takes place as wells as male-female pairing just prior to sexual maturity → retrograde migration of pairs into mesenteric veins or to the vesical plexus.

**Pathogenesis and clinical manifestations:** The infection can be divided into the following phases: Cutaneous phase: penetration of cercariae into the skin, either without reaction or especially in cases of repeated exposure with itching and skin lesions (erythema, papules), which disappear within a few days. Acute phase: about two to 10 weeks after a severe initial infection, the symptoms may include fever, headache, limb pains, urticaria, bronchitis, upper

abdominal pain, swelling of the liver, spleen and lymph nodes, intestinal disturbances, and eosinophilia (Katayama syndrome). Due to release of Schistosoma antigens, the serum antibody levels (IgM, IgG, IgA) rise rapidly and immune complexes are formed that can cause renal glomerulopathies. These symptoms persist for several days to several weeks. Normally, Schistosoma eggs are not yet excreted at the beginning of this phase (see prepatent periods). In low-level infections this phase is usually in apparent or subclinical. Chronic phase: the most significant phase in pathogenic terms begins after an incubation period of about two months with oviposition by the Schistosoma females. A large proportion (up to 50%) of the eggs laid remain in human body tissues, not only near the worms (urinary bladder, intestine), but also in more distant localizations due to hematogenous spreading (mainly to the liver and lungs, more rarely to the CNS, the skin, and other organs), where they lodge in small vessels.

- The main forms of schistosomiasis are differentiated according to the localization of the lesions:
  - Intestinal schistosomiasis (intestinal bilharziasis). The agents are mainly S. mansoni and S. japonicum, also S. mekongi (rare). Incubation period is from four to 13 weeks (acute phase) or months to years (chronic phase). The course of an initial infection is rarely symptomatic, in apparent and subclinical courses being the rule. Manifestations in the chronic phase are restricted almost entirely to large intestine with hyperemia, granulomatous nodules, papillomas (bilharziomas), ulcerations, hemorrhages, and increasing fibrosis, abdominal pain and bloody diarrhea.
  - Other forms: hepatosplenic form the causative agents are mainly S. japonicum, less frequently S. mansoni. The fibrotic form is caused by eggs deposited around the branches of the portal vein in the liver (“pipestem” fibrosis according to Swimmers) and results in circulatory. Anomalies, portal hypertension, splenomegaly, ascites, hemorrhages in the digestive tract, and other symptoms. Pulmonary schistosomiasis is observed mainly in severe S. mansoni infections, more rarely in infections with other species (including S. haematobium). Cerebral schistosomiasis is relatively frequent in S. japonicum infections (2–4%).
- Cercarial dermatitis. Cutaneous lesions (itching, erythema, urticaria, papules) in humans, caused by (repeated) skin penetration of cercariae parasitizing birds. The infection occurs worldwide in freshwater or brackish water and is known as “swimmer’s itch.” The symptoms generally abate after a few days. The cercariae of schisotosomes from humans can cause similar, although usually milder, symptoms.

- Urinary schistosomiasis (urinary bilharziosis). Causative agent: S. haematobium. Incubation 10–12 weeks or longer, morbidity rate as high as 50–70%. Hematuria (mainly in the final portion of urine), micturition (the discharge of urine) discomfort, hyperemia, increasing fibrosis, 1–2 mm nodules, necroses, ulcers and calcification of the bladder wall, pyelonephrosis and hydronephrosis, urethral strictures, lesions in the sexual organs. In some endemic areas, an increased incidence of urinary bladder cancer has been associated with the S. haematobium infection.
- Laboratory Diagnosis: Following the prepatent period, i.e., four to 10 weeks incubation period at the earliest, the eggs can be detected in stool specimens or in urine sediment. The eggs can also be found in intestinal or urinary bladder wall biopsies.
  - Immunodiagnostic methods are particularly useful for detecting infections before egg excretion begins (important for travelers returning from tropical regions!). Detection of microhematuria with test strips is an important diagnostic tool in bladder schistosomiasis.
  - Clinical examination with portable ultrasonic imaging equipment has proved to be a highly sensitive method of detecting lesions in the liver and urogenital tract in epidemiological studies.

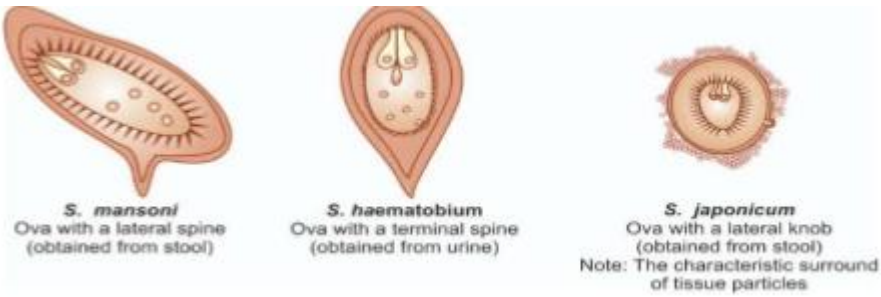



***Fig. 1.34. Eggs of schistosoma spp.***

- **Treatment:** The drug of choice for treatment of schistosomiasis is praziquantel, which is highly effective against all Schistosoma species and is well tolerated.
- **Prevention and Control:** Current schistosomiasis control strategies are based mainly on regular drug therapy of specific population groups. Morbidity, mortality, and egg excretion rates are clearly reduced by such programs. Hygienic and organizational measures (construction of latrines, improvement of water supply quality, etc.) aim to reduce Schistosoma egg dissemination and contact with contaminated bodies of water. Individual preventive measures in Schistosoma-contaminated areas include avoidance of skin contact with natural or artificial bodies of water (freshwater). Drinking water that

could be contaminated with cercariae must be decontaminated before use by boiling, chlorination, or filtration.

## 4.20. Medical Protozoology

- **General characteristics:** Parasitic protozoa are eukaryotic, single-celled microorganisms belonging to kingdom protista. They are varied from 1–150 µm in size and enclosed by a trilaminated cell membrane. The single protozoal cell performs all functions. They possess one, rarely two nuclei (and multinuclear reproductive forms). Reproduction is asexual by binary or multiple fission of the cell, or sexual. The cellular construction of the protozoa is generally the same as in other eukaryotes but they also exhibit some special features. During the course of evolution some protozoa (Giardia, Entamoeba) have lost the mitochondria secondarily, except several genomic traits that were laterally transferred to the nuclei.

  The apicoplast, present in some species of Apicomplexa, is a residual of a former plastid typical for their ancestors. Some protozoa contain specialized organelles, such as glycosomes (exclusively in trypanosomatids), hydrogenosomes (in trichomonads).

  Most of the protozoa are completely nonpathogenic but few may cause major diseases such as malaria, leishmaniasis, and sleeping sickness. Protozoa like *Cryptosporidium parvum* and *Toxoplasma gondii* are being recognized as opportunistic pathogens in patients affected with human immunodeficiency virus (HIV) and in those undergoing immunosuppressive therapy.

  Protozoa exhibit wide range of size (1–150 µm), shape, and structure; yet all possess essential common features.The differences between protozoa and metazoa are given in table 1 below.

***Table 1. Difference between protozoa and Helminths***

| **Variable** | **Protozoa** | **Metazoa** |
|---|---|---|
| Morphology | Unicellular;a single 'cell-like unit' | Multicellular;a number of cells, makingup a complex individual |
| Physiology | A single cell performsall the functions:reproduction,digestion, respiration,excretion | Each special cell performs aparticular function |
| **Example** | Amoeba | Tapeworm |

- **The special and common terms in protozoology:**
  - Chromatic body: extra nuclear chromatin material (e.g., as found in Entamoeba histolytica cyst).
  - Karyosome: it is a DNA containing body, situated peripherally or centrally within the nucleus and found in intestinal amoeba, and E. coli.
  - Kinetoplast: non-nuclear DNApresent in addition to nucleus is called kinetoplast. It is seen in trypanosomes. Flagellum originates near the kinetoplast.point of origin of flagellum is called as basal body
  - Cilia: these are fine, needle-like filaments, covering the entire surface of the body and are found in ciliates, e.g. balantidium coli
  - Trophozoite: active feeding and growing stage of the protozoa is called the trophozoites. It derives nutrition from the environment by diffusion, pinocytosis and phagocytosis

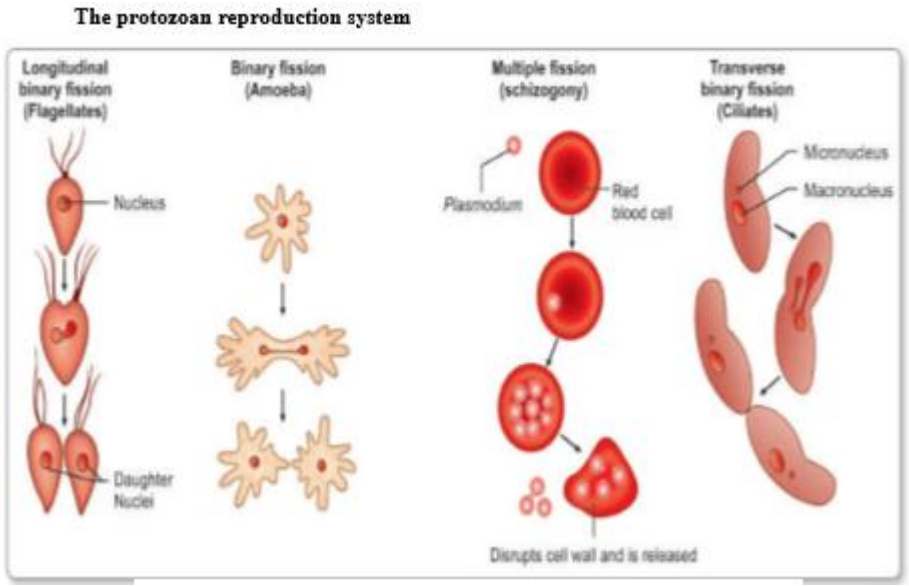



***Fig. 1.34. Modes of reproduction of protozoa***

Classification of protozoa

| Phylum | Subphylum | Superclass | Class | Subclass | Order | Suborder | Genus |
|---|---|---|---|---|---|---|---|
| **Sarcomastigophora** | **Mastigophora (having one or more flagella)** | | Zoomastigophorea | | Kinetoplastida | Trypanosomatina | *Trypanosoma*<br>*Leishmania* |
| | | | | | Retortamonadida | | *Retortamonas*<br>*Chilomastix* |
| | | | | | Diplomonadida | Enteromonadina | Enteromonas |
| | | | | | | Diplomonadina | *Giardia* |
| | | | | | Trichomonadida | | *Trichomonas*<br>*Dientamoeba* |
| | **Sarcodina (pseudopodia present)** | Rhizopoda | Lobosea | Gymnamoebia | Amoebida | Tubulina | *Entamoeba*<br>*Endolimax*<br>*Iodamoeba* |
| | | | | | | Acanthopodina | *Acanthamoeba* |
| | | | | | Schizopyrenida | | *Naegleria* |
| **Apicomplexa** | | | Sporozoea | Coccidia | Eucoccidia | Eimerlina | *Cryptosporidium*<br>*Isospora*<br>*Sarcocystis*<br>*Toxoplasma* |
| | | | | | | Haemosporina | *Plasmodium* |
| | | | | Piroplasmia | Piroplasmida | | *Babesia* |
| **Ciliophora** | | | Kinetofragminophorea | Vestibuliferia | Trichostomastida | Trichostomatina | *Balantidium* |
| **Microspora** | | | Microsporea | | Microsporida | Apansporoblastina | *Enterocytozoon*<br>*Encephalitozoon*<br>*Microsporum* |

### *4.20.1. Entameba histolytica*

Of the various amebic species that parasitize the human intestinal tract, *Entamoeba histolytica* is significant as the causative agent of the worldwide occurring entamebosis, a disease particularly prevalent in warmer countries. The vegetative stages (trophozoites) of *E. histolytica* live in the large intestine and form encysted stages (cysts) that are excreted with feces. The infection is transmitted by cysts from one human to another. The trophozoites of *E. histolytica* can penetrate into the intestinal wall and invade the liver and other organs hematogenously to produce clinical forms of amebosis, most frequently intestinal ameboses (amebic dysentery) and hepatic amebosis ("amebic liver abscess"). Diagnosis of an intestinal infection is primarily confirmed by detection of the parasites in stool. If an invasive, intestinal or extraintestinal infection with *E. histolytica* is suspected, a serological antibody test can also provide valuable information. Morphologically, *E. histolytica* is indistinguishable from the apathogenic *Entamoeba dispar* (collective term for both species: *E. histolytica*/*E. dispar* complex).

**The Parasites:** the causative agent of amoebiasis is the pathogenic Entamoeba histolytica. This species is morphologically identical with a non-pathogenic Entamoeba dispar. They can be differentiated by means of zymodeme and DNA analysis and with monoclonal antibodies.

- **histolytica Occurs in 3 forms:**
    - Trophozoites
    - Precyst
    - Cyst

**Trophozoites:** is the vegetative or growing stage of the parasite (Fig. 33 ). It is the only form present in tissues. It is irregular in shape and varies in size from 12–60 µm (average 20 µm). It is large and actively motile in freshly-passed dysenteric stool. The parasite, as it occurs free in the lumen as a commensal is generally smaller in size, about 15–20 µm and has been called the minuta form. **Cytoplasm:** Outer ectoplasm is clear, transparent, and retractile. Inner endoplasm is finely granular, having a ground glass appearance. The endoplasm contains nucleus, food vacuoles, erythrocytes, occasionally leucocytes, and tissue debris.

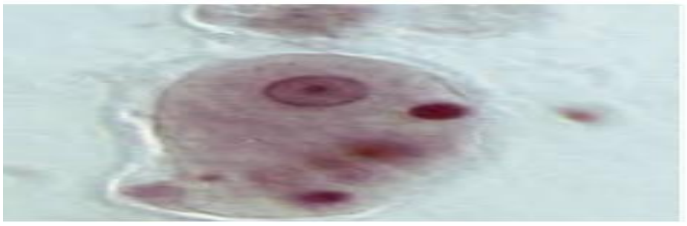


***Fig1.35 E. histolytica trophozoite***

**Pseudopodia** are finger-like projections formed by sudden jerky movements of ectoplasm in one direction, followed by the streaming in of the whole endoplasm. Typical amoeboid motility is a crawling or gliding movement and not a free swimming one. The direction of movement may be changed suddenly, with another pseudopodium being formed at a different site, when the whole cytoplasm flows in the direction of the new pseudopodium. The cell has to be attached to some surface or particle for it to move. Pseudopodia formation and motility are inhibited at low temperatures.

**Nucleusis** spherical 4–6 µm in size and contains central karoyosome, surrounded by clear halo and anchored to the nuclear membrane by f ne radiating fibrils called the linin network, giving a cartwheel appearance. The nucleus is not clearly seen in the living trophozoites, but can be clearly demonstrated in preparations stained with ironhemotoxylin. The nuclear membrane is lined by a rim of chromatin distributed evenly as small granules.

The trophozoites from acute dysenteric stools often contain phagocytized erythrocytes. This feature is diagnostic as phagocytized red cells are not found in any other commensal intestinal amoebae. The trophozoites divide by binary fission in every 8 hours. Trophozoites

survive up to 5 hours at 37°C and are killed by drying, heat, and chemical sterilization. Therefore, the infection is not transmitted by trophozoites.

Even if live trophozoites from freshly-passed stools are ingested, they are rapidly destroyed in stomach and cannot initiate infection.

- Precystic Stage: Trophozoites undergo encystment (cyst formation) in the intestinal lumen. Encystment doesn't occur in the tissues nor in feces outside the body. Before encystment, the trophozoites extrudes its food vacuoles and becomes round or oval, about 10–20µm in size. This is the precystic stage of the parasite (Fig. 33 B).It contains a large glycogen vacuole and two chromatid bars. It then secretes a highly retractile cyst wall around it and becomes cyst.

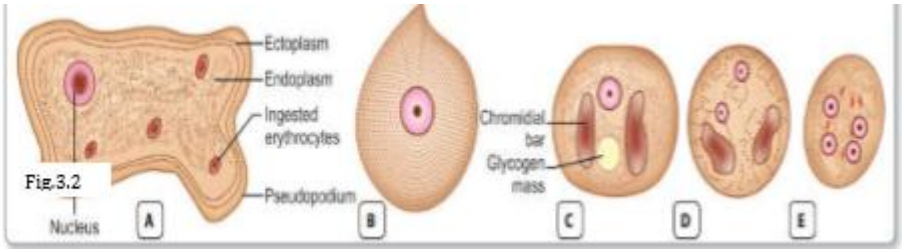


Fig 3.2

***Fig 1. 36.*** **A *E.histolytica trophozoite B.* precystic stage C. uninuclear cyst D. binuclear cyst E. mature quadrinuclear cyst**

**Cystic Stage:** The cyst is spherical in shape about 10–20 µm in size. The early cyst contains a single nucleus and two other structures—a mass of glycogen and 1–4 chromatoid bodies or chromidial bars, which are cigar-shaped refractive rods with rounded ends (Fig. 33 B.). The chromatoid bodies are so called because they stain with hematoxylin, like chromatin. As the cyst matures, the glycogen mass and chromidial bars disappear and the nucleus undergoes 2 successive mitotic divisions to form 2 (Fig. 33 B above) and then 4 nuclei. The mature cyst is, thus quadri-nucleate(Fig. 33 B above). The cyst wall is a highly refractive membrane, which makes it highly resistant to gastric juice and unfavorable environmental conditions. The nuclei and chromidial bodies can be made out in unstained films, but they appear more prominently in stained preparations. With iron hemotoxylin stain, nuclear chromatin and chromatoid bodies appear deep blue or black, while the glycogen mass appears unstained. When stained with iodine, the glycogen mass appears golden brown, the nuclear chromatin and karysome bright yellow, and the chromatoid bodies appear as clear space, being unstained.

**Life cycle:** E. histolytica passes its life cycle only in 1 host-human. - Infective form: Mature quadri-nucleate cyst passed in feces of convalescents (patients) and carriers. The cysts can remain viable under moist conditions for about 10 days.

**Mode of transmission:** Man acquires infection by swallowing food and water contaminated with cysts. As the cyst wall is resistant to action of gastric juice, the cysts pass through the stomach undamaged and enter the small intestine.

**Excystation:** When the cyst reaches caecum or lower part of the ileum, due to the alkaline medium, the cyst wall is damaged by trypsin, leading to excystation. The cytoplasm gets detached from the cyst wall and amoeboid movements appear causing a tear in the cyst wall, through which quadri-nucleate amoeba is liberated. This stage is called the metacys. Metacystic **trophozoites:** The nuclei in the metacyst immediately undergo division to form 8 nuclei, each of which gets surrounded by its own cytoplasm to become 8 small amoebulae or metacystic trophozoites. If exystation takes place in the small intestine, themetacystic trophozoites do not colonize there, but are carried to the caecum. In most of the cases, E. histolytica remains as a commensal in the large intestine without causing any ill effects. Such persons become carriers or asymptomatic cyst passers and are responsible for maintenance and spread of infection in the community. Sometimes, the infection may be activated and clinical disease ensues. Such latency and reactivation are the characteristics of amoebiasis.

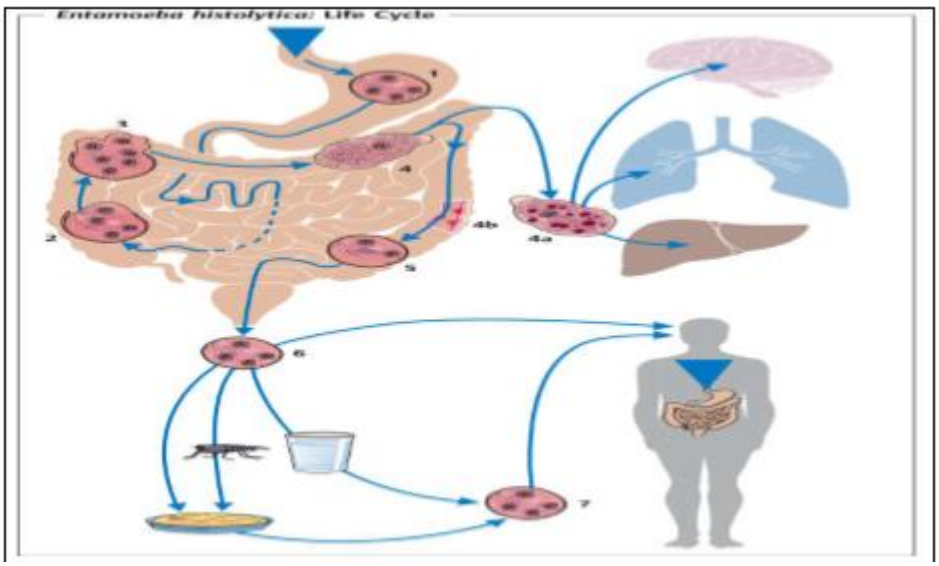
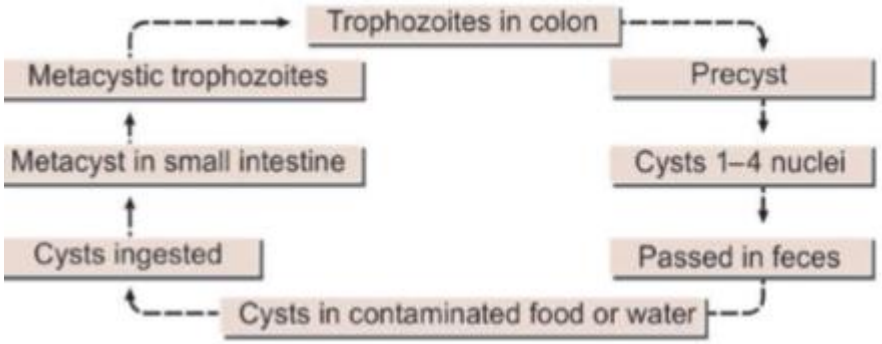



***Fig 1. 37. C. Life cycle of E. histolytica.***




***Flow chart 1.5 Life cycle of E. histolytical***

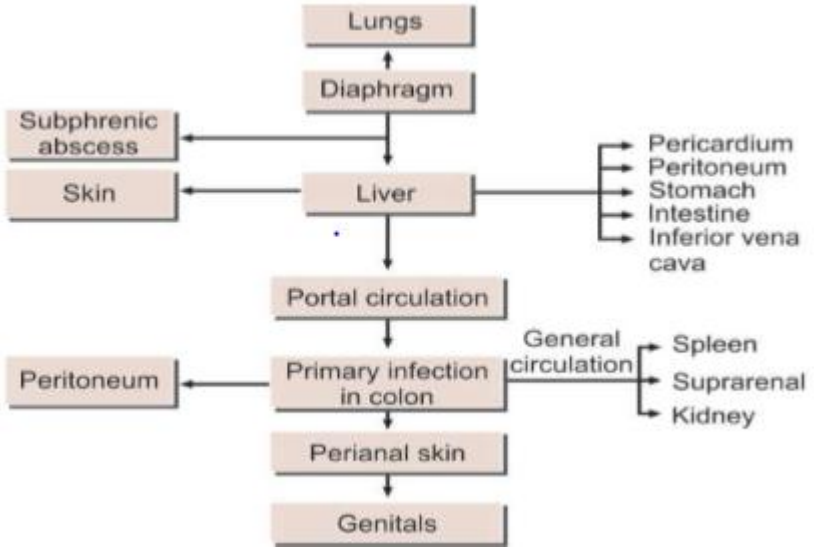
**Important points on life cycle stages**: Trophozoites are the multiplying, feeding and pathogenic stage of E histolytica. And cysts are transmission (infective stages), dormant, none dividing and none pathogenic stages E histolytica. Both trophozoites and cysts are diagnostic stages. Clinical manifestation:E. histolytica causes intestinal and extra-intestinal amoebiasis. Incubation period is highly variable. On an average, it ranges from 4 days to 4 months. Amoebiasis can present in deferent forms and degree of severity, depending on the organ affected and the extent of damage caused.

**Intestinal Amoebiasis:** The lumen-dwelling amoebae do not cause any illness. They cause disease only when they invade the intestinal tissues. This happens only in about 10% of cases of infection, the remaining 90% being asymptomatic. The following table shows summary of intestinal amoebiasis:

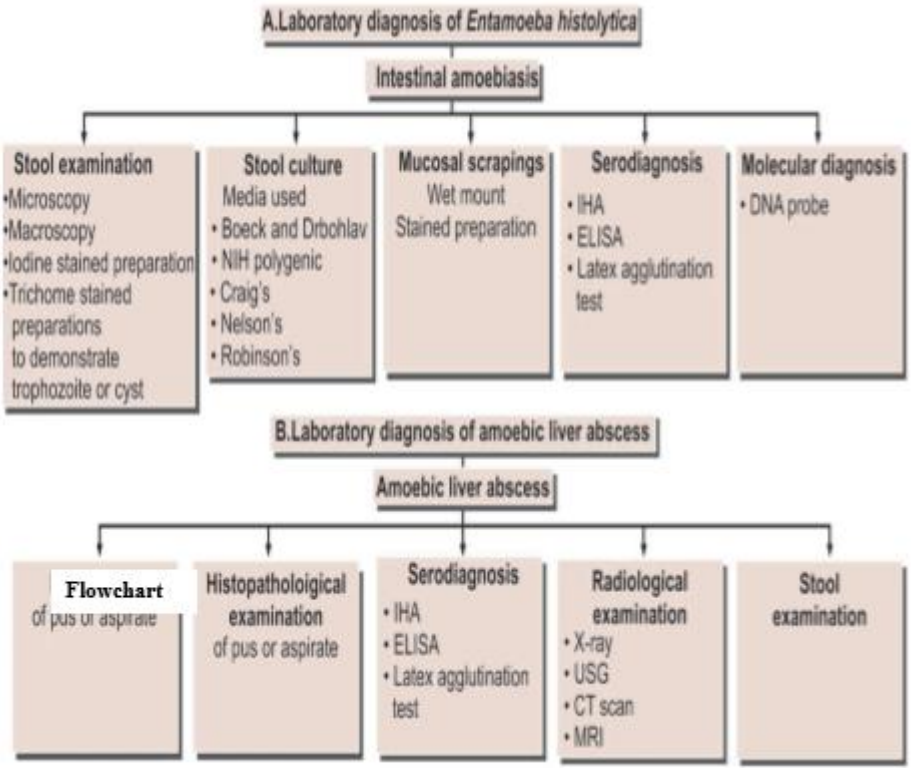
**Lesions in chronic intestinal amoebiasis**

- Small superficial ulcers involving only the mucosa.
- Round or oval-shaped with ragged and undermined margin and flask-shaped in cross-section.
- Marked scarring of intestinal wall with thining, dilatation, and sacculation.
- Extensive adhesions with the neighboring viscera.
- Formation of tumor-like masses of granulation tissue (amoeboma).

Extra-intestinal Amoebiasis: the following shows sites of extra-intestinal amoebiasis:




**Laboratory Diagnosis of Amoebiasis**




***Flow chart 1.6 laboratory diagnosis of Amoebiasis***

Table 2. Differential features of *E.histolytica and other non- pathogenic intestinal Entameoba*

| | E. histolytica | E. coli | E. hartmanni |
|---|---|---|---|
| **Trophozoite** | | | |
| Size (μm) | 12–60 | 20–50 | 4–12 |
| Motility | Active | Sluggish | Active |
| Pseudopodia | Finger-shaped, rapidly extruded | Short, blunt slowly extruded | Finger-shaped, rapidly extruded |
| Cytoplasm | Clearly defined into ectoplasm and endoplasm | Differentiation not distinct | Clearly defined into ectoplasm and endoplasm |
| Inclusions | RBCs present, no bacteria | Bacteria and other particles, no RBCs | Bacteria and other particles, no RBCs |
| Nucleus | Not clearly visible in unstained films | Visible in unstained films | Not visible in unstained films |
| Karyosome | Small, central | Large, eccentric | Small, eccentric |
| Nuclear Membrance | Delicate, with fine chromatin dots | Thick, with coarse chromatin granules | Coarse chromatin granules |
| **Cyst** | | | |
| Size (μm) | 10–15 | 10–30 | 5–10 |
| Nuclei in mature cyst | 4 | 8 | 4 |
| Glycogen mass | Seen in uninucleate, but not in quadinucleate stage | Seen up to quadrinucleate stage | Seen in uninucleate, but not in quadinucleate stage |
| chromidial | 1–4 with crounded ends | Splinter like with angular ends | Many with irregular shape |

### 4.20.2. Luminal flagellates

Most luminal flagellates are nonpathogenic commensals. Two of them cause clinical diseases:

- *Giardia lamblia*, which can cause diarrheal diseases and
- *Trichomonas vaginalis*, which can produce vaginitis and arthritis. They are grouped under **phylum Sarcomastigophora**. The following **note** focuses on intestinal lumen dwelling flagellate and hemoflagellates.

| Group | Parasites | Habitat |
|---|---|---|
| Lumen-dwelling flagellates | *Giardia lamblia* | Duodenum and jejunum |
| | *Trichomonas vaginalis* | Vagina and urethra |
| | *Trichomonas tenax* | Mouth |
| | *Trichomonas hominis* | Large intestine (caecum) |
| | *Chilomastix mesnili* | Large intestine (caecum) |
| | *Enteromonas hominis* | Large intestine (colon) |
| | *Retortamonas intestinalis* | Large intestine (colon) |
| | *Dientamoeba fragilis* | Large intestine (caecum and colon) |
| Hemoflagellates | *Leishmania* spp. | Reticuloendothelial cells |
| | *Trypanosoma brucei* | Connective tissue and blood |
| | *Trypanosoma cruzi* | Reticuloendothelial cells and blood |

4.20.2.1. **Giardia lambilia:** A protozoan parasites first described by Dutch scientist Antonie von Leeuwenhoek in his own stools in 1681.It is named 'Giardia' after Professor Giard of Paris and 'lamblia' after Professor Lamble of Prague, who gave a detailed description of the parasite.

**Habitat:***G. lamblia* lives in the duodenum and upper jejunum and is the only protozoan parasite found in the lumen of the human small intestine.

**Morphology:** It exists in 2 forms.

- trophozoites (or vegetative form): it is in the shape of a tennis racket (heart shaped or pyriform shaped) and is rounded interiorly and pointed posterior. It is bilaterally symmetrical and possesses 1 pair of nuclei, 4 pairs of flagella, 4 pairs of Blepharoplast from which the flagella arise, 1 pair of axostyles run along the mid line and two parabasal or median bodies, lying transversely posterior to the sucking disc. The trophozoites are motile, with a slow oscillation about its long axis, often resembling falling leaf.
- Cyst (or cystic form): it is the infective form of the parasite. The cyst is small and oval, measuring 12 µm x 8 µm and is surrounded by a hyaline cyst wall. Its internal structure includes 2 pairs of nuclei groupedat one end. A young cyst contains 1 pair of nuclei. The axostyle lies diagonally, forming a dividing line within cyst wall. Remnants of the flagella and the sucking disc may be seen in the young cyst.

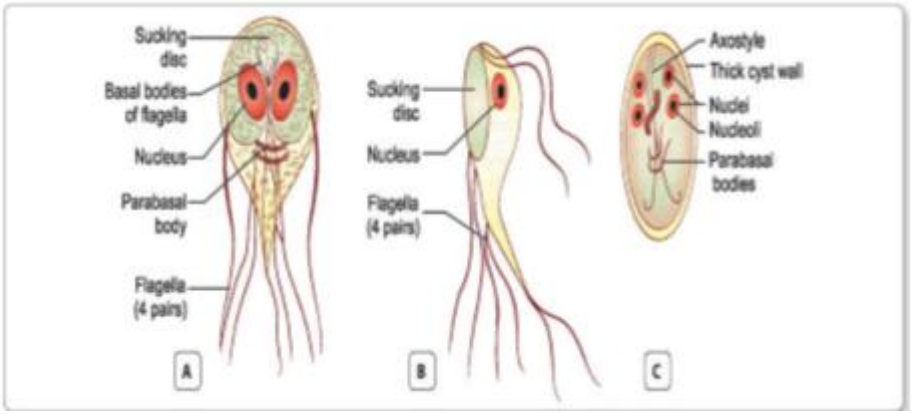



***Fig. 1.37. A. trophozoite of G. lambalia B. lateral view C. cyst***

**Life cycle:** The trophozoites live on the small intestine mucosa (less frequently on the gallbladder mucosa as well). They resemble a pear split lengthwise, are their dorsal side is convex; the anterior part of the ventral side forms a concave adhesive disk. Reproduction is by means of longitudinal binary fission of the trophozoites, which are able to produce variant specific surface proteins. G. lambilia produces oval cysts with four nuclei, flagella, and clawshaped median bodies. The cysts (and, less frequently, trophozoites) are excreted in stool.

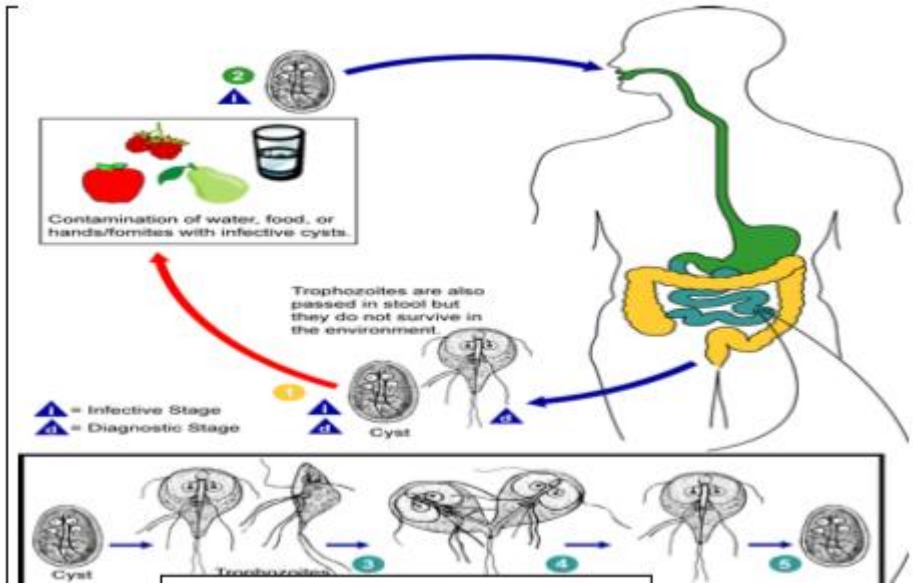
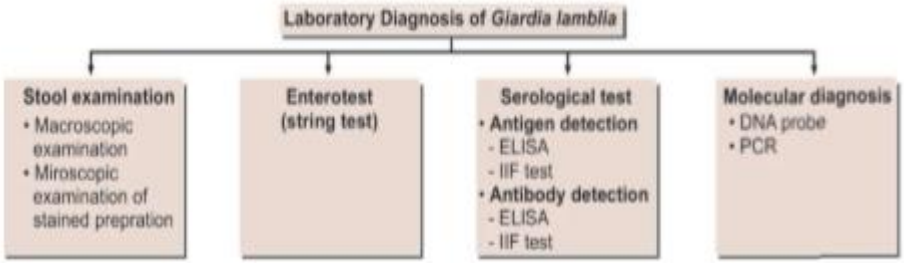



***Fig. 1.38. Description of life cycle of G. lambalia***

**Clinical features**: It does not invade the tissue, but remains tightly adhered to intestinal epithelium by means of the sucking disc. They may cause abnormalities of villous architecture by cell apoptosis and increased lymphatic infiltration of lamina propria. Diarrheal diseases (mucus diarrhea, fat malabsorption (steatorrheic), flatulence and adnominal discomfort.

Laboratory Diagnosis:




***Flow chart 1.7. Laboratory diagnosis of G.lambalia***

**Treatment:** Metronidazole (250 mg, thrice daily for 5–7 days) and tinidazole (2 g single dose) are the drugs of choice. Cure rates with metronidazole are more than 90%. Tinidazole is more effective than metronidazole. Furuzolidone and nitazoxamide are preferred for children, as they have fewer adverse effects.

### *4.20.3. Trichomonas vaginalis*

*Trichomonas vaginalis* is a frequent flagellate species that occurs worldwide and is transmitted mainly by sexual intercourse. It causes vaginitis in women and urethritis in men.

**Morphology:** T. vaginalis exists only in trophozoites stage. Trichomonas vaginalis is a pear shaped protozoon about 10–20 lm long and 2–14 lm wide. Five flagella emerge

from a basal body at the anterior pole, four freely extend forwards and one extends backwards, forming the outer edge of the undulating membrane, which reaches back only just beyond the middle of the cell. T. vaginalis colonizes the mucosa of the urogenital tract and reproduces by longitudinal binary fission. It is a motile with a rapid jerky or twitching type movement.

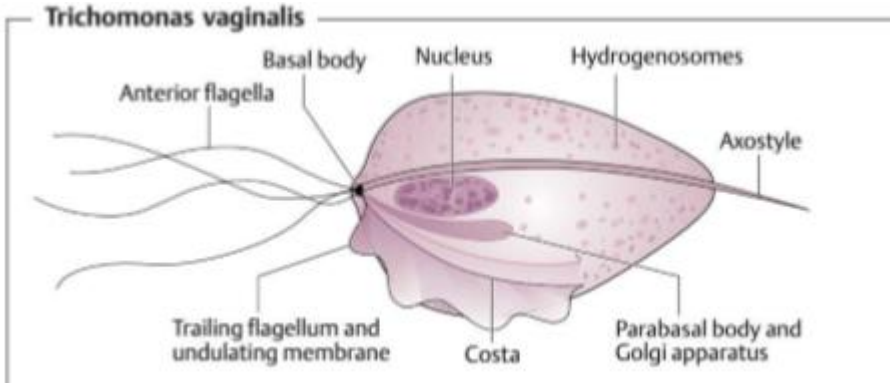



***Fig. 1.39. Morphology of T.vaginalis***

**Habitat:** In females, it lives in vagina and cervix and may also be found in urethra, and urinary bladder. In males, it occurs mainly in the anterior urethra, but may also be found in the prostate. Diagnosis: A fresh specimen of vaginal or urethral secretion is mixed with physiological saline solution and examined under a microscope for trichomonads. The trichomonads are readily recognized by their typical tumbling movements. The round trichomonad forms, by contrast, are hardly distinguishable from leukocytes. Can be easily identified as jerky like movement.

**Treatment:** It is always necessary for both sexual partners to receive treatment. Effective drugs include metronidazole preparations for oral application. In women vaginal application includes metronidazole and tinidazole. These substances are contraindicated in early pregnancy. Preventive measures include loyalty and use of condoms during sexual intercourse.

### 4.20.4. Hemoflagellates

General Characteristics: The blood and tissue flagellates belong to the family Trypanosomatidae. The family consists of 6 genera, of which 2 genera Trypanosoma and Leishmania are pathogenic to humans.

| Zoological Classification of Flagellates | | |
|---|---|---|
| Phylum | : | Sarcomastigophora |
| Subphylum | : | Mastigophora |
| Class | : | Kinetoplastidea |
| Order | : | Trypanosomatida |
| Family | : | Trypanosomatidae |

They live in the blood and tissues of man and other vertebrate hosts and in the gut of the insect vectors. Members of this family have a single nucleus, a kinetoplast, and a single flagellum. Nucleus is round or oval and is situated in the central part of the body. Kinetoplast consists of a deeply staining parabasal body and adjacent dot like blepharoplast. Flagellum is a thin, hair like structure, which originates from the blepharoplast. The portion of the flagellum, which is inside the body of the parasite and extends from the blepharoplast to surface of the body, is known as axoneme. A free flagellum at the anterior end traverses on the surface of the parasite as a narrow undulating membrane. Hemo-flagellates exist in two or more of four morphological stages. The names are **amastigote, promastigote, epimastigote and trypomastigote**. The names of the stages are formed by the suffix mastigote, combined with various prefixes, referring to the arrangement of the flagella in relation to the position of the nucleus and point of emergence from the cells.

Staining characteristics of trypanosomes: For smears of body fluids, Wrights stain, Giemsa stain, and Leishman's stain are suitable for identifying internal structures. The cytoplasm appears blue, the nucleus and flagellum appear pink, and the kinetoplast appears deep red. For tissue section, hematoxylin-eosin staining is done for demonstrating structures of the parasite. All members of the family have similar life cycles. They all require an insect vector as an intermediate host. Multiplication in both the vertebrate and invertebrate host is by binary fission. No sexual cycle is known.

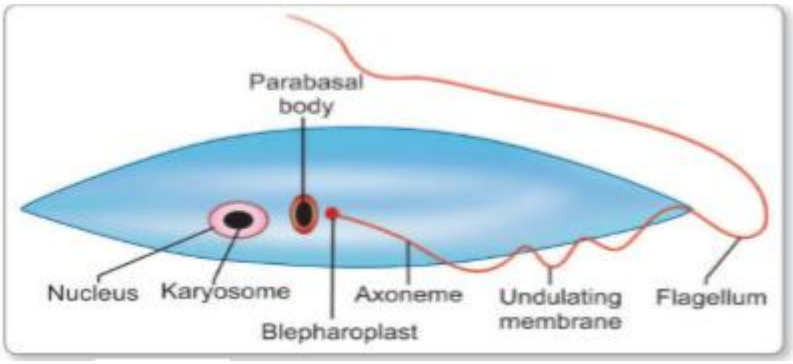
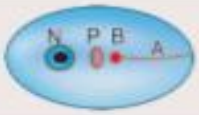
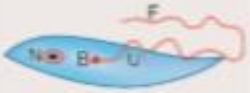
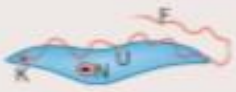



***Fig. 1.40. Basic morphology of hemoflagellates.***

The following table shows different between different morphological stages of Hemoflagellates.

***Table: 3 differences between various stages of Hemoflagellates.***

| | Amastigote | Promastigote | Epimastigote | Trypomastigote |
|---|---|---|---|---|
| Morphological characteristics | Rounded or ovoid, without any external flagellum. The nucleus, kinetoplast, and axial filaments can be seen. The axoneme extends upto the anterior end of the cell | Lanceolate in shape. Kinetoplast is anterior to the nucleus (antenuclear kineloplast) near the anterior end of the cell, from which flagellum emerges. There is no undulating membrane | Elongated, with the kinetoplast placed more posteriorly, though close to and in front of the nucleus (juxtanuclear kinetoplast). The flagellum runs alongside the body as a short undulating membrane, before emerging from the anterior end | This stage is elongated, spindle-shaped with a central nucleus. The kinetoplast is posterior to the nucleus (postnuclear kinetoplast) and situated at the posterior end of the body. The flagellum runs alongside the entire length of the cell to form a long undulating membrane before emerging as a free flagellum from the anterior end |
| Seen in | *Trypanosoma cruzi* and *Leishmania* as intracellular form in vertebrate host | It is the infective stage of *Leishmania*, found in the insect vector as well as in cultures *in-vitro* | It is the form in which *Trypanosoma brucei* occur in salivary gland of the vector tsetse fly and *Trypanosoma cruzi* in the midgut of the vector reduviid bug. **Note:** This stage is lacking in *Leishmania*. | This is the infective stage of trypanosomes found in arthropod vector and in the blood of infected vertebrate. **Note:** This stage is lacking in *Leishmania* |
| Schematic illustration |  |  |  |  |

**Three distinct Kinetoplastida cause human disease:**

- Leishmania species (leishmaniasis)
- African trypanosomes (African sleeping sickness)
- Trypanosoma cruzi (Chagas' disease), and)

### 4.20.4.1. Leishmania species (leishmaniasis)

They are the causative agent of Leishmaniasis and are obligate intracellular protozoa of the genus Leishmania. They were named after Leishmania, who first described it in London in May 1903. They are reproduces by longitudinal binary fission. Biological insect vectors as intermediate hosts & human as definitive host. The species are morphologically indistinguishable, but they can be differentiated on the basis of on their clinical features, geographical distribution, and serologic tests. Generally, human infection is caused by about 21 of 30 species that infect mammals.

These includes:

*L. donovani* complex:

- *L. donovani,*
- *L. infantum,*
- *L. chagasi*

*L. mexicana* complex:

- *L. mexicana*
- *L. amazonensis*
- *L. venezuelensis*

*L. braziliensis* complex:

- *L. braziliensis*
- *L. Peruviana*

*L. guyanensis* complex:

- *L. Guyanensis*
- *L. panamensis*

*L. tropica,*

*L. major*

*L. aethiopica*

Leishmaniasis can easily classified clinically as **visceral leishmaniasis, cutaneous leishmaniasis, Mucocutaneous leishmaniasis and Diffused cutaneous leishmaniasis**.

In Ethiopia: Four species of Leishmania are found, namely:

- *L. aethiopica,*
- *L.major*
- *L. tropica*
- *L. donovani*

In Ethiopia: – L donovani is the causative agent of visceral leishmaniasis

– Cutaneous leishmaniasis is caused by three parasites:

- *L. aethiopica,*
- *L. major*
- *L. tropica*

| **Promastigote** - Elongated, with flagella (10-20 µm long)<br>- Occur extracellularly in the insect midgut & in artificial culture<br>- Motile | **Amastigote** - Round (3-7 µm diameter)<br>- Occurs intracellularly, inmammalian<br>- Non-motile |
|---|---|

**Transmission and life cycle**

- Common mode of transmission.
  - Bite of infected female sandfly
    - Genera *Phlebotomus* ***in*** **Old world**
    - *Lutzomyia in* **New world**
- Uncommon modes of transmission:
  - Congenital transmission,
  - Blood transfusion,
  - Rarely, inoculation of cultures.


- Infective stage: Promastigotes
- Diagnostic stage: amastigotes
- Definitive host: human
- Intermediate host: sand flies (Genus Phlebotomus)

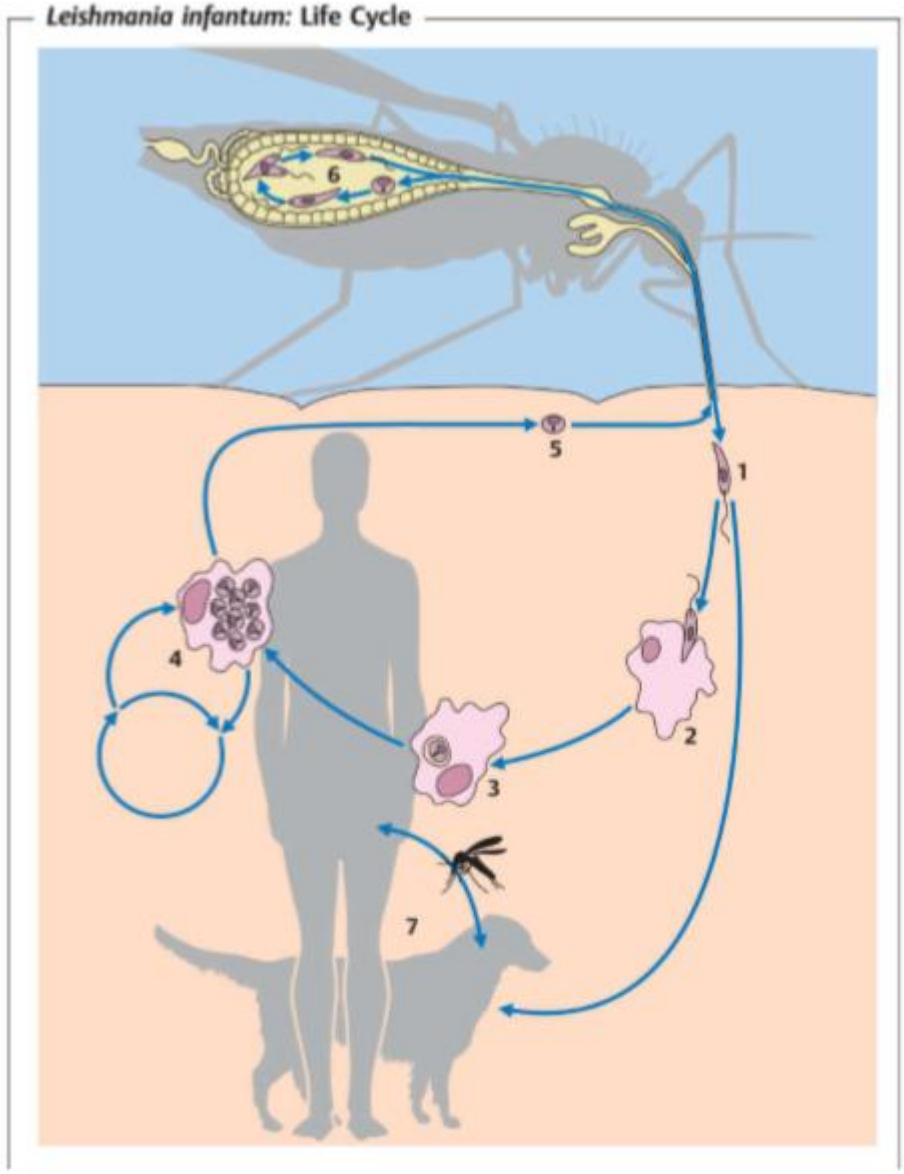
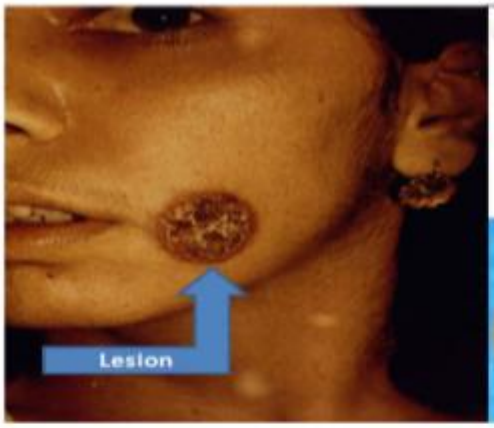



*Fig. 1.41. .**Inoculation of promastigotes stages (infective stage) by sand fly; 2 ingestion of parasites by phagocytes (Langerhans cells, dendritic cells, macrophages); 3 amastigote form in parasitophorous vacuole of a macrophage; 4 reproduction of amastigotes forms in a macrophage; 5 ingestion of amastigote forms by sand fly with blood meal (diagnostic stage); 6 transformation into promastigote form and multiplication in insect; 7 dog as reservoir host.***

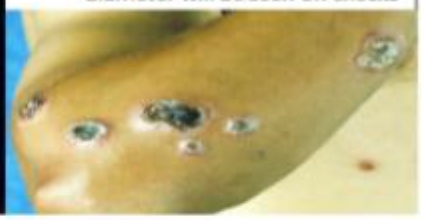
## Clinical manifestations:

1. Old World cutaneous Leishmaniasis caused by

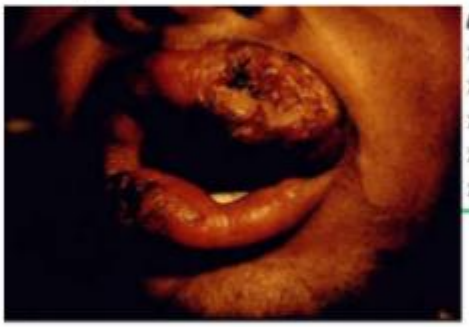



***L. Tropica***

- Dog are reservoir hosts
- It is called dry ,urban ,chronic, old world oriental sore
- A 'dry painless lesion' 5-70mm diameter will be seen on checks

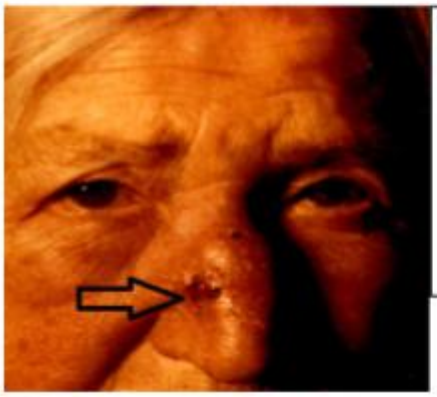


*2. L. major*



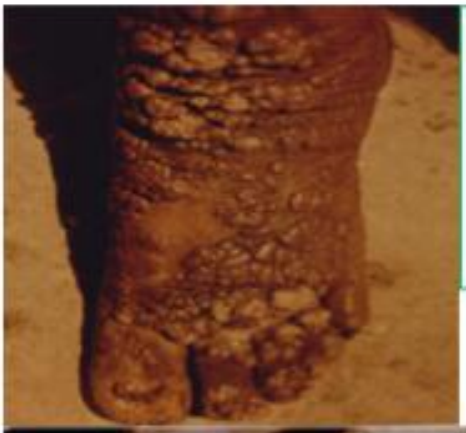
***L. Major***

- rural (rodent reservoir)
- wet oriental sore
- Early papules is inflamed (5-10mm)
- Develop to large uneven ulcer
- Self-healing (3-6mths)

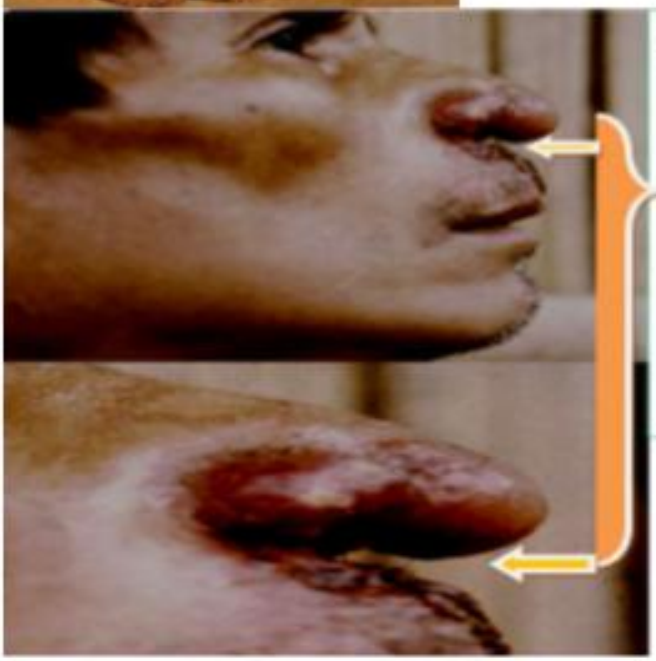


- *L. aethiopica*
  - highlands of Kenya and Ethiopia
  - Similar to oreintal sore
  - Self-heal 1-3 yrs
  - Can cause DCL in patients
  - also cause MCL

- **Diffuse Cutaneous Leishmaniasis:**

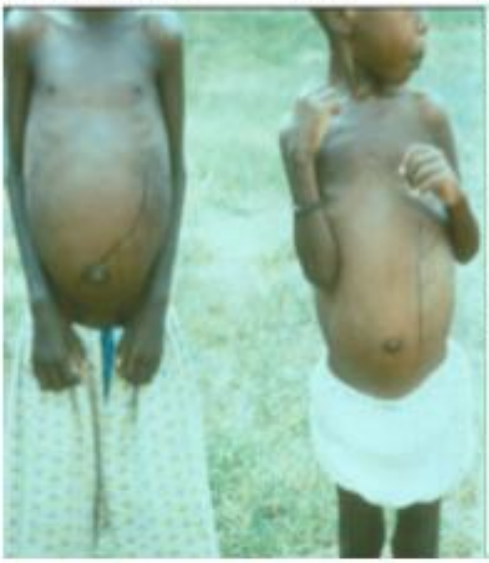


- Caused by *L.aethiopica* and *L. amazonensis*
- Skin lesion develop over large areas of the body
- Scaly, not ulcerated, nodules
- Chronic and painless



- Disfiguration is often extreme with complete destruction of the
- nasal septum, perforation of the palate and damage of the tissues of the lips and larynx

- **Visceral Leishmaniasis (VL)**: Caused by *L. donovani*. Reticuloendothelial system affected:spleen, liver, bone marrow, lymph nodes.



**Symptoms include:**

- Fever, malaise (as mild sickness or depression), weakness
- wasting despite good appetite
- Spleen and hepatomegaly, enlarged lymph nodes
- Epistaxis (nose bleeding) and bleeding of gums
- Depressed hematopoiesis (blood cell production abnormalities) severe anemia
  - leucopenia
  - Thrombopenia

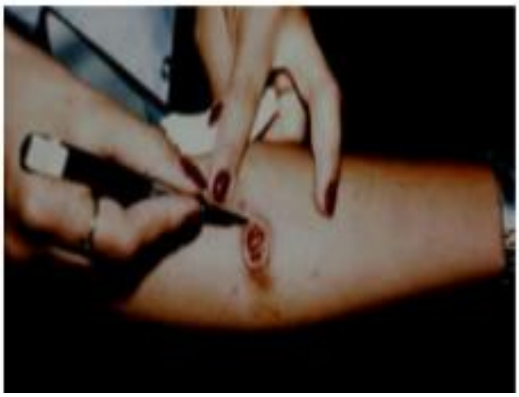
**Laboratory Diagnosis:**

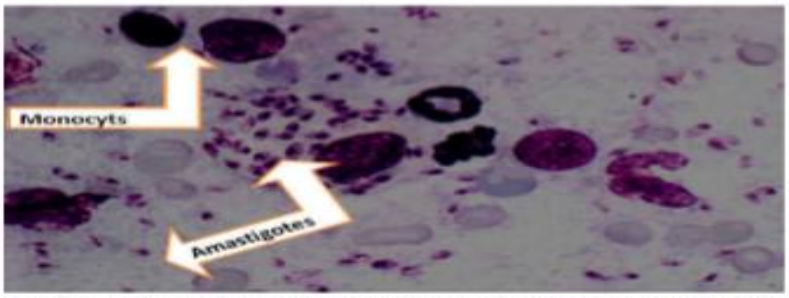
Cutaneous, Mucocutaneous and diffused cutaneous Leishmaniasis

Four Laboratory investigation methodes:

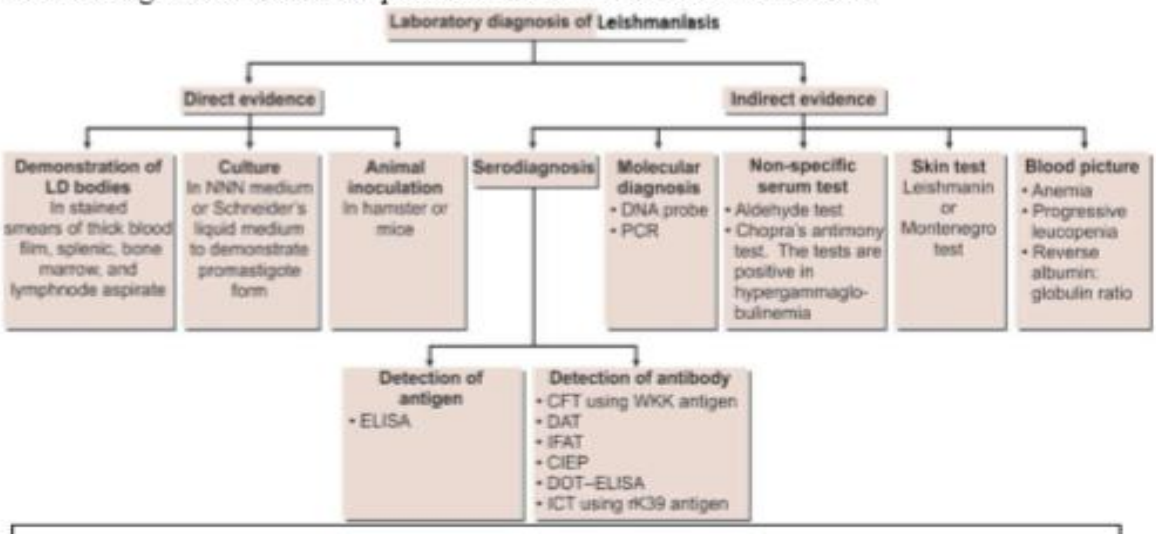
1. Detecting amastigotes from scrapings, biopsy, aspirates
2. Culture from ulcer material
3. Leishmanial test
4. Serology

**1. Detecting amastigotesfrom scrapings, biopsy, aspirates:** Collection and examination of skin slit smears for amastigotes. It should be taken from the inflamed raised swollen edge of an ulcer or nodule, not from its base or center which usually contains only necrotic tissue.





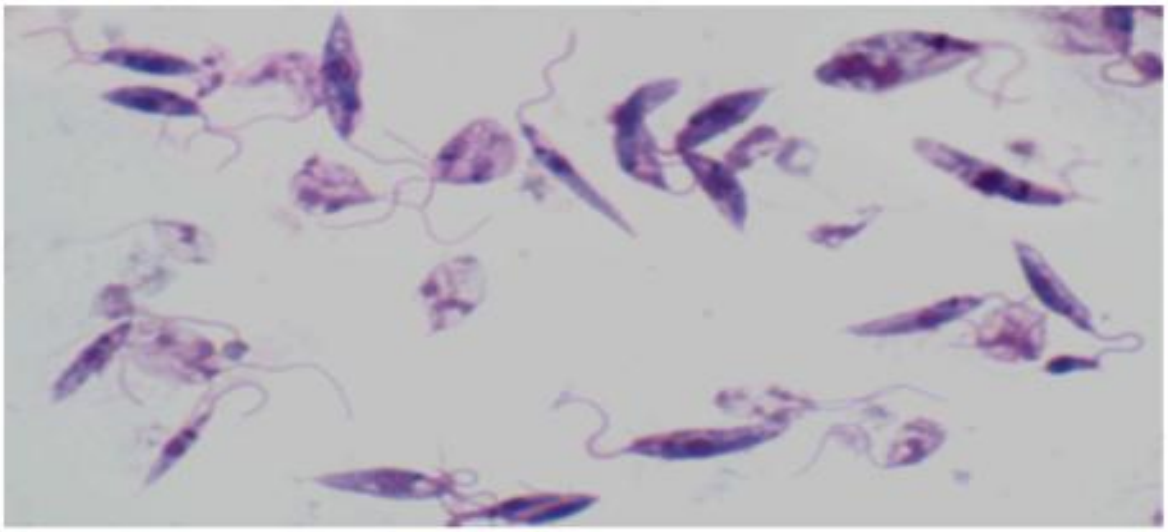

The above figure shows Microscopic examination of slides after Giemsa stain




***Flow chart 1.8. Laboratory diagnosis of leishmaniasis***

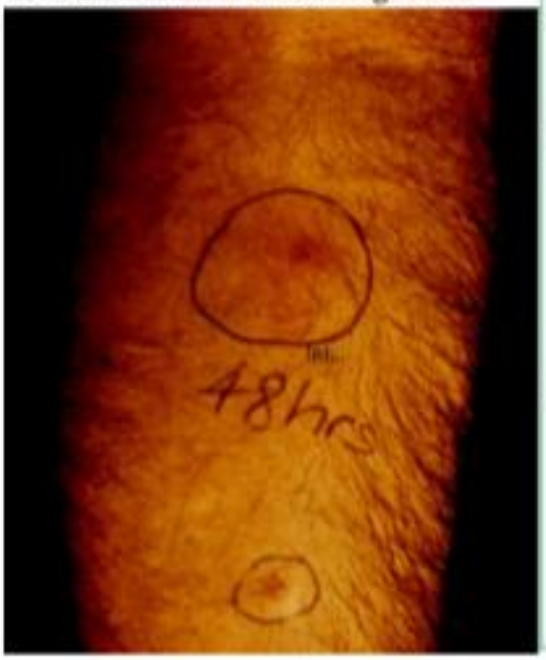
## 2.Culture **from ulcer material**

- Done when cutaneous leishmaniasis is suspected and parasites cannot be found in smears
- Material for culture is best obtained by injecting and then aspirating a small quantity **of sterile physiological saline in and out of** the hardened margin of the ulcer
- A few drops of the final aspirate is used to inoculate the culture medium
  - **Novy –Nicolle- MacNeal (NNN)**



Promastigote stages seen following *in vitro* culture.

### 3. Leishmainin or Montenegro test




- It is delayed type hypersensitivity skin test
- intradermal inoculation of leishmanin
  - suspension of whole or killed promastigotes
  - preferably from local area
  - include negative control
- *Positive reaction:* when the area of indurations ± erythema of 5mm in diameter or more indurations in 48-72 hours

**4. Serological Tests**

Because of the poor antibody response in cutaneous leishmaniasis, serological tests have no advantages or little value in diagnosis. This is due to cellular response which is the basis of the leishmanin skin test. In muco-cutanous leishmaniasis, antibodies can be found in the serum.

Diagnosis **visceral leishmaniasis**

1. Demonstration of parasite in tissues by
   - light microscopic examination of the stained specimen,
   - culture
   - animal inoculation
2. Detection of parasite DNA in tissue samples
3. Immunodiagnosis by detection
   - Antigen detection
   - antibody detection

**Demonstration of amastigotes from different specimens**

light microscopic examination of the stained specimen to recover amasigote from aspirates

| | Aspirate | %positive |
|---|---|---|
| 1. | Spleen | 95-98% |
| 2. | Bone marrow | 64-86% |
| 3. | Enlarged lymph node | About 64% |
| 4. | Buffy coat (India) | 67-99% |
| 5. | Buffy coat (Africa) | About 50% |

So, it's important to note that the positivity rates of aspirates are more blood smears for leishmaniasis diagnosis.

**Immunodiagnosis**

| 1. Antigen detection | 2. Antibody detection |
|---|---|
| ➢ more specific than Antibody based tests | ➢ Direct agglutination test (DAT) |
| ➢ Ag detected quite early | ➢ is a rapid and reliable screening test for VL |
| ➢ the amount of detectable Ag tends to decline rapidly following chemotherapy | ➢ sensitivity of 91-100% and specificity of 72-100% |
| ➢ useful in the diagnosis of disease in cases where there is deficient antibody production (as in AIDS patients) | ➢ some cross reaction with leprosy, Chagas disease, malaria and schistosomiasis may occur |
| | ➢ may also yield positive result for long time after complete cure |

**3. New K39 antigen strip test:** to diagnose visceral leishmaniasis has high sensitivity and specificity for active kala-azar. The nitrocellulose strips used in the test are impregnated with recombinant K39 antigen can be used to test peripheral (finger prick) blood samples.

**4. Rapid latex agglutination tests:** are quicker and easier to perform and interpretable than the Direct agglutination Teats. Equal volumes of test serum and sensitized dyed latex particles are mixed on a cavity microscope slide and rotated for up to 2 minutes. A positive test is shown by agglutination. Its sensitivity is around 100% & specificity 98%.Thus, yields positive result for long time after complete cure and some cross reaction with malaria, Tuberculosis or typhoid fever may also occur.

**5. Hematological investigations including:**

- Measurement of the hemoglobin
- Total and differential white cell (leucocytes) count: leucopenia-low WBCs,
- Platelet (thrombocyte) count: thrombocytopenic- low platelet count
- a raised ESR( erythrocyte sedimentation rates)
- Plasma albumin levels are greatly reduced, total protein raised in patients with VL(visceral Leishmaniasis)

**Treatment:** Sodium stibogluconate (Pentostam), Pentamidine isethionate,amphotericin B

**Prevention and control:** Early detection by serological diagnosis (VL) and treatment of infected persons:

- Health information dissemination
- Personal protection from sand fly bites by insect replants and avoiding endemic areas especially at times when sand flies are most active
- Use of pyrethroid impregnated bed nets and curtains
- Vector control etc.

### 4.20.5. Trypanosomiasis

Etiologic agents:

- Trypanosoma brucei complex
- African trypanosomiasis (sleeping sickness)
- Trypanosoma cruzi – American trypanosomiasis (Chagas' disease)

Important features: These species may have amastigote, promastigote, epimastigote, and trypomastigote stages in their life cycle. In human trypanosomes of the African form, however, the amastigote and promastigote stages of development are absent. Typical

trypanosome structure is an elongated spindle-shaped body that more or less tapers at both ends, a centrally situated nucleus, a kinetoplast posterior to nucleus, an undulating membrane arising from the kinetoplast and proceeding forward along the margin of the cell membrane and a single free flagellum at the anterior end.

African trypanosomiasis Trypanosoma gambiense & Trypanosoma rhodesiene are causative agents of the African typanosomiasis, transmitted by insect bites. The vector for both is the tsetse fly.

- **Life cycle**: During a blood meal on the mammalian host, an infected tsetse fly (genus Glossina) injects metacyclic trypomastigotes into skin tissue
    - The parasites enter the lymphatic system and pass in to the blood stream transform into blood stream trypomastigotes , are carried to other sites throughout the body, reach other body fluids (e.g., lymph, spinal fluid), and continue the replication by binary fission.
    - The tsetse fly becomes infected with blood stream trypomastigotes when taking a blood meal on an infected mammalian host
    - In the fly's midgut, the parasites transform into procyclic trypomastigotes, multiply by binary fission , leave the mid gut, and transform into epimastigotes
    - The epimastigotes reach the fly's salivary glands and continue multiplication by binary fission and transform into metacyclic trypomastigotes .

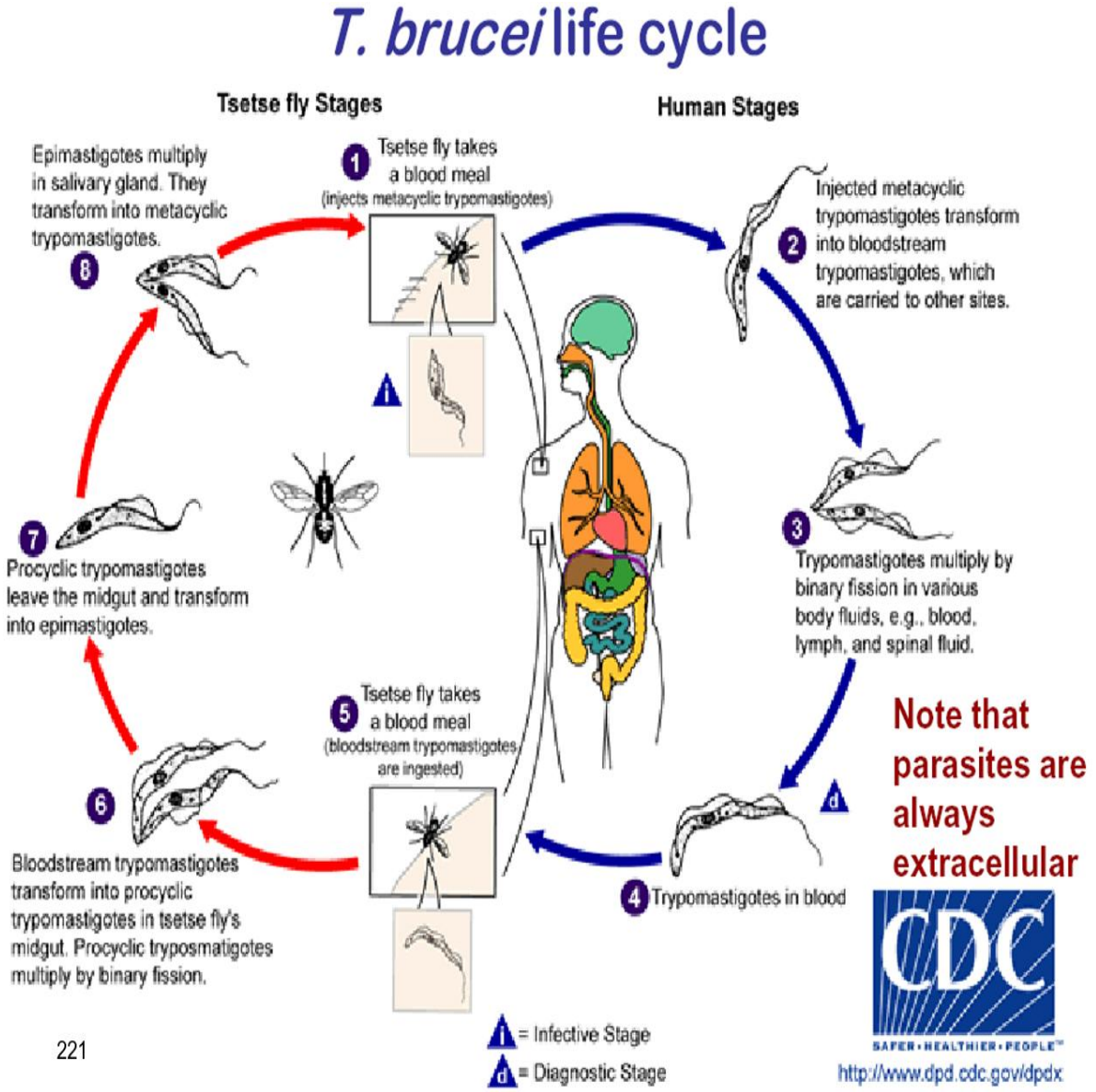
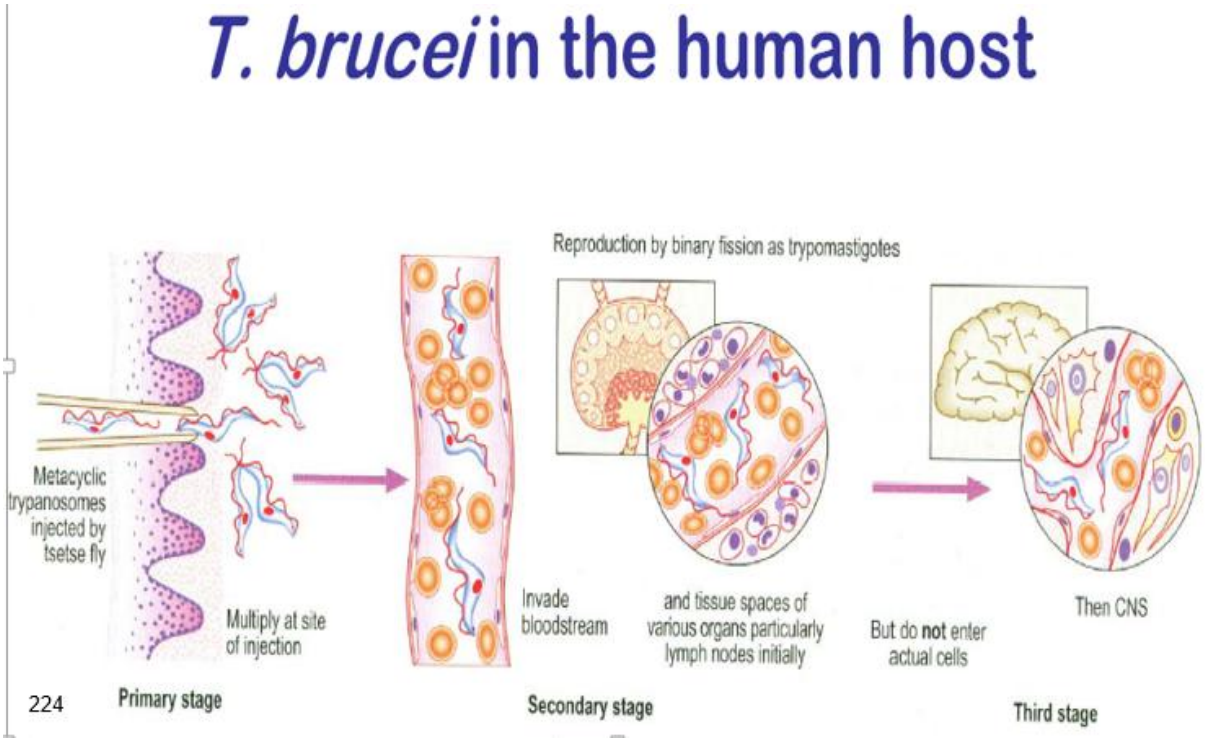



***Fig 1. 42. Lifecycle African trypanosomiasis***

- **Pathogenesis:**
  - An exact pathogenesis of sleeping sickness is not known, although immune complexes and inflammation have been suspected to be the mechanism of damage to tissues
  - The immune response against the organism help to eliminate the parasite but it is not protective
- Antigenic variation
  - Trypanosomes are covered with a glycoprotein surface coat called variable surface coat glycoprotein (VSG)
  - VSG is major component 'surface coat " covering blood steam trypomastigotes

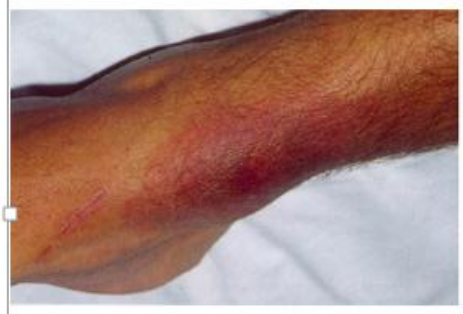
- Immunoglobulin directed against these VSG recognize the trypanosomes and destroy it until the organism vary their VSG
- Periodically (every 5-7 days ) the parasite will express a different VSG gene which is antigenically distinct from the previously expressed VSG
- It also causes severe depression of cell mediated and humoral immunity to other antigens
- Results in death

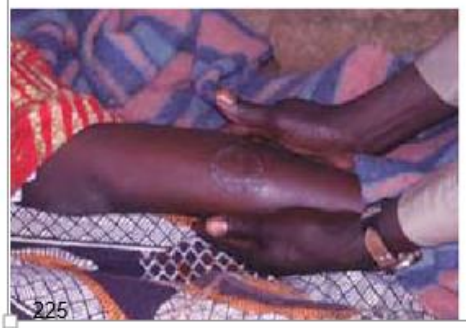
- **Clinical feature:**



# Sleeping sickness

## Disease course and symptoms





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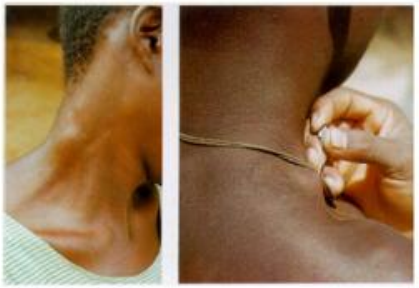
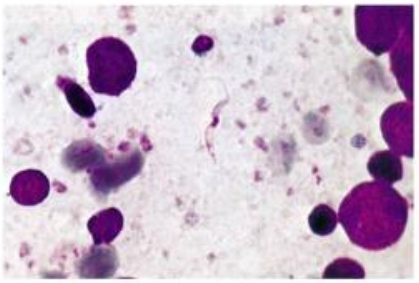
### First stage

- Trypanosomes multiply in the tissue around the initial bite site
- This often results in a characteristic local inflammation the trypanosomal **"chancre".** Usually not painful
- From there they enter the blood and lymphatic system

# Sleeping sickness

## Disease course and symptoms

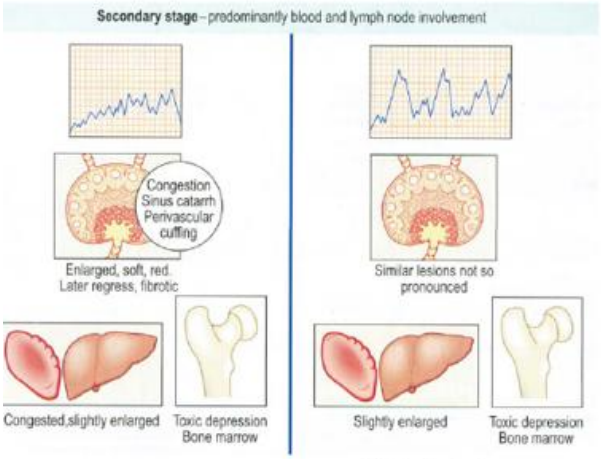




- Enlargement of the lymphatic glands (especially in the posterior triangle of the neck) can be an early sign of the disease (**Winterbottom sign**, not as common in rhodesiense infection).
- Aspiration of swollen gland often reveals parasites.

# Sleeping sickness

## Disease course and symptoms



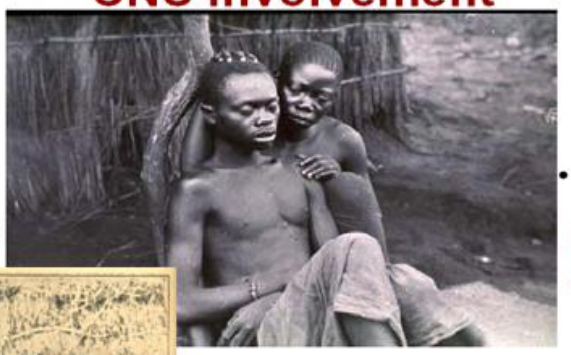

- After 1-2 week period of asymptomatic incubation, parasites invade blood leading to fever and headache
- Once parasites enter blood stream fever sets in (low and irregular in gambiense and high and periodic in rhodesiense)
- General toxic symptoms include headache, facial edema, nausea and vomiting, back and bone pain
- Symptoms at this stage are rather mild in gambiense but can be severe in rhodesiense with often fatal outcome

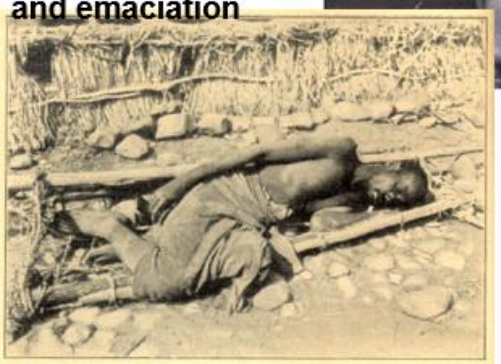
# Sleeping sickness

## Disease course and symptoms

### CNS involvement

**Fatigability , confusion , drowsiness , somnolence (uncontrollable urge to sleep day time which may alternate with night time insomnia ), wasting and emaciation**





- In later stages of infection parasites pass the blood brain barrier and infect the CNS
- Presence of parasites leads to **meningo-encephalitis** with progressive neurological involvement, which ultimately ends in coma (sleeping sickness)
- Untreated trypanosomiasis is always fatal

# Sleeping sickness

## Disease course and symptoms

Neurological complications can occur as a result of infection and, as seen here, patients may be immobilized for their own safety.

- The progressive encephalitis can cause severe dementia with sometimes aggressive behavior
- Disease progression especially CNS invasion is much faster in rhodesiense
- Gambiense can take a year or two while rhodesiense usually passes the blood brain barrier within a month

# Gambiense and Rhodesiense Sleeping sickness

- **West African**
  - *T.b.gambiense*
  - West & Central Africa
  - Tsetse fly (*Glossina*, palpalis group)
  - Reservoir = human
  - Parasitemia = low/variable
  - Clinical features
    - Early: fever, myalgias, lymphadenopathy, edema,
    - Late: CNS; seizures, coma
    - Duration; months-years
  - Mortality; 100% if unRx
  - Asymptomatic carriers common

- **East African**
  - *T.b. rhodesiense*
  - Rift valley & eastern Africa
  - Tsetse fly (*Glossina*, morsitans group)
  - Reservoir = wild animals
  - Parasitemia = high
  - Clinical features
    - Early: severe fevers, edema, weakness, emaciation
    - Late: rapid demise
    - Duration; weeks - months
  - Mortality; 100% if unRx
  - Asymptomatic carriers rare

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# Laboratory diagnosis

## 1. Microscopy

A. Examination of blood for trypanosomes

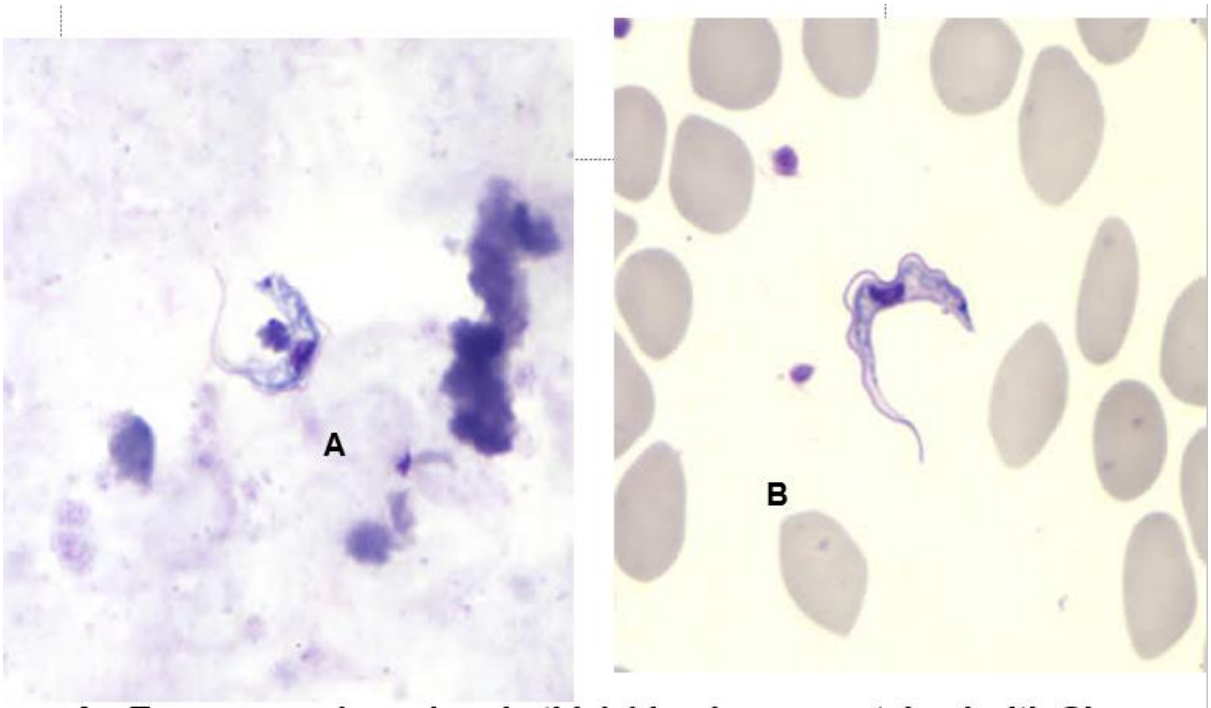
B. Examination of lymph gland aspirates

C. Examination of chancre fluid for trypanosomes

D. Examination of CSF for trypanosomes

## 2. Serology

## 3. Molecular techniques : polymerase chain reaction (PCR).




**A: *Trypansoma brucei* sp. in thick blood smears stained with Giemsa.**
**B: *Trypanosoma brucei* sp. in thin blood smears stained with Giemsa**
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**Treatment :** Pentamidine isethionate or Suramin

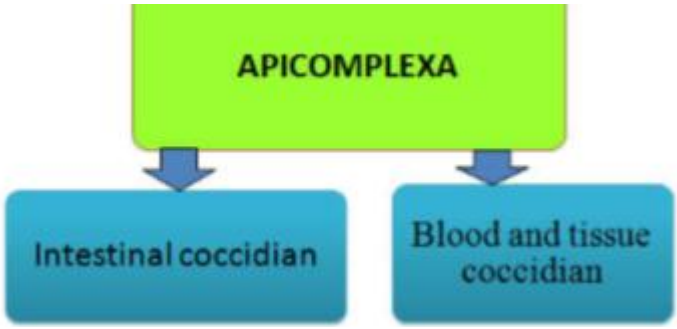
**Preventionand Control**

1. Detecting and treating human infections at early stage
2. Identifying and killing/ restricting movement of animal reservoir hosts in endemic areas
3. Health information dissemination
4. Sterile breeding technique
5. Vector control:

- ☐ By spraying vehicles with insecticide as they enter and leave the tsetse fly infested area,
- ☐ By selectively clearing the bush and wood areas especially around water holes, bridges, and along river blanks
- ☐ By using and maintaining insecticide impregnated tsetse fly traps

### 4.20.6. Blood and Tissue Coccidians

**General features:** they are **Coccidians** are **apicomplexa** parasites




- During part of their life cycles, most apicomplexans invade and replicate within the host cells
- Both asexual and sexual reproduction are involved
- The basic life cycle may be said to start when an infective stage (sporozoite) enters a host cell, and divides repeatedly to form numerous merozoites
- Micronemes and rhoptries are specialized membrane bound apical organelles that play a major role in interactions with and invasion of host cells
- Micronemes contain adhesive proteins that are important in attaching to host cells or the substratum
- The force needed for zoite motility and invasion is provided by a motor protein complex including a myosin unique to apicomplexans
- The apicomplexa have complex life cycles that are characterized by three distinct processes: **Sporogony, merogony and gametogony**
- Sporogony occurs immediately after a sexual phase and Consists of an asexual reproduction that culminates in the production of sporozoites
- Sporozoites are an invasive form that will invade host cells and develop into forms that undergo another asexual replication known as merogony.
- Therefore, blood and tissue sporozoa are found inside blood, blood forming organs or tissues.

Seven species infecting humans are but our main focus is Plasmodium species and we give short description on C. parvum and T. gondii.

*1. Plasmodium species 2. Babesia 3. Cryptosporidium parvum 4. Isospora belli*

*5. Cyclospora cayetenesis 6. Sarcocystis 7. Toxoplasma gondii*

### 4.20.6.1. Plasmodium species

They are causative agent of Malaria: an acute and/or chronic infection caused by protozoans of the genus Plasmodium.

Five plasmodium species causing human malaria

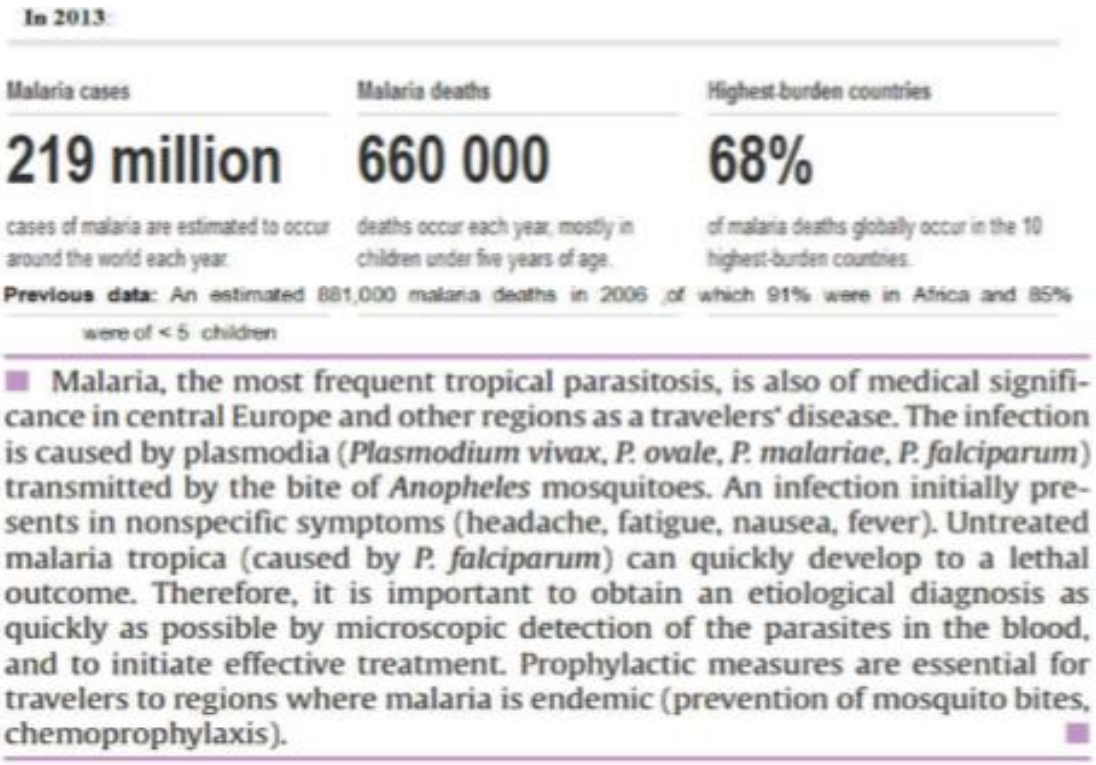
1. *Plasmodium falciparum*
2. *P. vivax*
3. *P. malariae*
4. *P. ovale*
5. *P. knowlesi*

**General feature of Plasmodium species:**

They are intracellular obligate parasites (liver cell & RBC). The life cycle is alternation of generation by alternation of hosts and requires two hosts:

- Sexual and asexual reproduction

Malaria is a highly fatal disease of all known to kill human beings.




**Morphological Stages**

1. Sporozoite: develops in the mosquito salivary gland

2. Hepatic schizont: actively dividing, multinucleated, parasite form in hepatocytes
3. Trophozoite: metabolically active form living within the RBC. Sometimes called the ring form
4. Erythrocytic schizont: multinucleated stage in RBC resulting from asexual multiplication of trophozoites. Each schizont contains a species determined number of merozoites
5. Merozoite: infective of stage for RBC and its schizont components that break out of hepatocyte or RBC
6. Gametocyte: morphologically distinctive sexual (male or female) form which develops from some trophozoites in RBCs

**The life cycle**

**The infective stage:** sporozoites.

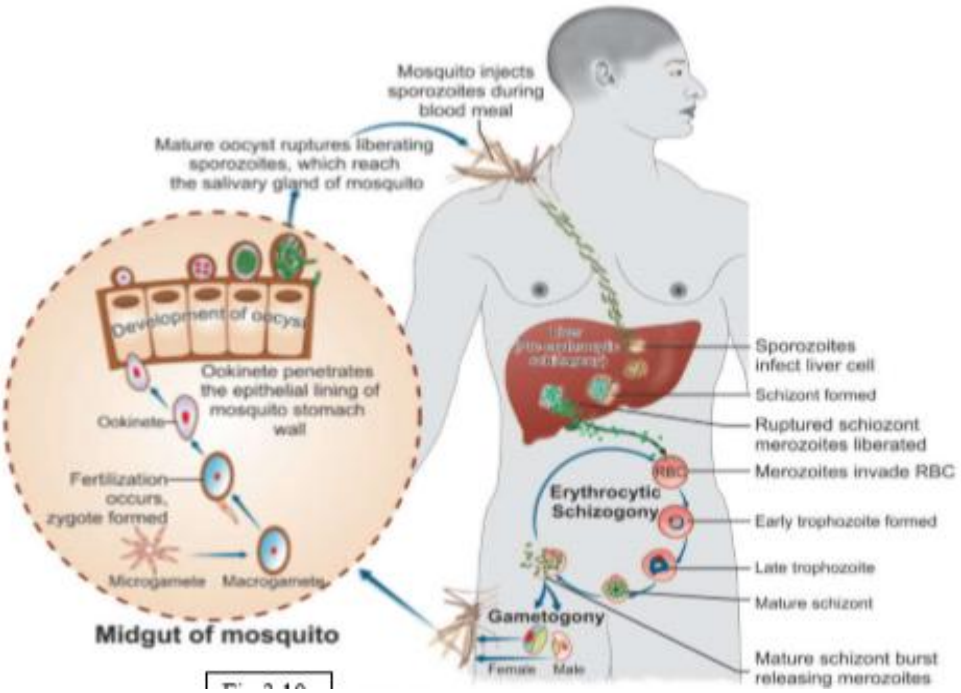
**The diagnostic stages:** Trophozoites, schizonts and gametocytes.

- Human as intermediate hosts-asexual reproduction takes place
- Female Anopheles mosquitoes are definitive hosts-sexual reproduction takes place.
- Male anopheles mosquitoes never take blood because

(1) They rely on flower nectars to feed on

(2) Their proboscis are blunt so that cannot pierce human skin.

Therefore, it is only the female one which is responsible for the transmission of malaria from infected person to non-infected person.

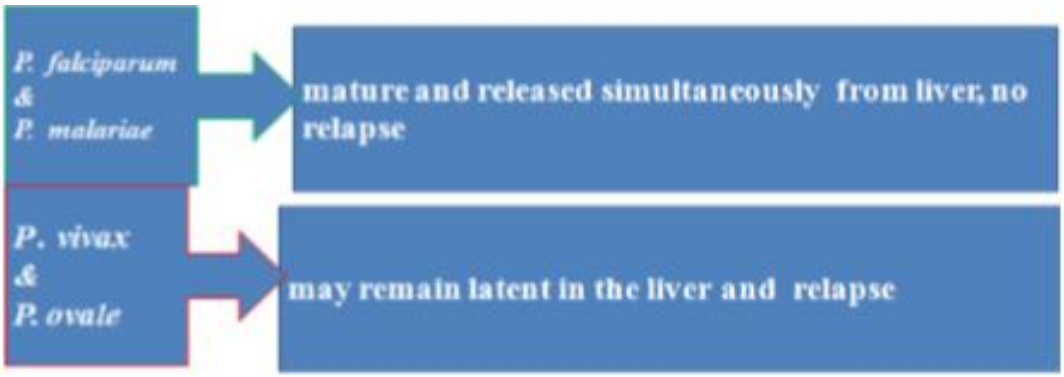



*Fig, 1.43.* ***Life cycle of plasmodium spp.***

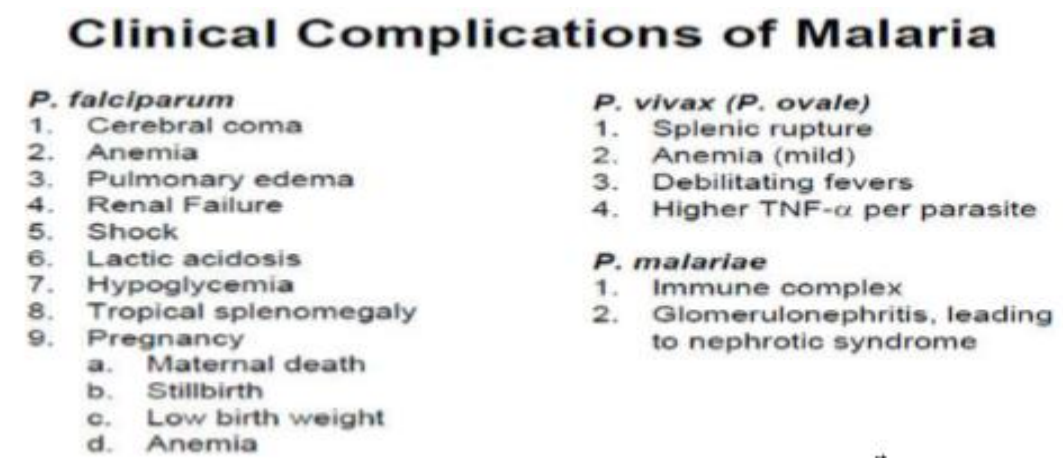
### Development in the Mosquito (Sexual Development and Sporogony)

This developmental stage is shown in detail in Fig. 9.**17b** and will only be described briefly here. In the mosquito midgut, each microgamont develops into (in most cases) eight uninucleate, flagellate microgametes and the macrogamont is transformed into a macrogamete → fusion of a microgamete and macrogamete to form a motile zygote (ookinete) → the ookinetes occupy the space between the epithelial layer and basal membrane of the midgut → morphological transformation into oocysts (40–60 µm) → in oocyst nuclear proliferation and production of thousands of sporozoites → sporozoites emerge into the hemolymph and migrate through the body cavity to the salivary glands, from where they can be transmitted to a new host. The duration of the cycle in the mosquito depends on the plasmodial species and the ambient temperature; at 20–28 °C, it takes eight to 14 days.

**Note that:**




**The major clinical manifestations of malaria are as follows:**

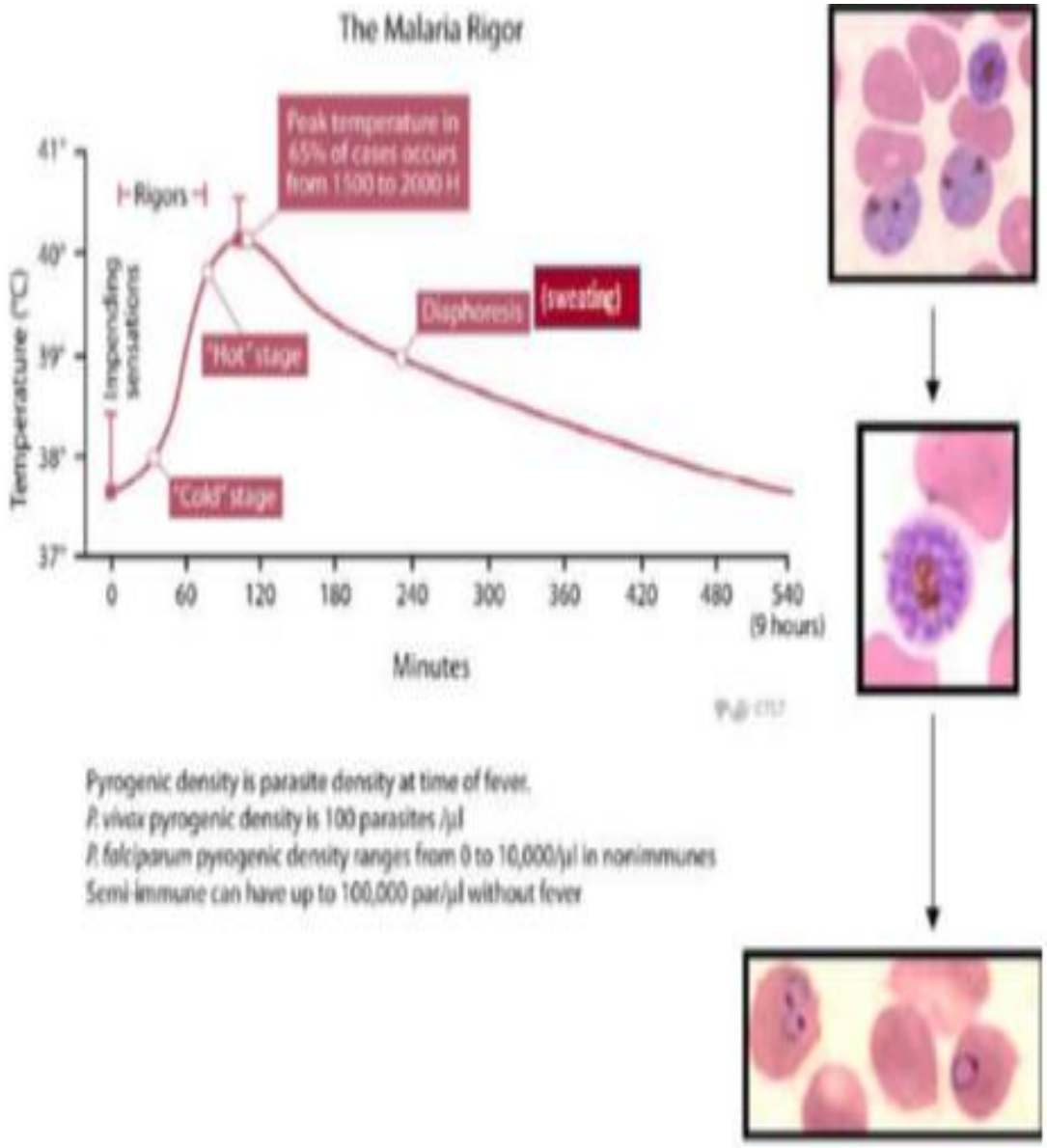



Malaria paroxysm preceded by prodromal period from 2-3 days before 1stparoxysm. This includes: malaise, fatigue, headache, muscle pain, nausea, anorexia (i.e., flu-like symptoms) can range from none to mild to severe.

When generalized, the manifestations can be of three alternative episodes:

- Prodromal Symptoms (the cold stage, hot/heat stage and the sweating stage).

This cab ne presented by the following graph:




1. The cold stage: manifestations of cold and shivering for some minutes to an hour.
    - feeling of intense cold
    - vigorous shivering, rigor
    - lasts 15-60 min
2. Hot stage
    - feeling of intense heat
    - dry burning skin will be observed
    - severe headache
    - Lasts 2-6 hours
3. Sweating stage: this is the final stage when our body takes action to equilibrate the internal and external temperature by reducing excess heat via sweating. It is called homeostasis. **Symptoms include:**
    - profuse sweating, declining temperature, exhausted, weak

- sleep which lasts for 2-4 hours Among malaria parasites known to infect human, P. falciparum is the dangerous one to kill human beings on the earth. The following shows its complications.

**Severe Falciparum Malaria**

| Complications | Features Indicating Poor Prognosis |
|---|---|
| ➢cerebral malaria | ➢impaired consciousness |
| ➢blackwater fever | ➢repeated convulsions |
| ➢anemia | ➢respiratory distress |
| ➢hypoglycemia | ➢shock |
| ➢GI and liver syndromes | ➢acidosis/hyperlactemia |
| ➢pulmonary edema | ➢hypoglycemia |
| ➢algid malaria (shock) | ➢jaundice or other liver malfunctions |
| | ➢renal impairment |
| | ➢high parasitemia (>500,000/mm³) |

The rest species are not life threatening and some are self-limiting.

**Table 4. Comparison of the characteristics of plasmodia causing human malaria**

| | *P. vivax* | *P. falciparum* | *P. malariae* | *P. ovale* |
|---|---|---|---|---|
| Hypnozoites | Yes | No | No | Yes |
| Erythrocyte preference | Reticulocytes | Young erythrocytes, but can infect all stages | Old erythrocytes | Reticulocytes |
| Stages found in peripheral blood | Rings, trophozoites, schizonts, gametocytes | Only rings and gametocytes | As in vivax | As in vivax |
| Ring stage | Large, 2.5 µm, usually single, prominent chromatin | Delicate, small, 1.5 µm, double chromatin, and multiple rings common, Accole forms found. | Similar to vivax, but thicker | Similar to vivax, more compact |
| Late trophozoite | Large irregular, actively amoeboid, prominent vacuole | Compact, seldom seen in blood smear | Band form characteristic | Compact, coarse pigment |
| Schizont | Large filling red cell | Small, compact, seldom seen in blood smear | Medium size | Medium size |
| Number of merozoites | 12–24 in irregular grape-like cluster | 8–24 grape-like cluster | 6–12 in daisy-head or rosette pattern | 6–12 irregularly arranged |
| Microgametocyte | Spherical, compact, pale blue cytoplasm, diffuse nucleus | Sausage or banana-shaped pale blue or pink cytoplasm, large diffuse nucleus | As in vivax | As in vivax |
| Macrogametocyte | Large, spherical, deep blue cytoplasm, compact nucleus | Crescentic, deep blue cytoplasm, compact nucleus | As in vivax | As in vivax |
| Infected erythrocyte | Enlarged, pale, with Schuffner's dots | Normal size, Maurer's clefts, sometimes basophilic stippling | Normal, occasionally Ziemann's stippling | Enlarged, oval fimbriated, prominent Schuffner's dots |
| Duration of schizogony (days) | 2 | 2 | 3 | 2 |
| Prepatent period (days) | 8 | 5 | 13 | 9 |
| Average incubation period (days) | 14 | 12 | 30 | 14 |
| Appearance of gametocyte after parasite patency (days) | 4–5 | 10–12 | 11–14 | 5–6 |
| Duration of sporogony in mosquito (25°C) (days) | 9–10 | 10–12 | 25–28 | 14–16 |
| Average duration of untreated infection (years) | 4 | 2 | 40 | 4 |

**Laboratory diagnosis Techniques:** two methods are routinely performed for malaria diagnosis:

1. Microscopy examination: Collection of Blood Specimen: Collect sufficient quantity of blood (50 µl or one drop of finger prick blood). The collection sites include:

- Capillary blood from finger prick , toes, or ear lobes are best ones
- Venous blood.(EDTA anticoagulant)
- In obstetric practice, cord blood and placental impression smears can be used

**Time of collection**

- Collect when the patients feels febrile.
- Collect before anti-malaria drugs are given to the patients
- Note: that a negative test DOES NOT rule out malaria and repeated tests are recommended in all doubtful cases
- **Thin and thick blood film**s can be prepared.

**Immuno-chromatographic tests for malaria antigens**

- Are based on the capture of the parasite antigens from the peripheral blood
- Uses either monoclonal or polyclonal antibodies against the parasite anti targets.
- RDTs do not require a laboratory, electricity, or any special equipment.
- **Targets**
  1. Histidine-rich protein 2 of *P. Falciparum,*
  2. Pan-malarial *plasmodium* aldolase, and
  3. Parasite specific lactate dehydrogenase (pldh)

1. Histidine-rich protein of P. falciparum (PfHRP2): – It is a protein produced by the asexual stages and gametocytes of P. falciparum which remain in the blood for at least 28 days after the initiation of antimalarial therapy. Thus, detection of this protein from patient's blood may not indicate current infection. Because this proteins are released from dead RBCs and parasites.
2. Parasite lactate dehydrogenase (pLDH)
   - Is a soluble glycolytic enzyme produced by the asexual and sexual stages of the live parasites
   - Present in and released from the parasite infected erythrocytes
   - Found in all 4 human malaria species, and different isomers of pLDH for each of the 4 species exist. With pLDH as the target, a quantitative immuno-capture assay
3. Plasmodium aldolase
   - Is an enzyme of the parasite glycolytic pathway
   - Expressed by the blood stages of P. Falciparum as well as the non-falciparum malaria parasites

- Monoclonal antibodies against plasmodium aldolase are pan-specific in their reaction
- Have been used in a combined 'P.F / P.V' ICT test that targets the pan malarial antigen (PMA) along with pfhrp2

2. RDTS general Procedures 50 µl of blood from finger prick(one drop) is required.
   A blood specimen is mixed in a buffer. The labeled Ag – Ab complex migrates up the test strip.
   - Labeled antibodies pre deposited –
   - Finger prick blood is mixed with buffer solution with haemolysing compound & specific antibody
   - If the target Antigen is present, antigen /antibody is formed
   - Antigen –antibody complex migrates up the test strip by capillary action towards test specific reagents, which have been pre-deposited during manufacture –
   - Buffer added to wash hemoglobin and permit visualization of any colored line on the strip
   - Performance of the test is assessed in divers clinical situation:
   - some RTD detect two parasites
   - Advantages over microscopy:
     * Simpler to perform & interpret
     * Do not require electricity, special equipment & training in microscopy
     * Community volunteers can be taught the procedure in hrs.
     * RDTs detect circulating antigen may detect Pf antigens even when the parasites are sequestered
   - Disadvantages over microscopy:
     * Highly sensitive, false positive result
     * Cannot detect density of the parasite
     * Expensive
     * Positive for circulating antigens
     * Not quantitative method, doesn't tell us the number of parasites
     * Do not differentiate between species: P. vivax , P. ovale P. malariae
     * Specific to malaria parasite only, cannot detect other haemoparasites

**Prevention and Control**

- Treatment of infected individuals
- Use of bed nets

- Use of Personal repellents and use of insecticides and Health education

**Key points of *Plasmodium* and *Babesia***

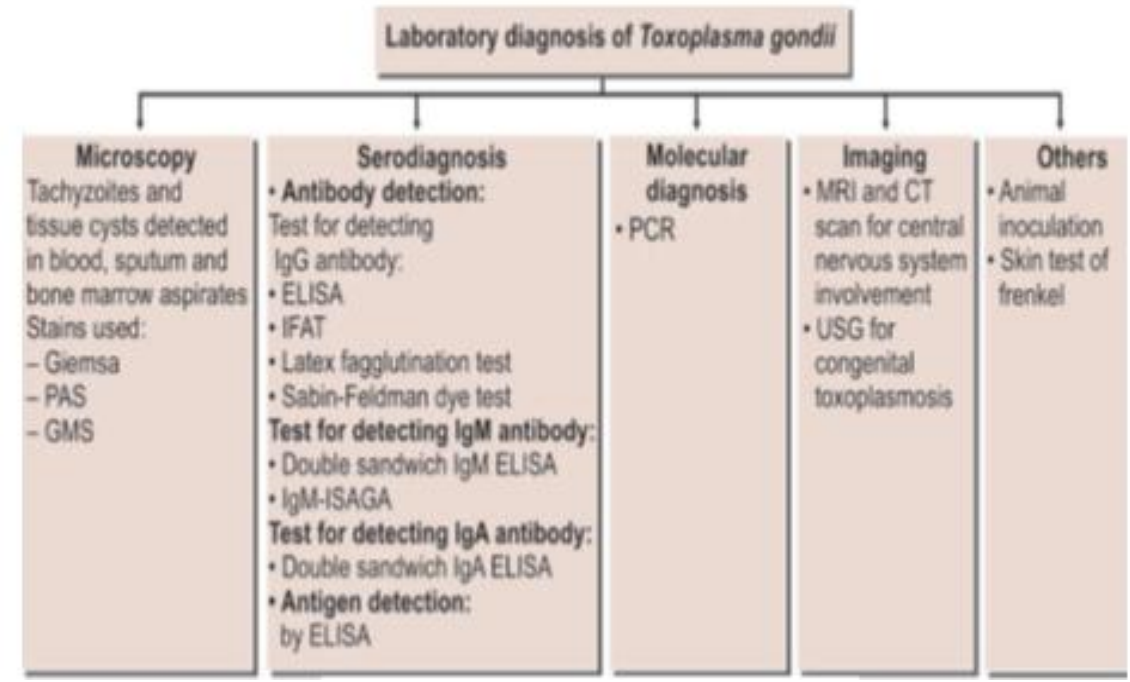
- **Malaria parasite** belongs to the genus *Plasmodium*.
- Four species of *Plasmodium* cause malaria in man—*P. vivax, P. falciparum, P. malariae*, and *P. ovale*.
- **Definitive host:** Anopheles mosquito (sexual phase of life cycle)
- **Intermediate host:** Man (asexual phase of life cycle)
- **Infective form**: Sporozoites present in salivary gland of mosquito.
- *P. vivax* and *P. ovale* cause benign tertian malaria, *P. falciparum* causes malignant tertian malaria and *P. malariae* causes benign quartan malaria.
- Acute falciparum malaria is the most dangerous and fatal form and is due to heavy parazitization of RBCs which cause blockage of capillary and venules by cytoadherence.
- **Clinical features:** Typical picture of malaria consist of periodic bouts of fever with rigor followed by anemia and splenomegaly. Febrile pasoxysms comprise of cold stage, hot stage, and the sweating stage.
- TSS is a chronic benign condition resulting from abnormal immunological response to malaria.
- Relapse of malaria occurs in *P. vivax* and *P. ovale* infection due to persistence of dormant stage hypnozoites in liver. Recrudescence occurs commonly in *P. falciparum* and *P. malariae* due to persistance of parasite in circulation at a subclinical level.
- **Diagnosis**: By demonstration of parasite in thick and thin smear of peripheral blood and also by detection of malaria antigen by rapid ICT.
- **Treatment**: Chrooquine sulfadoxine, and pyrimethamine along with primaquine. In chlroquine resistance, quinine or artemesinin are used.
- *Babesia species* comprising *B. microti, B. divergens*, and *B. bovis*, are intraerythrocytic sporozoan parasite resembling plasmodia. They cause opportunistic infections in humans.
- **Mode of transmission:** Through bite of Ixodid ticks.
- **Reservoirs:** Rodents and cattle.
- **Clinical features**: Mild and self-limiting. In immunocompromised patients, it causes anemia, jaundice, hemoglobinuria, respiratory failure, etc.
- **Diagnosis**: By examination of stained blood films for intraerythrocytic parasites, reticulocytosis, increased SGPT, alkaline phosphatase, hemoglobiniuria.
- **Treatment**: Atovaquine + azithromycin. Alternatively, clindamycin and quinine may be given.

### 4.20.7. *Toxoplasma gondii*

*Toxoplasma gondii* is the causative agent of a zoonosis that occurs worldwide with high prevalences (up to 80% depending on region and age). Humans are infected by ingesting oocysts excreted by the definitive hosts (cats) or by eating unprocessed meat containing *Toxoplasma* cysts. If a women contracts toxoplasmosis for the first time during pregnancy, diaplacental transmission of the pathogen to the fetus is possible with potential severe consequences (for example malformations, eye damage, clinical symptoms during childhood). There is, however, no risk to the fetus from mothers who had been infected before their first pregnancy and have produced serum antibodies (about 35–45%). Latent infections can be activated by immunodeficiencies (e.g., in AIDS patients) and may result in cerebral or generalized symptomatic toxoplasmosis. Serological surveillance in pregnant women is important to prevent prenatal infections.

**Key points of *Toxoplasma gondii***

- Obligate intracellular parasite.
- Exists in 3 forms: trophozoite, tissue cyst, and oocyst.
- **Definitive host:** Cat family (enteric cycle).
- **Intermediate host:** Human (exoenteric cycle).
- Human infection occurs by ingestion of food containing oocyst and tissue cyst.
- Congenital infection can also occur.
- **Clinical feautes:** Acute encephalopathy, fever, chorioretinitis, lymphadenopathy, myocarditis, hepatosplenomegaly.
- Disseminated infection in AIDS.
- **Diagnosis:** By demonstration of parasite in tissue specimen, ELISA, IFAT, Sabin-Feldman dye test IgM-ISAGA.
- **Treatment:** Congenital infection is treated with pyrimethamine and sulfadiazine. For primary prophylaxis Trimethoprim-sulfamethoxazole is the drug of choice




***Flow chart 1.8. Laboratory diagnosis of T.gondii***

## 4.20.8. Isospora

The causative agent of human isosporiosis is *Isospora belli*. After peroral ingestion of sporulated oocysts and release of sporozoites, further development (schizogony, gamogony) takes place in the epithelium of the upper small intestine, leading finally to oocyst formation. In AIDS patients, encysted sporozoites have been found in various extraintestinal organs (lymph nodes, liver, gallbladder, spleen).

*I. belli* can cause severe clinical symptoms, especially in AIDS patients, for example persistent diarrhea, steatorrhea, cholecystitis, weight loss, and fever. Diagnosis is made by detection of unsporulated oocysts (20–30 µm long) in stool ( , ) or of developmental stages in intestinal biopsies. High-dosed cotrimoxazole is the recommended therapy.

**Key points of *Cryptosporidium parvum***

- Sexual and asexual cycle in single host.
- **Infective form:** Sporulated oocyst in food and water.
- **Clinical features:** Self limited diarrhea with abdominal pain in healthy persons. Chronic persistent watery diarrhea in immunocompromised hosts.
- **Diagnosis:** Demonstration of round oocyst in stool by direct microscopy, fluroscent microscopy, and modified acid-fast stain.
- **Treatment:** Supportive therapy with electrolytes and fluids and early antiretroviral therapy in AIDS patients.

## 4.20.9. Microspora

| Genus | Species | Habitat and infection caused |
|---|---|---|
| *Enterocytozoon* | *E. bieneusi* | Small intestine epithelium (leading to diarrhea, and wasting). Also found in biliary tract of patients with cholecystitis. Rarely spreads to respiratory epithelium |
| *Encephalitozoon* | *E. intestinalis* | Small intestine epithelium (causing diarrhea and wasting). Also causes sinusitis, cholangitis, and bronchiolitis |
| | *E. hellem* | Conjuctival and corneal epithelium (causing keratoconjuctivitis). Also causes sinusitis, respiratory tract disease, and disseminated infection |
| | *E. cuniculi* | Small intestine epithelium (causing diarrhea).<br>Corneal and conjuctival epithelium (causing keratoconjuctivitis). Rarely, may cause hepatitis and renal infection |
| *Pleistophora* | *P. ronneafier* | Skeletal muscle (causing myositis) |
| *Brachiola* | *B. vesicularum*<br>*B. connori* | Skeletal muscle (causing myositis)<br>Muscles (smooth and cardiac) |
| *Trachipleistophora* | *T. hominis*<br>*T. anthropophtheria* | Cornea and conjuctival epithelium (leading to keratoconjuctivitis). Also causes myositis<br>Brain |
| *Vittaforma* | *V. corneae* | Corneal stroma (causing stromal keratitis) |
| *Nosema* | *N. ocularum* | Corneal stroma (causing stromal keratitis) |
| *Microsporidium* | *M. ceylonensis*<br>*M. africanum* | Corneal stroma (causing stromal keratitis) |

| Self-check 4 | Multiple choice |
|---|---|

***Directions:*** choose the best answer for all the questions listed below. Use the Answer sheet provided in the next page:

1. One is **not true** characteristic of helminthes:
   A. Majority occur in parasitic forms or adults
   B. They are metazoans
   C. diagnosis is by detecting larva, eggs
   D. life cycle simple or complex
2. Which of the following is/are not class of helminthes?
   A. Cystodes
   B. Nematodes
   C. Trematodes
   D. None
3. The large intestine can be infected by adult stage of _____and _____.
   A. Trichuri trichuria
   B. Hook worm
   C. Ascaris lumbricoids
   D. Loa loa
4. For which of the following parasites the larval stage undergo heart lung migration.
   A. Trichuri trichuria
   B. Entribium vermicularis
   C. whip worm
   D. none
5. One of the following **doesn't** belong to geo helminthes:
   A. Ascaries lumbricoids
   B. Strongliodes stercoralis
   C. Hook worm
   D. Entribium vermicularis
6. Egg stage are extremely resistant to adverse environments and chemicals, and remain viable in soil and dust for up to 10 years for which nematode?
   A. Ascaries lumbricoids
   B. Strongliodes stercoralis
   C. Hook worm
   D. Entribium vermicularis
7. An infective stage for hook worm is;
   A. Rhabidity form larva
   B. Filariform larva
   C. embriyonated eggs
   D. adult
8. One of following is hematophagous:
   A. S.stercolaries
   B. A. lumbricoides
   C. T. trichuria
   D. pinworm
9. Egg stage **is not** a diagnostic stage for one of the following:
   A. *S.stercolaries*
   B. A. lumbricoides
   C. T. trichuria
   D. pinworm
10. Diagnostic stage for *S.stercolaries* is:
    A. Rhabidity form larva
    B. Filariform larva
    C. embriyonated eggs
    D. adult
11. Close relationship between two different organisms from which one benefits and the other neither benefits nor suffers.

    A. Commensalism B. Mutualism C. Parasitism D. Relationship

12. Any animal that carries a parasite that can cause infections in humans

    A. Intermediate host B. Definitive host C. Reservoir host D. None

13. Sources of Exposure to most intestinal parasitic Infections is though

A. Direct skin penetration B. Sexual intercourse C. Feco-oral route D. Blood transfusion

14. Vertical Direct Mode of Transmission of the parasite is from the mother to child through:

A. Direct skin penetration C. Congenital

B. Sexual intercourse D. Blood transfusion

15. Which of The following is **not** the reason to study Life cycles of Parasites?

A. Control D. Fundamental research

B. Treatment E. None

C. Epidemiology

16. Which of The following is not Parasite factors to cause the disease

A. Strain of the parasite to human host C. Metabolic processes of the parasite

B. Parasite load Site D. None

17. How do Parasites Cause Inquiry to their Host?

A. Competition for the host's nutrients C. Toxins and secretions

B. Destruction of host tissues andTissue changes D. ALL

18. Which of the following is the infective stage of plasmodium to human?

A. Gametocyte C. Merozoite

B. Sporozoite D. trophozoite

19. Which of the following an infective stage of plasmodium to female anopheles mosquito?

A. Gametocyte C. Merozoite

B. Sporozoite D. trophozoite

20. Which of the following are not the basics properties of protozoan?

A. Movement B. Feeding C. Reproduction D. None

21. Trophozoites stage of the protozoa is?

A. Changed to vegetative form C. Resístant form

B. Commonly the pathogenic form D. None

22. Cysts ingested by the definitive host has to be changed to vegetative form is ?

A. Excystation B. life cycle C. Encystation D. Resístant form

23. Which of the following are not properties of Amoebic Trophozoites State?

A. Non-Motile C. Multiplying form

B. Actively feeding D. susceptible to destruction

24. Is the trophozoite which emerges from the cyst?

A. Encystation B. Excystation C. Metacyst D. Pre-Cyst

25. Finger like structure used for amoeboid motility in fresh warm stool specimen is ?

A. Cilia B. Flagella C. Pseudopodia D. Gilding

26. Transmission of Entamoeba histolytica is by Ingestion of -

A. Cyst from B. immature cyst from C. mature cyst from D. None

27. The Most common form of extra intestinal amebiasis Primarily infects….

A. Kidney B. Liver C. Lung D. Spleen

28. The infective stage of schistosomia is:

A. Cercaria C. rabidity form larva

B. Filariform larva D. eggs

29. A definitive host for plasmodium is

A. *Human* C. tsetse fly

B. *Female anopheles mosquito* D. sand fly

30. Which of the following exists only in trophozoite form?

A. *G. lambalia* C. *T. vaginalis*

B. *Leshmania* D. plasmodium

31. Which of the following is a diagnostic stage for the leshmania?

A. Promostigote C. epimastigote

B. Amastigote D. metamastigote

32. Which of the following stage doesn't exist for trypanosomes?

A. Promostigote C. epimastigote

B. Amastigote D. metamastigote

33. The most prevalent form of malaria in Ethiopia is caused by

A. P. vivax

B. P. malariae

C. P. ovale

D. P. falciparum

34. The most common prevention and control method of vector borne disease is/are:

A. Treating infected individuals C. vector control

B. Health education D. all

35. "Falling leaf" motility in fresh warm stool specimen is Trophozoite stage of?

A. *Giardia lamblia* *C.Trichomonas hominis*

B. *Enteromonas hominis* D. *Trichomonas tenax*

36. Which of the following are TRUE about *Giardia* transmition is?

A. ingestion of fecally contaminated food or water with cyst
B. Person to person
C. sexually
D. All
E. None

37. Which of the following is TRUE about susceptiblelity of *Giardia* Cysts are susceptible to…

A. 1% sodium hypochlorite C. ammonium disinfectants
B. 2% glutaraldehyde or quaternary D. boiling water

38. Which of the following is associated with the more chronic stage of*Giardia?*

A. Vitamin B12 malabsorption
B. Disaccharidase deficiency
C. Lactose intolerance
D. All

*39.* Which of the following is drug used for the Treatment *Giardia* is?

A. Mebindalzole B. Metronidazole C. Metazole D. ALL

40. *Trichomonas vaginalis* trophozoite stage transmitted by Which of the following ways:

A. sexual intercourse D. mother to child during birth
B. communal bathing, sharing of wash clothes E. ALL
C. toilet equipment seats

41. Common mode of transmission of *Leishmaniasis* is byBite of infected female:

A. Mosquito B. Tsetse fly C. Sand fly D. Bean E. None

42. Which of the following is the diagnostic stage of trypanosomesis?

A. Promastigotes D. Trypomastigote
B. Amastigotes E. Metacyclic Trypomastigote
C. Epimastigote

43. Which of the following drugs are used for the Treatment of *Leishmaniasis?*

A. Sodium stibogluconate (Pentostam) D. cryotherapy and thermotherapy
B. Pentamidine isethionate E. ALL
C. amphotericin B

44. Common mode of transmission of African Trypanosomiasis is byBite of infected …
A. Mosquito B. Tsetse fly C. Bug D. Sand fly E. None

45. Common mode of transmission of American Trypanosomiasis is byBite of infected…

A. Mosquito B. Tsetse fly C. Bug D. Sand fly E. None

46. The developemental form of trypanosomiasis which is found in blood vessels & intercellular spaces of Lymph nodes, spleen, liver, Brain, CSF of vertebrate host is?
    A. Promastigotes
    B. Amastigotes
    C. Epimastigote
    D. Trypomastigote
    E. Metacyclic Trypomastigote
47. The developemental form of trypanosomiasis which is found in the mid and fore gut of the tsetse flies is?
    A. Promastigotes
    B. Procyclic trypomastigotes
    C. Amastigotes
    D. Epimastigote
    E. Metacyclic Trypomastigote
48. The developemental form of trypanosomiasis which is seen in the salivary gland of tsetse flies is?
    A. Procyclic trypomastigotes
    B. Promastigotes
    C. Epimastigote & Metacyclic trypomastigotes
    D. Amastigotes
49. During a blood meal on the mammalian host, an infected tsetse fly (genus Glossina) injects which stage of trypanosomiainto skin tissue is?
    A. metacyclic trypomastigotes
    B. amastigotes
    C. Epimastigote
    D. Promastigotes
50. The tsetse fly becomes infected with blood stream, when taking a blood meal on an infected mammalian host the stage of trypanosomia is?
    A. Trypomastigotes B. Amastigotes C. Epimastigote D. Promastigotes

***Note:*** **Satisfactory rating - 25 points** **Unsatisfactory - below 25 points**
You can ask you teacher for the copy of the correct answers

Score = _____
Rating: _____

| Instruction Sheet | LG44:Process samples and associated request details |
|---|---|

This learning guide is developed to provide you the necessary information regarding the following content coverage and topics –

**Process samples for microscopic identification of parasites**

2. Parasitological Sample Collection and Processing
    - 2.1. Checking of request papers and samples
    - 2.2. Sorting of specimens according to its urgency
    - 2.3. Acceptance and rejection criteria
    - 2.4. Sample log and labeling
    - 2.5. Processing of samples
    - 2.6. Storage of sample and its components

This guide will also assist you to attain the learning outcome stated in the cover page. Specifically, upon completion of this Learning Guide, you will be able to –

- Check details of request form and sample before they accepted
- Sort specimens according to tests requested
- Comply reasons to rejected samples and request forms to their sources with reasons for rejection
- Log accepted samples and request forms to easily tracking mechanisms
- Process samples for testing
- Store samples appropriately for testing.

**Learning Instructions:**

1. Read the specific objectives of this Learning Guide.
2. Follow the instructions described in number 3 to 19.
3. Read the information written in the “Information Sheets 1”. Try to understand what are being discussed. Ask your trainer for assistance if you have hard time understanding them.
4. Accomplish the “Self-check 1” in page 7.
5. Ask from your trainer the key to correction (key answers) or you can request your trainer to correct your work. (You are to get the key answer only after you finished answering the Self-check 1).
6. If you earned a satisfactory evaluation proceed to “Information Sheet 2”. However, if your rating is unsatisfactory, see your trainer for further instructions..
7. Submit your accomplished Self-check. This will form part of your training portfolio.

| Information Sheet-1 | Parasitological Sample Collection and Processing |
|---|---|

## 2. Parasitological Sample Collection and Processing

### Introduction

Parasite disease continue to be a significant threat throughout the word. These disease are usually brought about by climate conditions desirable for parasitic survival as well as poor sanitation and personal hygiene practices of the inhabitants. Certain population are more at risk of contracting parasitic infections, including forging visitors and those traveling and emigrating to other countries. Successful laboratory identification of parasite requires the knowledge and practice of laboratory testing in the pre-analytic, analytic and post analytic steps. For example, in pre-analytic phase, a specimen received in the laboratory that is compromised because of improper collection, labeling or transport should be rejected and anew specimen requested. Similarly laboratory techniques performed in the analytic phase of testing of these samples should be completed with care to ensure that the accurate results are obtained. Interpretation and reporting of the results obtained, completed in the post analytic phase of the testing, should be accurately reported in the timely manner.

- **Definitions Specimen:**A piece or portion of a sample selected for examination. The specimen may, or may not be representative, whereas the sample may have been selected to be representative.

- **Stool specimen**

  Adult healthy human defecate three times a day. The common pattern is once a day. Stool tends to be soft and bulky on diet high in vegetables and small and dry on a diet high in meat. 2/3 of stool is water.

- **Stool sample is composed of**
  - Waste residue of indigestible material in food
  - Bile pigment and salts
  - Intestinal secretions, including mucous
  - Leucocytes that migrate from the blood stream.
  - shed epithelial cells
  - Large number of bacteria (1/3 of total solid)
  - Inorganic material chiefly calcium and phosphate
  - Digested food present in very small quantities.

- **Blood specimen**

Systemic or blood-borne parasitic infections are diagnosed by demonstrating the diagnostic stage(s) of the responsible parasite(s) in a blood specimen. The proper collection and handling of blood specimens is essential to obtain adequate smears for examination. There are some parasites that can be detected by observing motility in a wet preparation of a fresh blood sample under low- and high-power magnification. Blood smears can be prepared from fresh whole blood without anticoagulant (fingertip or earlobe) or from venipuncture collection with anticoagulant.

- **Cerebrospinal Fluid and Other Sterile Fluids**

Cerebrospinal fluid (CSF) specimens may be collected for the diagnosis of amebic conditions as well as African sleeping sickness. The CSF must be examined promptly to detect the motility of these parasites. Special stains can also be performed on CSF including Giemsa, trichrome, and modified trichrome stains. The specimen can be cultured on non-nutrient agar seeded with Escherichia coli. The CSF sediment is inoculated to the medium, sealed, and incubated at 35' C. The plate is then examined for evidence of the amebae feeding on the bacteria. Other pathogens that might be recovered from the central nervous system include *Toxoplasma gondii*. Sterile fluids other than CSF include several specimen types, such as fluid present in cysts, aspirates, peritoneal fluid, pleural fluid, and bronchial washings. All these samples may be examined using wet preps and/or permanent stains.

- **Tissue and Biopsy Specimens**

Tissue and biopsy specimens are recommended for the recovery of a number of parasites, including intracellular organisms such as Leishmania spp. and T. gondii. Surgical removal of the specimen followed by the preparation of histologic tissue sections and impression smears is the preferred method for handling these Samples. Hepatic abscess material is the specimen of choice for patients suspected of liver abscesses caused by E. histolytica.

- **Sputum**

Sputum is typically collected and tested from patients suspected of being infected by the lung fluke. Other parasitic infections that may be found in sputum samples include E. histolytica, Entdmoebagingiualis, Ascaris lumbricoides, and hookworm. An early-morning specimen is best and should be collected into a wide mouthed container with a screw cap lid. Saliva should not be mixed with the specimen. The

sample may then be examined directly via wet preps. Microscopic examination of the sediment can include wet preps and permanent stains.

- **Urine and Genital Secretions**

  Urine is the specimen of choice for the detection of Schistosoma haematobium eggs and may also yield *Trichomonas vaginalis*. The specimen should be collected into a clean container with a watertight Lid. The sample should be centrifuged on arrival at the laboratory. Microscopic examination of the sediment should reveal the parasites' if they are present. Vaginal and urethral specimens, as well as prostatic secretions, are typically collected and examined for the presence of T. vaginals trophozoites. These specimens may be collected on a swab or in a collection cup equipped with a lid. Saline wet preparations are the method of choice for demonstrating the motile trophozoites. Culture methods are available, including a commercial product that uses a culture pouch. All these methods are highly successful for diagnosing this sexually transmitted parasite.

- **Skin Snips**

  Skin snips may be made using one of two collection techniques. The objective of both procedures is to obtain skin fluid without bleeding. One of the methods involves making a firm (scleral) punch into skin with a specially designed tool. The other technique uses a razor blade with which a small cut into the skin is made. The resulting material obtained by both techniques may then be placed in approximately 0.2 mL of saline. After a 30-minute incubation period, the sample may be microscopically examined.

| Self-check 1 | Written test |
|---|---|

**Write True if the statement is correct and False if it is incorrect**

1. Specimen is the representative of the whole, but sometimes it may not represent the whole. (2 points)
2. Skin snip is a specimen that has to be taken without fluid. (2 points)
3. *Trichomonas vaginalis*is a protozoa that diagnosed by taking stool specimen.( 2 points)
4. Pre analytical phase the most important in clinical laboratory.(2 points)
5. Blood specimen is collected in the lab for diagnosing tissue parasites. (2 points)

**Note: satisfactory rating is 5 points, unsatisfactory <5 points. You can ask your instructor for copy of correct answer.**

**Answer Sheet**

**1.**_____

**2.** _____

**3.** _____

Score = _____

Rating: _____

Name: _____ Date: _____

## 2.1. Checking of request papers and samples

**Pre-analytical variables** refers to any and all procedures that occur during sample collection, prior to sample analysis. This involves patient identification, physical sample collection, sample transportation to the testing site and sample preparation. Patient samples are sometimes collected by the patient themselves, for example, faecal parasitology samples. It is important that the laboratories have set protocols to ensure that appropriate collection kits with instructions for collection, safety precautions, and labeling are available for their patients. It is suggested that instructions for the patients be in the languages for the community the laboratory is serving or presented as simple easy-to-understand graphics.

Proper patient identification is mandatory to produce quality test result in the laboratory. Some of the pre-analytical activities in the lab are the following.

- **Patient preparation:** Some tests require that the patient be fasting. There may also be special timing issues for tests such as blood glucose, drug levels, and hormone tests. The client from whom sample is to be taken has right to know the type of the sample to be collected, the reasons why we collect the sample and the procedure applied to collect the sample.
- **Patient identification:** we have to properly check whether we have collected a sample from appropriate patient and the request paper and sample must be labeled correctly with some informations such as; name of the patient, age, sex, ward address of the patient, required test. The person collecting the sample must accurately identify the patient. This might be done by questioning the patient, by questioning an accompanying family member, or by the use of an identifying wrist band or other device.
- **Sample collection:** appropriate procedures must be applied to collect the sample. Some of the important informations to be considered here are:
    - Specimen container
    - Volume of the specimen
    - Time of collection
    - Type of anticoagulants for blood specimens
    - Preservatives to be considered etc.…
- **Sample transportation:** specimens can be transported to reference laboratories for more specialized tests or for quality control purpose. Here proper labeling, packing,

and correct preservative selection are mandatory. Generally the pre-analytical phase is the phase where the laboratory has no direct control on the process. Pre-analytical factors that can affect results include: sample type, sampling time, sample handling, patient's preparation and the nutritional status of the patient.

| Self-check 2 | Written test |
|---|---|

**Choose the best answer from give alternatives**

1. One of the following is **Not** pre analytical activity in the laboratory?
   A. Patient identification
   B. Patient preparation
   C. Sample collection
   D. Sample processing
2. One of the following is **Not**informations to be considered during specimen collection
   A. Specimen container
   B. Volume of the specimen
   C. Time of collection
   D. Reporting result

**Note: satisfactory rating is 2points, unsatisfactory 1 points. You can ask your instructor for copy of correct answer.**

**Answer Sheet**

**1.**_____

**2.** _____

Score = _____

Rating: _____

Name: _____ Date: _____

## 2.2. Sorting of specimens according to its urgency

Once a sample enters the laboratory, there are a number of steps needed prior to testing. These pre-examination steps include:

- Verifying the sample is properly labelled, adequate in quantity, in good condition, and appropriate for the test requested. The test request must be complete and include all necessary information;
- Recording sample information into a register or log;
- Enforcing procedures for handling sub-optimum samples, including sample rejection, when necessary.

The objectives of the sorting concepts are to monitor and control the sample flow regarding the whole laboratory cycle.

The advantages of sorting sample are:

- Control and monitor sample material from delivery to disposal
- Documentation of know-how and regulations in the system
- Flexible assignment of the expert staff and quick integration of new employees.
- Defined & structured process
- Continuous sample cycle time
- Continuity regarding sample flow and capacity utilization by recursive sample sorting and defined buffer zones (to smooth peaks)
- Preparation of sample material for automation
- Automated handling of standard samples
- Sorting of special material on manual work places
- Programming of special rules by the maintenance personnel
- Special workflows can be configured
- Daily analysis of the order data

Collection of sufficient quantity is important to permit detection of organisms and to prevent rapid drying. The stool specimen should contain at least 4 ml.

Process & examine stool specimen immediately after collection, if not, preserve stool specimen.

- Liquid stool: < 30 minutes of passage at Room Temperature.
- Semi-formed stool: < 1 hour of passage at Room Temperature.

- Formed stool: < 24 hours of passage, 4 at $^{0}C$.

Collect approximately 100g of faeces in a clean, dry container without preservatives for parasitological examination. A screw-top container labeled with full information identifies the patient is most suitable. Make sure that any adult worms or segments passed are included. For collection of stool specimens for bacteriological examination (e.g. for culture of cholera and other bacteria that cause dysentery).

| Self-check 3 | Written test |
|---|---|

Answer the following question

8. List advantages of sorting laboratory specimen. (7 points)

**Note: satisfactory rating is 3.5 points, unsatisfactory <3.5 points. You can ask your instructor for copy of correct answer.**

Score = _____

Rating: _____

**Answer Sheet**

**1.**_____

_____

_____

_____

_____

_____

Name: _____ Date: _____

## 2.3. Acceptance and rejection criteria

The laboratory should establish rejection criteria and follow them closely. It is sometimes difficult to reject a sample, but remember that a poor sample will not allow for accurate results. It is the responsibility of the laboratory to enforce its policies on sample rejection so that patient care is not compromised. Management should regularly review the number of rejected samples and reasons for rejections, conduct training on sample collection, and revise written procedures for sample management as needed.

The following are examples of samples that should be rejected:

- unlabeled sample;
- stool contaminated with urine during collection
- broken or leaking tube/container;
- insufficient patient information;
- sample label and patient name on the test request form do not match;
- If it is transported sample collected in wrong tube/container; for example, using the wrong preservative
- Inadequate volume for the quantity of preservative;
- insufficient quantity for the test requested;
- Prolonged transport time, or other poor handling during transport.

***N.B. Record the reason for rejection in the log book and include all pertinent information***.

| Self-check 4 | Written test |
|---|---|

Answer the following questions

1. List the characteristics of laboratory specimen that should be rejected. (9 points)

**Note: satisfactory rating is 4.5 points, unsatisfactory <4.5 points. You can ask your instructor for copy of correct answer.**

Score = _____

Rating: _____

## 2.4. Sample log and labeling

The label must contain the following legible information**:**

- Patient name.
- Patient medical record number,
- Patient location.
- Collection date and time.
- Specimen type and/or source.
- The initials of the person collecting the sample.
- Test required (note any special handling required)
- Ordering physician.

Potential outcomes of collection and labeling errors:

- delays in reporting test results
- unnecessary re-draws/re-tests
- decreased customer satisfaction
- increased costs
- incorrect diagnosis / treatment
- injury
- Death.

**During Labeling:**

- Make sure that container label & the requisition match.
- Label should be on the container not on the lid, since the lid can be mistakenly placed on a different container.
- Ensure the labels on the containers are adherent under refrigerated conditions.


***Fig, 2.1. Labeling specimen***

The laboratory should keep a register (log) of all incoming samples. A master register may be kept, or each specialty laboratory may keep its own sample register.

Assign the sample a laboratory identification number – write the number on the sample and the requisition form. If computers are used for reports, enter the information into the computer.

The register should include:

- date and time of collection;
- date and time the sample was received in laboratory;
- sample type;
- patient name and demographics, as required;
- laboratory assigned identification (e.g., number 276_01_06_2009);
- tests to be performed

The laboratory needs a system to allow for tracking a sample throughout the laboratory from the time it is received until results are reported.

This can be done manually by careful keeping of records.

- confirm receipt of samples, include date and time;
- label samples appropriately; keep with the test requisition until laboratory ID is assigned;
- Track aliquots–traceable to the original sample**.**

If computers are available, maintain a database for tracking. The following information about each sample should be entered into the database:

- identification number;
- patient information;
- collection date and time;
- type of sample: for example, urine, throat, cerebrospinal fluid for culture;
- tests to be performed;
- name of ordering physician (or other health care provider);
- location of patient, such as ward, clinic, outpatient;
- diagnostic test results;
- Time and date results are reported.

**Table 2.1. Sample parasitology log book**

| Date | Specimen no. | Patient | Sent by | Specimen provided | Examination requested | Results | Results sent (date) |
|---|---|---|---|---|---|---|---|
| 2.1.01 | 1 | Mr F | Dr A | Stool | Intestinal parasites | Direct microscopy: moderate no. of *Ascaris lumbricoides* ova seen | 2.1.01 |
| 2.1.01 | 2 | Ms M | Dr C | Stool | Intestinal parasites | Direct microscopy: no ova or parasites seen<br>Concentration technique: no ova or parasites seen | 2.1.01 |
| 2.1.01 | 3 | Mrs L | Medical ward 1 | Skin snips | Onchocerciasis | No parasites seen | 3.1.01 |
| 3.1.01 | 4 | Mr S | Dr R | Stool | Parasites | Occult blood: positive<br>Direct microscopy: many trophozoites of *Entamoeba histolytica* and a few hookworm ova seen | 3.1.01 |

23

| Self-check 5 | Written test |
|---|---|

Answer the following questions

1. What are the informations that should be included during specimen labeling? (8 points)

**Note: satisfactory rating is 4 points, unsatisfactory <4 points. You can ask your instructor for copy of correct answer.**

Score = _____

Rating: _____

Name: _____ Date: _____

## 2.5. Processing of samples

2.5.1. **Stool sample processing :** Collect about 4 gm of fresh faces uncontaminated by urine or soil using wooden applicator stick or spatula to a container

A. The Container should be

- Wide necked
- Screw top
- Free from antiseptics
- Preferably, this is incinerated after use.

B. Label all the specimen with

- Patients name
- Date of collection
- Time of collection
- Ward number

C. Sample from patient with a confirmed or suspected diagnosis of certain infections disease such as cholera, shigella should be labeled “Risk of infection biohazard”

D. If delay is unavoidable use preservative. Note that stool sample should be

- Faces should be urine free
- Collect before antimicrobial agents are administered
- Examine diarrhea stool soon.
- Do not refrigerate stool specimen

❖ **Macroscopic examination stool specimen**

Is the primary step in the diagnosis of fresh stool specimen for parasitic infection and includes:

A. Consistency
B. Composition
C. Color and presence of adult parasites should be reported

**A. *Consistency***

The presence of protozoan trophozoites in the stools will depend on the consistency and passage rate of the faces.

i. Formed **(**normal shape)

- Water content is absorbed
- Few trophozoites principally the cyst stage protozoa is common

ii. Semi formed or unformed soft stool with no regular shape as in diarrheal

iii. Watery /liquid – contain and any flakes of mucous and blood

**B. *Composition***

The stool may contain blood mucus or pus, which is the evidence of ulceration due to pathogenic parasites.

**C. Color**

Pale yellowish stools are passed in statorrhoeaic conditions such as giardiasis.

**D. Adult parasites**

Faces may contain adult helminthes or segments present such as *A. lumbricoides*, *Entrobium vermicularis* or gravid segments of *Tenia species*.

Dysenteric and watery specimens must reach the laboratory as soon as possible after being passed (within 15 minute) otherwise motile parasites such as E.*histollytica&G.lambalia* may not be detected. Other specimen should reach the laboratory within 1 hour of being collected.

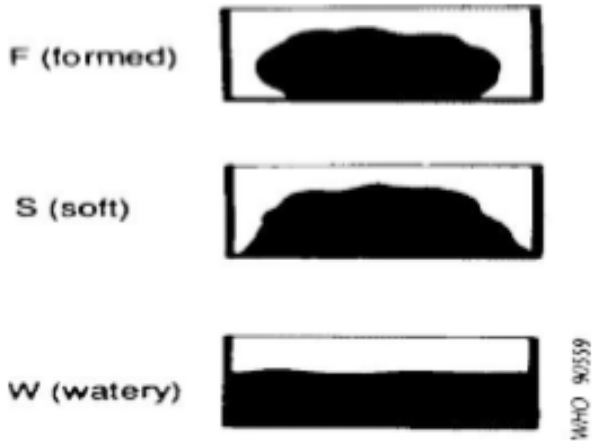



***Fig 2.2 consistency of stool sample***

❖ **Microscopic examination of faces**

- Necessary materials and reagents
- Gloves
- Normal saline
- Applicator stick
- Cover glass
- Slide

Microscopic examination of stool is used to observe cellular exudates and motile protozoan trophozoites, which are not easily seen by our naked eye.

❖ **<u>Concentration technique for fecal parasite</u>**

## ***<u>The two concentration techniques are</u>***

✓ Floatation concentration technique

✓ Sedimentation concentration technique

➢ ***Floatation Concentration Technique***

Uses high specific gravity of a solution to float the lighter ova and cyst

▪ **Zinc sulphate technique /Znso$_4$/**

- Is recommended for concentrating cysts of *Gardia lamblia*, *E. histolytica* and egg of *Trichuris trichuria*.
- Other nematode eggs are concentrated less well, while operculated trematodes egg are not concentrated because they are ruptured in the zinc sulphate solution.
- The technique is not suitable for concentrating egg or cysts in fatty faces.
- A zinc sulphate solution is used which has a specific gravity of 1.180 – 1.200: faces are emulsified in the solution and the suspension is left undisturbed for the eggs and cysts to float to the surface where they are collected on a cover glass.

**Required materials and reagents**

- Zinc sulphate solution 33% W/V specific gravity (1.80 – 1.200)
- Test tube of about 15 ml capacities, which has a completely smooth rim.

### 2.5.2. Blood sample processing

- Blood is the only fluid tissue that constitutes 6 – 8% of the total body weight and consists of cells suspended in fluid plasma the three types of blood cells are
  - Platelets ( thrombocytes)
  - White blood cells (leucocytes)
  - Red blood cells (erythrocytes)
- The fluid portion (plasma) forms 45 – 60% of the total volume of blood.
  - Red cell possess (occupy) most of the remaining volume.
  - Platelate and WBC occupy relatively small proportion of the blood mass.
- Obtaining blood from a finger (capillary blood)

  Some tests may be performed with a few drops of blood other test require several cubic centimeters of blood. When test call for a few drops of blood. The blood is obtained from a finger or lobe of the ear for blood film preparations.
- There are two types of blood films
  - Thick blood film
  - Thin blood film

- **Thick blood film preparation:** First step is identifying the patient and label the slide with request paper, prepare the blood film fig

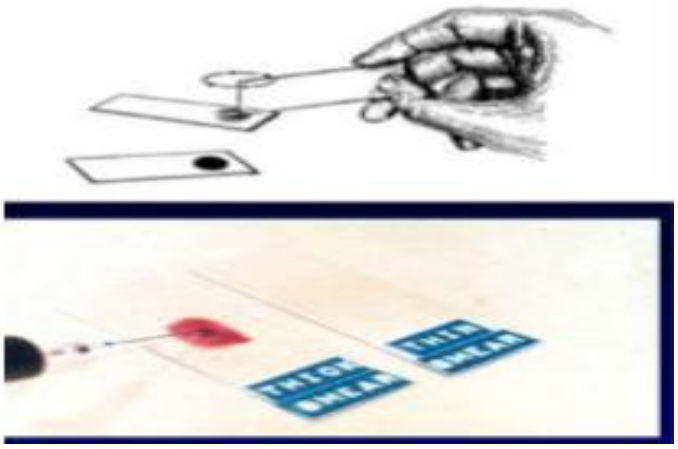



Fig 2.3. ***Preparation of thick blood film***

- **Preparation of Thin Films:** First step is identifying the patient and label the slide with request paper, prepare thin film as fig (2.4).

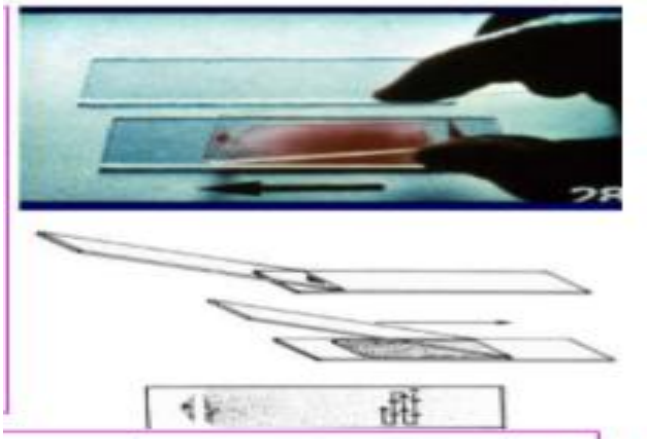


Fig. 2.4. ***Preparation of thin films***

- **Spreading a thin blood film:** This takes practice. A well-made thin blood film should have a smooth tail end (not ragged) and be free of vertical lines and ‘holes’. A poorly spread film is extremely difficult to report because the red cells, parasites, and white cells will appear distorted. It is essential to use a spreader which has a smooth ground glass polished edge.
- **Drying thick blood films:** It is good practice to keep a separate box or deep tray for drying malaria blood films. Cover it with a lid made from netting to protect the films from insects and dust (flies will rapidly ‘clean’ blood from a slide). If the box or tray is placed in a warm sunny place, the thick film will dry quickly (do not allow the blood to remain in the sun after it has dried). In humid climates, it may be necessary to use a hand dryer or an incubator to dry thick blood films.

- **Fixing thin blood films**: Use absolute methanol (methyl alcohol) or when not available, ethanol (ethyl alcohol) to fix thin blood films. The alcohol must be free from water otherwise it will not fix the cells properly. Always make sure the stock bottle of alcohol is kept tightly stoppered. For routine use, keep a small amount of alcohol in a dispensing bottle, which can be closed in between use.

| Advantage of thin blood film | Advantage of thick blood film |
|---|---|
| ➢ Required to confirm the *plasmodium* species | ➢ Good for rapid detection malaria parasites , particularly when they are few *P. malariae* parasitaemia is normally low |
| ➢ Enabling the parasites to be seen in the red cells | ➢ About 30 times more sensitive (detecting about 20 parasites/µl) |
| ➢ Greatly assists in the identification of mixed infection | ➢ In a thick film the blood is not fixed |
| ➢ Value in assessing whether a patient with falciparum malaria is responding to treatment | |
| ➢ Gives the opportunity to investigate **anemia** and **white cell abnormalities** | |

- **Staining malaria parasites:** Malaria parasites in thick and thin blood films require staining at pH 7.1–7.2 using a Romanowsky stain (contains azure dyes and eosin).
    - The stains most frequently used in district laboratories are:
        - **Field's stain**: This water-based Romanowsky stain is composed of two solutions, Field's stain A and Field's stain B. It is buffered to the correct pH and neither solution requires dilution when staining thick films. When staining thin films, Field's stain B requires dilution. Compared with Giemsa working stain, Field's stains are more stable. They stain fresh blood films well, particularly thick films. The rapid technique is ideally suited for staining blood films from waiting outpatients and when reports are required urgently.
        - **Giemsa stain:** This is an alcohol-based Romanowsky stain that requires dilution in pH 7.1–7.2 buffered water before use. It gives the best staining of malaria parasites in thin films. It also stains thick films well providing they are completely dry (overnight drying is recommended), the concentration of stain is low, and the staining time is sufficiently long. Less satisfactory results are obtained when the concentration of Giemsa stain is greatly increased to reduce the staining time. Care must be taken to prevent water from entering the stock stain. Giemsa stain is commonly

used in malaria survey work because many films can be stained at one time and differentiation of the different species in thin films is excellent.

**Notes**: the mature gametocytes of Pv, Po and Pm are hard to distinguish from mature troph of these species.

Artefacts

Potential source:

Vegetable spores, yeast, pollen, algae and bacteria in the stain or on the slide

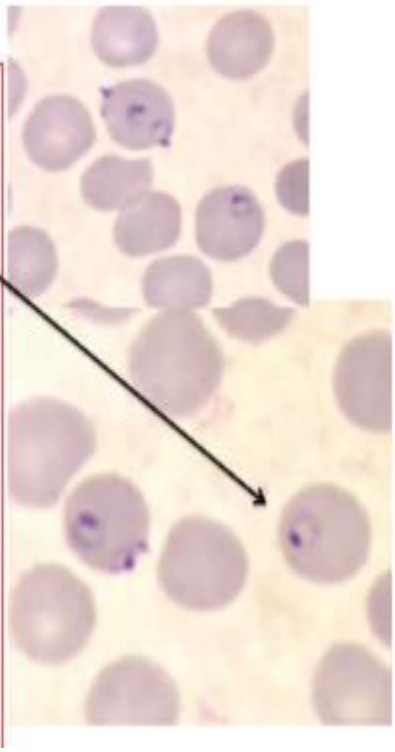
- Platelets
- Howell-jolly bodies in anaemic patients
- Ghosts of immature red cells mimicking schuffner's stippling

Examination

***P. falciparem***

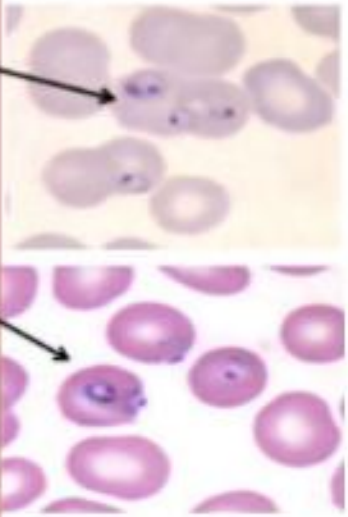
**Young Trophozoite (Ring forms)**

- Stage frequently found in blood film
- **Size**: Very delicate, 0.15-0.5 diameters of RBCs which is unaltered in size.
- **Shape**: small fine pale blue ring
- **Chromatin:** 1 or 2 small red dots.
- Often with double chromatin dot
- May lie on red cell membrane **(accole forms)**
- **Pigment**: absent



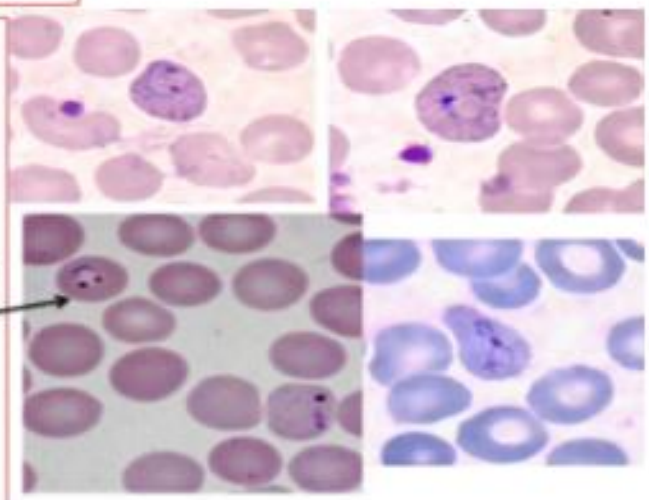
**Mature Trophozoite**

- Stage rarely seen in peripheral blood
- RBC unaltered in size, sometimes stippled, pale
- **Size**: small
- **Shape**: compact thin blue ring, comma or exclamation mark
- **Chromatin**: 1 or 2 red dots
- **Pigment**: black or dense brown mass
- Stippling Maurer's cleft



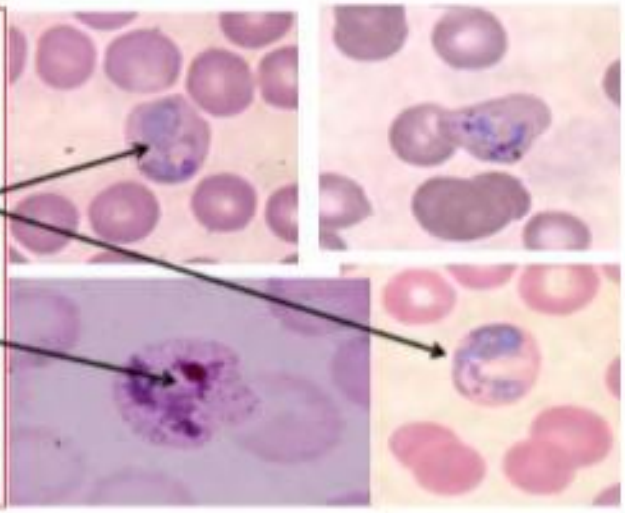
**Plasmodium vivax**

- **Young Trophozoites**
- Stage frequently seen
- Size : 0.3-0.5 diameter of RBC which is unaltered in size.
- Cytoplasm-irregular blue quite thick ring
- Chromatin : one rather large red dotted/ some times two
- Pigment: absent.
- RBCs are enlarged and distorted.
- Schüffner's dots may be seen



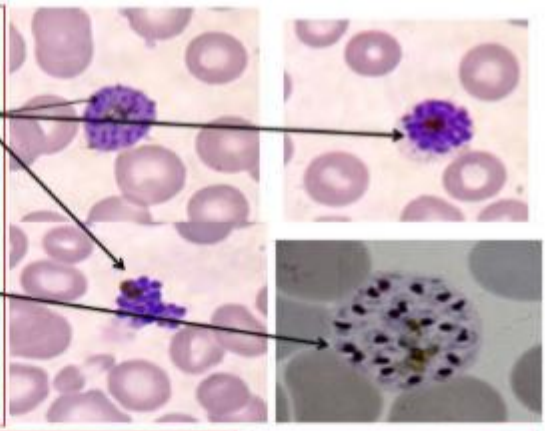
**Mature Trophozoite**

- Not frequently seen
- Size : large ( RBC enlarged, stippled)
- Parasite- large blue irregular/ amoeboid
- Chromatin: Dots or threads of red colour
- Pigment: Golden brown and scattered



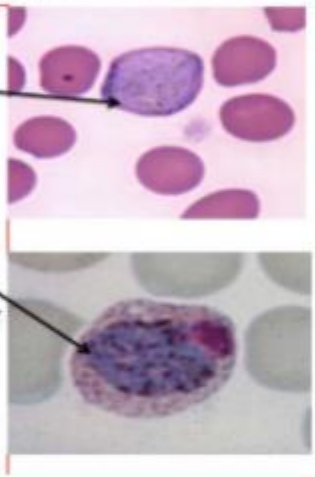
**Schizonts**

- quite frequently seen
- RBC much enlarged
- Size: Almost fills red blood cells
- Shape: amoeboid or segmented, parasite large, filling enlaged RBC
- Cytoplasm: pale blue
- Merozoites: 14-24; average 16
- Pigment: Golden brown central loose mass



**Gametocytes**

- Are **round to oval** with blue stain
- Scattered brown pigment
- Dense **red triangular nucleus** often at one end
- May almost **fill the red blood** cell (RBC).
- RBCs are enlarged and may be distorted.
- Under optimal conditions, **schüffner's dots** may appear more fine than those seen in *p. Ovale*.
- Difficult to differentiate from late trophozoites



***Plasmodium malariae***

***Young Trophozoite***

**Size**: Up to 1/3 Red blood cell.

**Cytoplasm**:thicker & dense (compact) blue ring

**Chromatin**: one large red dot

**Pigment**: *absent*

***Mature Trophozoite***

RBC unaltered
Parasite- compact often band or rounded s
Chromatin: round do or red band
Pigment: dark brown or black pigment, often concentrated along one edge of the band
Band forms can be seen in thin films
Occasionally "birds-eye" ring form may be seen 8-10 merozoites in mature schizont
- 'rosette'

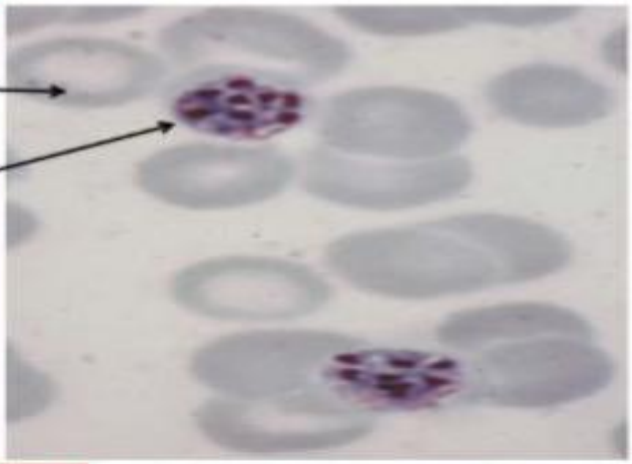
**Schizont**

RBC unaltered

**Size**: small compact nearly fills red cells

**Merozoites** 6-12; average 8; sometimes forming rosette

**Pigment**: Brown aggregated



**Gametocytes**

**Shape**- large, oval/round

Nucleus-1 round red chromatin at one edge

**Pigment**-black brown course

RBC unaltered, parasite small round filling RBC

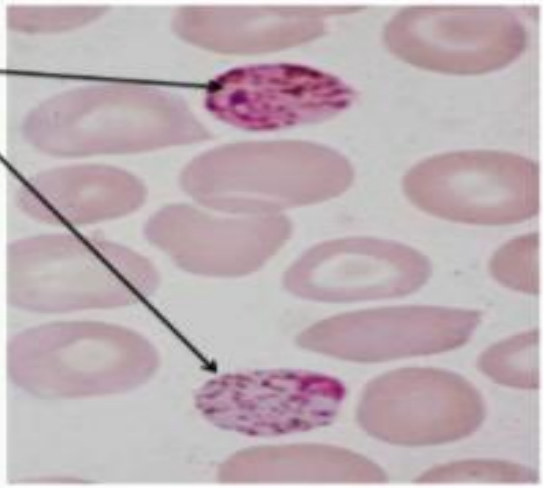
**Infected Red Blood Cells**

Oldest erythrocytes are infected

***Stippling***- None (**Ziemanns dots** often after prolonged leishmania staining

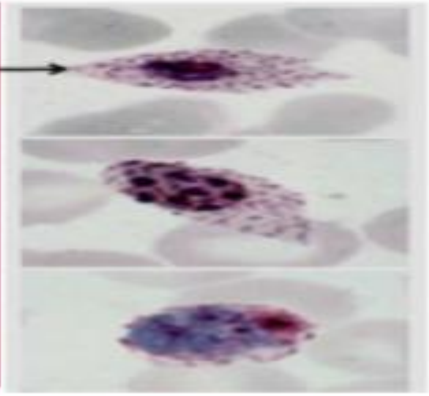
*Parasite Density*: low density,

Rarely more than 1% of cells infected (easily missed in Laboratory diagnosis



***P. ovale***

- Red cells enlarged.
- Comet forms common (top right
  - Schüffner's dots
  - Rings large and coarse.
- similar to *P. vivax*
- subtle differences
  - 'compact' trophozoite
  - fewer merozoites
  - elongated erythrocyte
- Mature schizonts similar to those of P. *malariae* but larger and more coarse



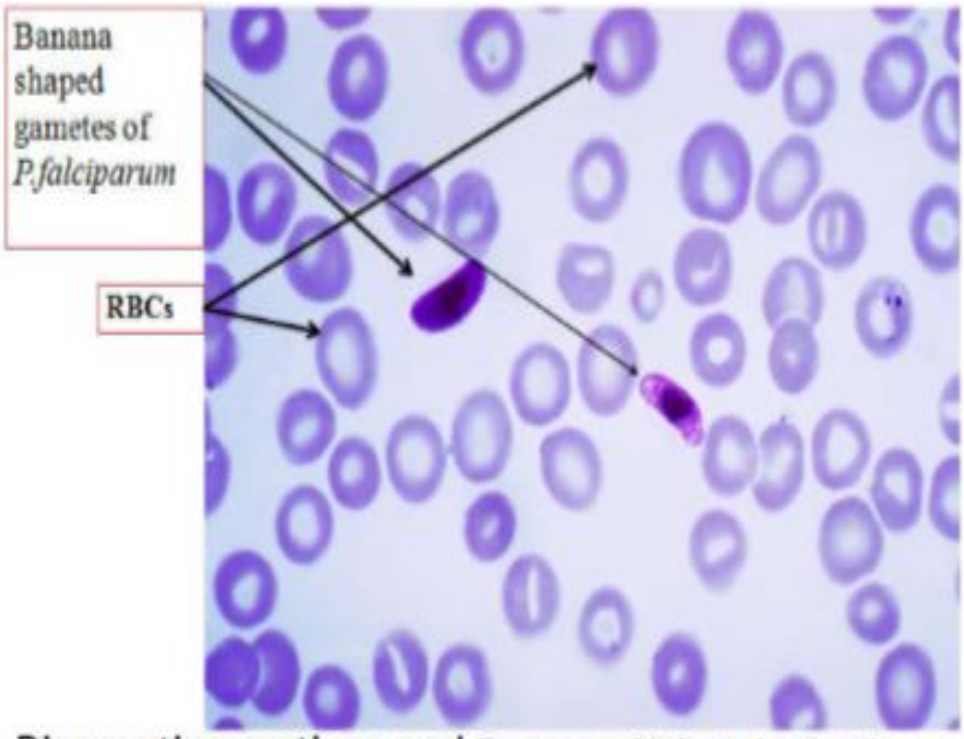
**Microscopic differentiation**

- Microscopic differentiation of species depend on Host cell and Parasite characteristics

1. Feature of infected red cell and ghosts
   - change in size, shape and colour
   - Presence of dots, maurer's clefts (not on ghost cell) on infected red cell
   - Single or multiple infection of each cell
2. Parasite morphology at specific stages
   - Number and size of chromatin beads
   - Shape and size of cytoplasm
   - Degree of pigmentation within cytoplas:
   - Stages of parasite seen together

**Microscopic features of three blood stages**

- trophozoites, schizonts, gametocytes
  - All these three stages have Red nuclear chromatin, blue cytoplasm and pigment (except young troph)
- What are the key features of :
- trophozoites?
  - single (Sometimes double) chromatin bead
  - Cytoplasm as a ring uniform or fragmented mass
  - Pigment absent from young (ring) form




## Diagnostic questions and Features of infected red cells

- Are some infected RED CELLS enlarged?

  YES…..
- Marked enlargement….PV
- Moderate enlargement…PO

  NO……
- Infected red cell occasionally smaller than normal…..PM
- Infected red cell show no change in size…PF

- Are some infected RED CELLS changed in shape?
- YES…
- Angular distortion is observed…..PV
- Enlarged or oval with a ragged edge (fimbriation)…..PO
- Sometimes crenation ……Pf

- No change of shape in infected cell ….Pm
- Is there marked loss of colour?
- YES……
- Decolourization is characteristics of Pv and PO
- NO…..
- Retention of colour is characteristics of Pf and Pm

- Multiplicity of infection and stippling or cleft
- Are some red cells infected with more than a single parasite?
- YES: Pf
- NO: Pm and Po, Pv typically does
- Is there evidence of red cell dots?
- Yes: schuffner's ….PV and Po

➢Maurer's cleft……Pf

➢Notes: The red cell ghosts in thick films can show schuffner's dots but do not show maurer's clefts

➢NO:….Pm

➢Thus Microscopy for Diagnosis of malaria:

➢An established method for the laboratory confirmation of malaria.

➢The careful examination of well prepared and stianed blood film by expert microscopist remaines currently the "GOLD STANDARD" for detecting and identifying malaria parasites

**Advantages of using a microscope are:**

➢It is senstive, when used by skilled and careful microscopist

➢Can detect densities as low as 5-10 parasites per µl of blood(WHO,1990)

➢It is informative, when parasites are found they can be identified in terms of their

➢Parasite densities can be quantified(from ratio of the parasites per number of leukocytes or erythrocyte)

➢Relatively inexpensive

**Reporting blood films for malaria parasites**

Reporting thick blood films: plus sign scheme

***Parasites***

1 – 10 per 100 high power fields ..... +

11 -100 per 100 high power field..... ++

1 – 10 in every high power field..... +++

More than 10 in every high power field ..... ++++

**Example**: *P. falciparum* trophozoites +++, gametocytes +, with malaria pigment in white cells

If no parasites are found after examining 100 fields (or if indicated 200 fields), report the film as: Malaria thick film: NPF (No parasites found

## 2. Immunologic/Biochemical techniques-Rapid Diagnostic Tests-RDTs

**Immuno-chromatographic tests for malaria antigens**

- Are based on the capture of the parasite antigens from the peripheral blood
- Uses either monoclonal or polyclonal antibodies against the parasite anti targets.
- RDTs do not require a laboratory, electricity, or any special equipment.
- **Targets**
  1. Histidine-rich protein 2 of *P. Falciparum,*
  2. Pan-malarial *plasmodium* aldolase, and
  3. Parasite specific lactate dehydrogenase (pldh)

| Self-check 6 | Written test |
|---|---|

Answer the following questions

1. Macroscopic examination of stool specimen includes _____, _____and _____. ( 3 points)
2. Discuss stool wet mount technique with necessary reagents & materials needed. (3 points)
3. Discuss concentration technique with necessary reagents & materials needed. (6 points).

**Note: satisfactory rating is 6 points, unsatisfactory <6 points. You can ask your instructor for copy of correct answer.**

Score = _____

Rating: _____

## 2.6. Storage of sample and its components

**Sample storage:**

Written policies should be developed that include:

- description of what samples should be stored;
- retention time;
- location–consider ease of access;
- conditions for storage, such as atmospheric and temperature requirements;
- System for storage organization, one method being to store samples by day of receipt or accession number.

**Sample retention:**

Set a laboratory policy for retention of each type of sample. Some samples can be quickly discarded, and others may need to be retained for longer periods. Monitor stored samples, and do not keep for longer than necessary, as refrigerator and freezer space may be limited. Sample freeze/thaw cycles must be monitored, as samples may deteriorate with these conditions.

Planning is required for samples that may need long-term storage. An organized, accessible system using computer tracking would be useful for these samples. The inventory of stored samples should be reviewed at specified intervals to determine when they should be discarded.

**Sample referral:**

When referring samples to other laboratories for testing:

- obtain a laboratory handbook with detailed procedures from each laboratory;
- ensure the sample is labelled correctly, in the correct container, accompanied by a requisition form that specifies the required test(s), and includes the sending laboratory's contact information;
- carefully monitor samples that are referred:
  - keep a record of all tests / samples referred, date of referral, name of person referring the test;
  - record and report results received for each referred sample;
  - Monitor turnaround times and record any problems encountered.

**Sample disposal:**

The laboratory is responsible for ensuring that disposal of all laboratory waste is handled in a safe manner. To ensure proper disposal of patient samples:

- develop a policy for sample disposal; apply local, as well as country regulations for disposal of medical waste;
- Establish and follow procedures to disinfect samples prior to disposa

| Self-Check 7 | Written Test |
|---|---|

***Directions:*** Answer all the questions listed below. Use the Answer sheet provided in the next page:

1. Discuss sample storage. (3 points).

***Note:*** **Satisfactory rating 1.5 points** **Unsatisfactory - below 1.5 points**
You can ask you trainer for the copy of the correct answers.

Name: _____ Date: _____

| Operation Sheet 1 | Procedures preparing stool wet mount Direct saline and Iodine mounts: |
|---|---|

1. With a wax pencil write the patient's name or number and the date at the left – hand end of the slide
2. Place a drop of saline in the center of the left half of the slide and place a drop of Iodine solution in the center of the right half of the slide.

   ***NB: use warm saline ($37^0$) if the presence of amoebic trophozoite is suspected***
3. With an applicator stick, pick up a small portion of the specimen (size of a match head) and mix with the drop of saline
4. Similarly, pick up a small amount of the stool and mix with the drop of iodine, to prepare an iodine mount
5. Cover the drop of saline and the drop of iodine with a cover slip. Hold the cover-slip at an angle, touch the edge of the drop, and lower gently on to the slide. This will reduce the chance of including air bubbles in the mount
6. Observe the preparation under the microscope with the condenser lowered and the light intensity adjusted, So that the opaque structure of the trophozoit adjusted and cysts can be seen.
7. Scan the total area of the cover slip using the l0x objective initially, and 4ox objective as required. Take note of any trophozoite and their motility and any red blood cells ingested by amoebae. *Note also the presence of cysts, ova, red blood cells polymorph nuclear cells and macrophages.*

| Operation Sheet 2 | **Procedures preparing stool Sedimentation concentration techniquewith Formol ether concentration technique** |
|---|---|

***2.*** Using a rod or stick, emulsify an estimated 1g (pea-size) of faeces in about 4ml of 10% formol water contained in a screw-cap bottle or tube. ***Note: Include in the sample, faeces from the surface and several places in the specimen.***

3. Add a further 3–4ml of 10% v/v formol water, cap the bottle, and mix well by shaking.
4. sieve the emulsified faeces, collecting the sieved suspension in a beaker.
5. Transfer the suspension to a conical (centrifuge) tube made of strong glass, copolymer, or polypropylene. Add 3–4ml of diethyl ether or ethyl acetate. ***Caution: Ether is highly flammable and ethyl acetate is flammable, therefore use well away from an open flame, e.g. flame from the burner of a gas refrigerator, Bunsen burner, or spirit lamp. Ether vapour is anaesthetic, therefore make sure the laboratory is well-ventilated.***

***6.*** Stopper* the tube and mix for 1 minute. If using a Vortex mixer, leave the tube unstoppered and mix for about 15 seconds (it is best to use a boiling tube). ****Do not use a rubber bung or a cap with a rubber liner because ether attacks rubber.***

7. With a tissue or piece of cloth wrapped around the top of the tube, loosen the stopper (considerable pressure will have built up inside the tube).
8. Centrifuge immediately at 750–1000g (approx. 3000 rpm) for 1 minute.
9. Using a stick or the stem of a plastic bulb pipette, loosen the layer of faecal debris from the side of the tube and invert the tube to discard the ether, faecal debris, and formol water. The sediment will remain.
10. Return the tube to its upright position and allow the fluid from the side of the tube to drain to the bottom. Tap the bottom of the tube to resuspend and mix the sediment. Transfer the sediment to a slide, and cover with a cover glass.
11. Examine the preparation microscopically using the 10X objective with the condenser iris closed sufficiently to give good contrast. Use the 40X objective to examine small cysts and eggs. To assist in the identification of cysts, run a small drop of iodine under the cover glass . Although the motility of Strongyloides larvae will not be seen, the non-motile larvae can be easily recognized.
12. If required, count the number of each species of egg in the entire preparation. This will give the approximate number per gram of faeces.

| Operation Sheet 3 | Procedures preparing stool concentration techniquewith zinc sulphate flotation technique |
|---|---|

1. Fill the tube about one quarter full with the zinc sulphate solution. Add an estimated 1 gram of faeces (or 2ml if a fluid specimen). Using a rod or stick, emulsify the specimen in the solution.
2. Fill the tube with the zinc sulphate solution, and mix well. Strain the faecal suspension to remove large faecal particles.
3. Return the suspension to the tube. Stand the tube in a completely vertical position in a rack.
4. Using a plastic bulb pipette or Pasteur pipette, add further solution to ensure the tube is filled to the brim.
5. Carefully place a completely clean (grease-free) cover glass on top of the tube. Avoid trapping any air bubbles.
6. Leave undisturbed for 30–45 minutes to give time for the cysts and eggs to float. *Note: Do not leave longer because the cysts can become distorted and the eggs will begin to sink.*
7. Carefully lift the cover glass from the tube by a straight pull upwards. Place the cover glass face downwards on a slide. *Caution: Avoid contaminating the fingers. Mature E. histolytica and G. lamblia cysts are infective when passed in the faeces.*
8. Examine microscopically the entire preparation using the 10X objective with the condenser iris closed sufficiently to give good contrast. Use the 40X objective, and run a drop of iodine under the cover glass, to identify the cysts.
9. Count the number of T. trichiura eggs to give the approximate number per gram of faeces. *Note: Parasites can also be recovered from the surface of the floatation fluid after centrifuging. If however, a centrifuge is available, the safer formol ether technique is recommended for concentrating eggs and infective cysts from faecal specimens.*

| Operation Sheet 4 | Procedures for preparing Capillary blood method (thick and thin films on same slide) |
|---|---|

1. Cleanse the lobe of the finger (or heel if an infant) using a swab moistened with 70% v/v alcohol. Allow the area to dry.
2. Using a sterile lancet, prick the finger or heel. Squeeze gently to obtain a large drop of blood. Collect the blood preferably in a small plastic bulb pipette.
3. Using a completely clean grease-free microscope slide and preferably a malaria slide card, add a small drop of blood to the center of the slide and a larger drop about 15mm to the right
4. Immediately spread the thin film using a smooth edged slide spreader (see fig 2.3 &2.4). Blood from anaemicpatients needs spreading more quickly with the spreader held at a steeper angle.
5. Without delay, spread the large drop of blood to make the thick smear. Cover evenly an area about 15 x15mm (see Fig. 2.3). It should just be possible to see (but not read) newsprint through the film. When spreading the blood, mix it as little as possible to avoid the red cells forming marked rouleaux which can cause the blood to be easily washed from the slide during staining.
6. Using a black lead pencil, label the slide with the date and the patient's name and number. If a slide having a frosted end is not used, write the information neatly on the top of the thin film (after it has dried).
7. Allow the blood to air-dry with the slide in a horizontal position and placed in a safe place.

| Operation Sheet 5 | Procedures for staining thin films with Field's staining technique |
|---|---|

1. Place the slide on a staining rack and cover the methanol-fixed thin film with approximately 0.5ml of diluted Field's stain B.
2. Add immediately an equal volume of Field's stain A and mix with the diluted Field's stain B. Leave to stain for 1 minute. Note: The stains can be easily applied and mixed on the slide by using 1ml graduated plastic bulb pipettes.
3. Wash off the stain with clean water. Wipe the back of the slide clean and place it in a draining rack for the film to air-dry.

   Results for malaria thin film

   Chromatin of parasite . . . . . Dark red
   Cytoplasm of parasite . . . . . Blue
   Schuffner's dots . . . . . Red
   Maurer's dots (clefts) . . . . . Red-mauve
   Malaria pigment in white cells. . . . . Brown-black
   Red cells. . . . . Grey to pale mauve-pink
   Reticulocytes. . . . . Grey-blue
   Nuclei of neutrophils . . . . . Dark purple
   Cytoplasm of mononuclear cells . . . . . Blue-grey
   Granules of eosinophils . . . . . Red

| Operation Sheet 6 | Procedures for staining thick films with Field's staining technique |
|---|---|

1. Holding the slide with the dried thick film facing downwards, dip the slide into Field's stain A for 5 seconds. Drain off the excess stain by touching a corner of the slide against the side of the container. ***Caution: Thick blood films are not fixed and the stains do not kill parasites, viruses, or other pathogens which may be present in the blood.***
2. Wash gently for about 5 seconds in clean water. Drain off the excess water.
3. Dip the slide into Field's stain B for 3 seconds. Drain off the excess stain.
4. Wash gently in clean water. Wipe the back of the slide clean and place it upright in a draining rack for the film to air-dry.

Results for malaria thick film

Chromatin of parasite . . . . . .Dark red

Cytoplasm of parasite . . . . . .Blue-mauve

Schuffner's dots around . . . . . .Pale red

P. vivax and P. ovale parasites

Malaria pigment . . . . . .Yellow-brown or brown-black

Nuclei of small lymphocytes . . . . . .Dark purple

Nuclei of neutrophils . . . . . .Dark purple

Granules of eosinophils . . . . . .Red

Cytoplasm of mononuclear cells . . . . . .Blue-grey

Reticulum of reticulocytes . . . . . .Blue-grey stippling in background

| Operation Sheet 7 | Procedures for staining thick and thin films with Geimsa staining technique |
|---|---|

1. Immediately before use, dilute the Giemsa stain as required: 3% solution for 30 minute staining

   ***Measure 50ml of buffered water (or saline) pH 7.1–7.2. Add 1.5ml of Giemsa stain and mix gently. The stain can be measured using a dry graduated plastic bulb pipette or a small volume (2ml) plastic syringe. 10% solution for 10 minute staining Measure 45ml of buffered water, pH 7.1–7.2 in a 50ml cylinder. Add 5ml of Giemsa stain (to 50 ml mark) and mix gently.***

2. Place the slides face downwards (This is necessary to prevent fine particles of stain being deposited on the films). in a shallow tray supported on two rods, in a Coplin jar, or in a staining rack for immersion in a staining trough.

   Thick blood films must be thoroughly dried and thin blood films must be fixed (methanol for 2 minutes).

3. Pour the diluted stain into the shallow tray, Coplin jar, or staining trough. Stain as follows: 30 minutes if using a 3% stain solution 10 minutes if using a 10% stain solution

4. Wash the stain from the staining container using clean water (need not be distilled or buffered). Important: Flushing the stain from the slides and staining container is necessary to avoid the films being covered with a fine deposit of stain.

5. Wipe the back of each slide clean and place it in a draining rack for the preparation to air-dry.

   Results

   Chromatin of parasite . . . . . Dark red

   Cytoplasm of parasite . . . . . Blue

   Schuffner's dots . . . . . Red

   Maurer's dots (clefts) . . . . . Red-mauve

   Red cells . . . . . Grey to pale mauve

   Reticulocytes . . . . . Grey-blue

| Operation Sheet 8 | Procedures for reporting thick blood films |
|---|---|

1. When the thick film is completely dry, apply a drop of immersion oil to an area of the film which appears mauve coloured (usually around the edges).
2. Spread the oil to cover an area about 10mm in diameter (there is no need to add a cover glass). This is to enable the film to be examined first at a lower magnification.
3. Select an area that is well stained and not too thick. Change to the 100X objective (if required add a further small drop of oil).
4. Examine for malaria parasites and malaria pigment. Confirm the Plasmodium species by examining the thin blood film.
5. Report the approximate numbers of parasites (trophozoites, schizonts, and gametocytes) and also whether malaria pigment is present in white cells.
6. If no parasites are found after examining 100 fields (or if indicated 200 fields), report the film as: **Malaria thick film: NPF (No parasites found).**

| Operation Sheet 9 | Procedures for reporting thin blood films |
|---|---|

1. When the stained thin film is completely dry, apply a drop of immersion oil to the lower third of the film and spread the oil to cover most of this part of the film.
2. Examine the film first with 40x objective to check the staining, morphology, and distribution of the cells and to detect malaria schizonts, gametocytes, and trophozoites.
3. Change to the 100x objective to examine the parasites. Identify the different Plasmodium species.
4. If no parasites are found after examining 100 fields (or if indicated 200 fields), report the film as: **Malaria thick film: NPF (No parasites found).**

| Operation Sheet 10 | Procedures for diagnosing urine for *S. hematobium eggs* |
|---|---|

1. Collect 10–15ml of urine (between 10.00h and 14.00h) in a clean dry container.
2. Report the appearance of the urine. In moderate to heavy infections, the urine will usually contain blood and appear red or red-brown and cloudy. When visible blood is present, add 2 drops of saponin solution
3. Transfer 10ml of well mixed urine to a conical tube and centrifuge at RCF 500–1000g to sediment the schistotome eggs (avoid centrifuging at greater force because this can cause the eggs to hatch).
4. Discard the supernatant fluid. Transfer all the sediment to a slide, cover with a cover glass, and examine the entire sediment microscopically using the 10x objective with the condenser iris closed sufficiently to give good contrast.
5. Count the number of eggs in the preparation and report the number/10ml of urine

| Operation Sheet 11 | **Procedures for diagnosing skin snip for *O.Volvulus microfilariae*** |
|---|---|

1. Cleanse the skin using a spirit swab. Allow the area to dry.
2. Insert a sterile fine needle almost horizontally into the skin. Raise the point of the needle, lifting with it a small piece of skin measuring about 2mm in diameter.
3. Cut off the piece of skin with a sterile razor blade (or scalpel). Immerse the skin snip in a conical centrifuge tube containing about 1ml of fresh physiological saline and leave it at room temperature for up to 4 hours. Do not tease (pull apart) the skin because this is not necessary and can damage the microfilariae.
4. Using forceps, remove the skin snip, place it on a slide, and cover with a cover glass. Centrifuge the contents of the tube at medium to high speed, i.e. RCF 500–1000, for 5 minutes. Remove and discard the supernatant fluid. Transfer the entire sediment to a slide.
5. Examine both the skin snip and sediment microscopically for microfilariae using the 10x objective with the condenser iris closed sufficiently to give good contrast. Report the number of microfilariae as scanty, few, moderate numbers, or many.

| LAP Test | Practical Demonstration |
|---|---|

Name: _____ Date: _____

Time started: _____ Time finished: _____

*Instructions:* Given necessary materials, reagents and color plates you are required to perform the following tasks within --- hours.

Task 1: perform Stool wet mount procedure directly with saline and Iodine.

Task 2: perform stool sedimentation concentration technique with formol ether concentration technique.

Task 3: perform stool concentration technique with zinc sulphate flotation technique.

Task 4: prepare thick and thin blood films with capillary blood

Task 5: perform staining of thin films with Field's staining technique

Task 6: perform staining of thick films with Field's staining technique

Task 7: perform staining thick and thin films with Geimsa staining technique

Task 8: report thick blood films

Task 9: report thin blood films

Task 10: perform urinalysis for *S. hematobium eggs*

Task 11: perform skin snip for *O.Volvulus microfilariae*

| Instruction Sheet | LG45:Set up and use microscope |
|---|---|

This learning guide is developed to provide you the necessary information regarding the following content coverage and topics –

**Set up and use microscope**

3. Microscope
    - 3.1. Set up of a microscope
    - 3.2. Parts and functions of a microscope
    - 3.3. Adjust of a microscope light path
    - 3.4. Placing sample on a microscope
    - 3.5. Cleaning of microscope lenses

This guide will also assist you to attain the learning outcome stated in the cover page. Specifically, upon completion of this Learning Guide, you will be able to –

- optimize resolution by setting up the light path
- select appropriate objectives and examine samples
- Ensure that the lenses are made clean
- adjust settings and alignment of the light path to optimize performance microscope
- place sample correctly on the stage

**Learning Instructions:**

1. Read the specific objectives of this Learning Guide.
2. Follow the instructions described in number 3 to 19.
3. Read the information written in the "Information Sheets 1". Try to understand what are being discussed. Ask your trainer for assistance if you have hard time understanding them.
4. Accomplish the "Self-check 1" in page 9.
5. Ask from your trainer the key to correction (key answers) or you can request your trainer to correct your work. (You are to get the key answer only after you finished answering the Self-check 1).
6. If you earned a satisfactory evaluation proceed to "Information Sheet 2". However, if your rating is unsatisfactory, see your trainer for further instructions..
7. Submit your accomplished Self-check. This will form part of your training portfolio.
8. Read the information written in the "Information Sheet 2". Try to understand what are being discussed. Ask your trainer for assistance if you have hard time understanding them.

| Information Sheet-1 | Set up and use microscope |
|---|---|

## 3.1. Set up and use microscope

- **Definition of terms:**
  A. **Microscope:** a magnifying instrument, which use to see objects that cannot seen by the necked eye.
  B. **Object:** Material examined Under the Microscope.
  C. **Specimen:** the part which represent the characteristic of whole
- **What is Microscope:** The Microscope is a magnifying instrument, which use to see objects that cannot seen by the necked eye.A Microscope is the most expensive and important piece of equipment used laboratories, forms70–90% of the work in medical laboratory, a microscope is a magnifying instrument. The magnified image of the object (specimen) is first produced by lens close to the object called the objective .This collects light from the specimen and forms the primary image.A second lens near the eye called the eye piece enlarges the primary image, converting it into one that can enter the pupil of the eye.
- **Types of Microscope**
  - **Bright field Microscope**: is the type of Microscope commonly used in medical laboratory in which visible white light its source of illumination.
  - **Dark field Microscope:** this form of Microscope used when maximum contrast is required, E.g. to visualize transparent objects. In dark-field (dark-ground) Microscope ,a black patch stop below the condenser or a central black- out area in a special dark-field condenser prevents direct light from entering the objective and therefore the field of view is dark .Instead of passing through the center of the condenser the light is reflected to stops to match their own Microscopes. If however this useful accessory is not available,a dark-field stop can be made in the laboratory.

  Value of dark-field Microscope Dark-field Microscopes particularly useful for detecting:
  - Motile Treponema palladium in chancre fluid.
  - Motileborreliae in blood.
  - Motile leptospires in urine.
  - Pathogenic microfilaria in blood. The sheath and nuclei can be clearly seen.

- Cryptococcus in cerebrospinal fluid. The capsule surrounding the cells can be seen.
- Vibreos' in specimens and cultures.

- **FlorescenceMicroscope:** In fluorescence microscope,ultra-violetlight which has a very short wave length and is not visible to human eye (or just visible deep blue light)is used to illuminate organisms, cellsor particles which have been previously stained with fluorescing dyes calledflorocrome dye.
- **Electron Microscope**: The various components of the Microscope can be classified into four systems:
  - Support system
  - Magnification system
  - Illumination system
  - Adjustment system

| Self-check 1 | Written |
|---|---|

***Directions:*** Answer all the questions listed below. Use the Answer sheet provided in the next page:

1. What is Microscope? (2 points )
2. List different types of Microscope. (2 points )

**Note: satisfactory rating is 4 points, unsatisfactory <2 points. You can ask your instructor for copy of correct answer.**

**Answer Sheet**

**1.**_____

**2.** _____

Score = _____

Rating: _____

Name: _____ Date: _____

| Information Sheet-2 | Parts and functions of a microscope |
|---|---|

## 3.2.Parts and functions of a microscope

3.2.1. **Support system**:

This consists of:

- the foot
- the limb
- Revolving nosepiece (objective changer)
- Stage
- Mechanical stage, which gives a slow controlled movement to the object slide.

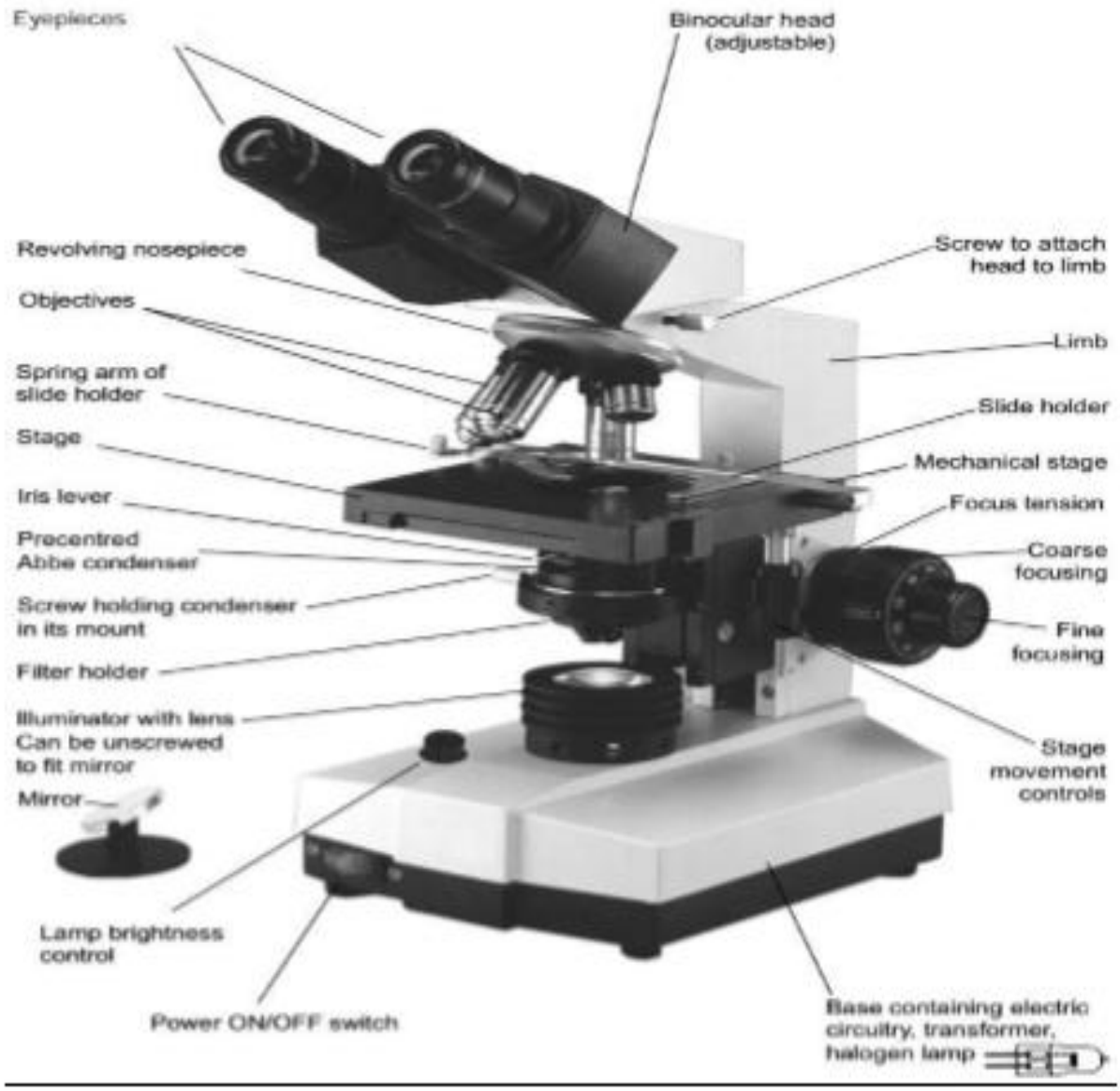


*Fig 3.1. Bright field Binocular Microscope with built in Illumination*

3.2.2. **Magnification system**: This consists of a system of lenses, the lenses of the Microscope are mounted in two groups, one at each end of the long tube or the body tube.

- First group of lenses is at the bottom of the tube, just above the preparation under examination (the object), and is called the objective.
- The second group of lenses is at the top of the tube and is called the eyepiece.
- Objectives Magnification the magnifying power of each objective is shown by a figure engraved on the sleeve of the lens 10x objective magnifies 10 times; 40x objective magnifies 40 times; 100x objective magnifies 100 times.
- The x100 objective is usually marked with a red ring to show that it must be used with immersion oil. Some Microscopes are fitted with x3 or x5 objective instead of x10 objective.


*Fig. 3.2. Objectives of Microscope*

- Numerical aperture (NA) it is relates to the resolving power of the objective. The higher the resolving power of an objective, the closer can be the fine lines or small dots in the specimen which the objective can separate in the image. The numerical aperture is also engraved on the sleeve, next to the magnification.
  - 0.25 on x10 objective
  - 0.65 on x40 objective
  - 1.25 On x100 objective.

  The greater the numerical aperture, the greater the resolving power. Moreover, the greater the numerical aperture, the smaller the front lens mounted at the base of the objective.

  The front lens of the x100 objective is the size of a pinhead, so handle it with care
  - Working with immersion Oil

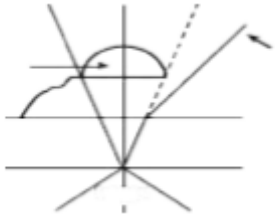


*Fig 3.3. Working principle of oil immersion objectives*

**The sleeve on objectives may also display:** The recommended length in millimeters of the tube (between the objective and the eyepiece) usually 160mm

- The recommended thickness in millimeters of the cover slip used to cover the object slide e.g. 0.16mm.
- The screw threads of all objectives are standard, so the objectives in the revolving nosepiece are interchangeable

A. Working distance the working distance of an objective is the distance between the front lens of the objective and the object slide when the image is in focus. The greater the magnifying power of the objective, the smaller the working distance.

- x10 objective: the working distance is 5 -6mm
- x40 objective: the working distance is 0.5 – 1.5 mm
- x100 objective: the working distance is 0.15 – 0.20mm


*Fig. 3.4. Working distance Objective*

B. **Resolving Power**: The resolving power of an objective is its ability to reveal closely adjacent details as separate and distinct. The greater the resolving power of the objective, the clear the image, The maximum resolving power of a good medical laboratory Microscopes about 0.25mm (the resolving power of the normal human eye is about 0.25mm).Immersion oil increases the resolving power by conserving many light rays that would be lost by refraction if a dry objective were used

C. **Objective Magnification**:

- Eyepiece Magnification The magnifying power of the eyepiece is marked on it.

- X5 eyepiece magnifies the image produced by the objective five times;
- X10 eyepiece magnifies the image 10 times.

D. If the object is magnified 40 times by the 40 objective, then by five times by the 5 eyepiece, the total magnification is: 5 x 40 = 200.

- To calculate the total magnification of the object observed, multiply the magnifying power of the objective by that of the eyepiece.
- Microscopes used in medical laboratories have a magnifying power of between x50 and x 1000.
- Certain eyepieces have a calibrated graticule. These eyepieces are used to measure the size of an object under the Microscope (e.g. protozoan cysts).

| Self-check 2 | Written |
|---|---|

***Directions:*** Answer all the questions listed below. Use the Answer sheet provided in the next page:

1. Explain component of Microscope. (2 points )
2. What is total magnification? (2 points )

**Note: satisfactory rating is 4 points, unsatisfactory <2 points. You can ask your instructor for copy of correct answer.**

Score = _____

Rating: _____

**Answer Sheet**

**1.**_____

_____

**2.**_____

_____

Name: _____ Date: _____

| Information Sheet-3 | Adjust of a microscope light path |
|---|---|

## 3.3.Adjust of a microscope light path

### 3.3.1. Illumination system Light source

- **An electric light source is preferable**, since it is easy to adjust. It is provided either by a lamp built into the Microscope beneath the stage, or by an external lamp placed in front of the Microscope.
- **Mirror:** The mirror reflects rays from the light source onto the object. One side has a plane surface, the other a concave surface, the concave side forms a low-power condenser and is not intended to be used if the Microscope already has a condenser.

*Fig 3.5. Microscope Mirror*

- **Condenser**: The condenser brings the rays of light to a common focus on the object to be examined. It is situated between the mirror and the stage. The condenser can be raised (maximum illumination) and lowered (minimum illumination). It must be centered and adjusted correctly.
- **Diaphragm**: The diaphragm which is found inside Condenser used to reduce or increase the angle and therefore also the amount of light that passes into the condenser.

**Fig. 3.6.** ***A when diaphragm is opened to allow maximum light for high power objective B when diaphragm is closed to allow low light for lower power objective***

3.3.2. Adjustment system*:* This consists of:

- coarse adjustment screw
- fine adjustment screw
- condenser adjustment screw
- condenser centering screws
- an iris diaphragm lever
- mechanical stage controls
    - ✓ Coarse adjustment screw, this is the largest screw. It is used first to achieve an approximate focus.
    - ✓ Fine adjustment screw, this moves the objective more slowly. It is used to bring the object into perfect focus.
    - ✓ Condenser adjustment screw, this is used to raise the condenser for greater illumination or to lower it to reduce the illumination.
    - ✓ Condenser centering screws, There may be three screws placed around the condenser:
        a. One in front,
        b. One on the left and,
        c. One on the right.

These are used to center the condenser exactly in relation to the objective.

- Iris diaphragm lever this is a small lever fixed to the condenser. It can be moved to close or open the diaphragm, thus reducing or increasing both the angle and the intensity of the light.
- Mechanical stage controls these are used to move the object slide on the stage: one screw moves it backwards and forwards and the other screw moves it to the left or right.

**N.B.**When a new Microscope is received in the laboratory, it is important to know how to set it up correctly. Remember to flow manufacture's manual.

Positioning the Microscope Place it on a firm level bench (check with a spirit level) of adequate size but not too high. The Microscope must be placed in the shade away from the window. Place a square felt pad under the Microscope. If no felt is available, use a piece of heavy cloth.

**Setting up a lamp for the Microscope:**

If the Microscope has a mirror, you can make a lamp to provide illumination. A porcelain holder for a light bulb is fixed on a wooden base and the whole is encased in a wooden or tin box with an opening for the light. Cut slits in the top of the box to enable the bulb to cool. Alternatively, a flap can be fitted above the opening to serve as a shutter. Use a 100W opaque electric bulb of the "daylight" type (blue–white).

***Fig. 3.7. Setting up a Lump for a Microscope***

- **Binocular adjustment**: When a binocular Microscope is used, the inter pupillary distance (the distance between the pupils of the eyes) can be adjusted to suit the operator.
- **Focusing the eyepieces**: one of the eyepiece holders (usually the left) has a focusing collar. If the collar is on the left eyepiece holder, close your left eye and, using the x40 objective, bring the image into focus for your right eye with the right eyepiece. Then close your right eye and look through the left eyepiece. If the image is in focus, no adjustment is needed. If the image is not clear, turn the focusing collar until it is in focus. The Microscope is now adjusted to suit your own binocular vision.

3.3.3. **Depth of the Microscope field**: The image is seen in depth when a low-power objective is used. When the high power objectives (x40, x100) are used, the depth of focus is small and the fine adjustment screw must be used to examine every detail from the top to the bottom levels of focus of the object observed (e.g. the different nuclei in a spherical amoeba cyst).

Images seen under the Microscope Remember that the circle of light seen in the eyepiece is called "the Microscopic field". Images observed in the Microscopic field can be placed in relation to the hands of a clock. For example, a schistosome egg is placed at "2 o'clock" in Fig.

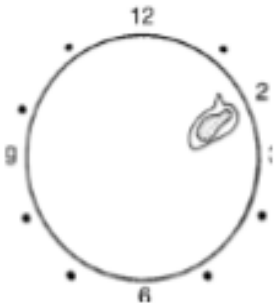



Fig. 3.8. ***Establishing the position of Image under the Microscope***

**The image seen is inverted by the lenses:**

- Objects seen at the bottom of the Microscopic field are actually at the top.
- Objects seen on the left side of the Microscopic field are actually on the right. If you move the slide in one direction, the object examined moves in the opposite direction

| | |
|---|---|
| Self-check 3 | Written |

***Directions:*** Answer all the questions listed below. Use the Answer sheet provided in the next page:

1. What is the use of condenser? (2 points )
2. What is the difference between monocular & binocular Microscope? (2 points )

**Note: satisfactory rating is 4 points, unsatisfactory <2 points. You can ask your instructor for copy of correct answer.**

Score = _____

Rating: _____

**Answer Sheet**

**1.**_____

_____

**2.**_____

_____

Name: _____ Date: _____

| Information Sheet-4 | Cleaning of microscope lenses |
|---|---|

### 3.4.Cleaning of microscope lenses

**Routine maintenance and care of Microscope**: Microscopes must be installed in a clean environment, away from chemicals. Workplaces should be well ventilated or permanently air-conditioned (intermittent use of air conditioners produces condensed water). The Microscope needs daily attention to keep it in good working order and thus to ensure reliable laboratory results. Optical instruments should not be kept for long periods in closed compartments since these conditions also favor fungal growth which can corrode optical surfaces. Special care is required in hot and humid climates.

- Cleaning the Microscope Microscopes are used to investigate biological tissues and fluids and must therefore be decontaminated and dirt must be cleaned at regular intervals, when no at work.
- Additional precautions to be taken in hot climates
    - Dry climates: In hot, dry climates the main problem is dust. Fine particles work their way into the threads of the screws and under the lenses. This can be avoided as follows:
        - ✓ Always keep the Microscope under an airtight plastic cover when not in use.
        - ✓ At the end of the day's work, clean the Microscope thoroughly by blowing air over it with a rubber bulb.
        - ✓ Finish cleaning the lenses with a soft camel-hair brush, a fine paintbrush or a blower. If dust particles remain on the surface of the objective, clean it with special lens tissue paper.
- **Humid climate:**In hot, humid climates and during the wet season in hot, dry climates, fungi may grow on the Microscope particularly on the surface of the lenses, in the grooves of the screws and under the paint, and the instrument will soon be useless. This can be prevented as described below. Always keep the Microscope under an airtight plastic cover, when not in use, together with a dish filled with blue silica to dry the air under the cover.(The silica will turn red when it has lost its capacity to absorb moisture from the air. It can be simply regenerated by heating in a hot-air oven or over a fire.) The Microscope must be cleaned daily to get rid of dust. These procedures must be carried out regularly, and are essential in conjunction with repair and maintenance procedures.

| | |
|---|---|
| Self-check | Written |

***Directions:*** Answer all the questions listed below. Use the Answer sheet provided in the next page:

1. What is the three different forms of Objective? (2 points )
2. What is the use of Immersion Oil? (2 points )
3. Discuss microscope cleansing and daily maintenance. ( 3 points)

***Note:*** **Satisfactory rating – 11 points** **Unsatisfactory - below 11 points**
You can ask you trainer for the copy of the correct answers.

**Answer Sheet**

Score = _____
Rating: _____

Name: _____ Date: _____

1. _____
   _____
2. _____
   _____
3. _____
   _____

| Operation Sheet 1 | Identify parts of a microscope |
|---|---|

The purpose of this activity is to enable you to practice. The purpose of this activity is to enable trainees to practice those skills necessary to Identify parts of Microscope of Microscope, and to achieve competency in these skills.

INSTRUCTIONS: This activity should be conducted in a training institution skills laboratory. Trainee's should review Learning Guide/check list for Identifying parts of Microscope before beginning the activity, the trainer should demonstrate the steps/tasks in each learning guide one at a time. Under the guidance of the trainer, trainees should then practice the steps/tasks in the Learning Guide/check for identifying parts of Microscope & of Microscope. Trainees should be able to perform the steps/tasks before skills competency is assessed using the Checklist for.

**Conditions or situation for the operations:**

This task should be performed in a well-organized skills laboratory which has an electric light source and water supply for accomplishment of the tasks at allowable period of time.

Resource/ materials

| Tools and materials | |
|---|---|
| ○ TTLM( Laboratory Log Book Learning module ,Laboratory Manual Checklists<br>○ Reporting format | ○ Cleaning materials |
| ○ Sample | ○ Microscope |
| ○ SOP, | ○ Slide |
| ○ Stationary (pen, pencil, marker, paper ...) | ○ Cover slides |
| | ○ Sample container |
| | ○ Applicator sticks |

Precaution: Operating with Microscope requires special care, because microscopy is Expensive material, and all universal precaution-in the medical laboratory should be followed.

Procedure-Learning guide/Checklist:

Rate the performance of each step or task observed using the following rating scale:

**1. Needs Improvement:** Step or task not performed correctly, out of sequence (if necessary), or is omitted

**2. Competently Performed:** Step or task performed correctly in proper sequence (if necessary) but participant/student does not progress from step to step efficiently

**3. Proficiently Performed:** Step or task performed efficiently and precisely in the proper sequence (if necessary)

| Ser.No | Steps/Tasks | Needs improvement | Competently performed | .Proficiently Performed | Remark |
|---|---|---|---|---|---|
| | **Get ready** | | | | |
| 1 | Wearing gown | | | | |
| 2 | Washing your hand with soap and water | | | | |
| 3 | Wearing glove | | | | |
| 4 | Cleaning the working area | | | | |
| 5 | Confirming the working area fit for purpose(i.e. safe to work) | | | | |
| 5 | Arrange necessary materials& microscopy in appropriate place | | | | |
| 6 | Identify Support component of Microscope | | | | |
| 7 | Identify Magnification part of Microscope | | | | |
| 8 | Identify Illumination part of Microscope | | | | |
| 9 | Identify Adjustment part of Microscope | | | | |
| 10 | Practice switching on/ of Microscope | | | | |
| 11 | Practice placing Microscope At safe protected place at the end of day work | | | | |
| 12 | Review SOP for operating Microscope | | | | |
| | Identify all parts, set up, adjustment or focus maintenance ,with form | | | | |

**Quality criteria**:

**During accomplishment Operating parts of Microscope the trainees should be :**

- Able to Identify support parts of Microscope
- Able to Identify magnification part Microscope
- Able to identify Illumination part of microscope

| Operation Sheet 2 | Operate Parts of Microscope |
|---|---|

Purpose: The purpose of this activity is to enable you to practice. The purpose of this activity is to enable trainees to practice those skills necessary to Operate parts of Microscope of Microscope , and to achieve competency in these skills.

INSTRUCTIONS: This activity should be conducted in a training institution skills laboratory. Trainee's should review Learning Guide/check list for Operating parts of Microscope before beginning the activity, the trainer should demonstrate the steps/tasks in each learning guide one at a time. Under the guidance of the trainer, trainees should then practice the steps/tasks in the Learning Guide/check for Operating parts of Microscope & of Microscope. Trainees should be able to perform the steps/tasks before skills competency is assessed using the Checklist for. Operating parts of Microscope of Microscope.

Conditions or situation for the operations:

This task should be performed in a well-organized skills laboratory which has an electric light source and water supply for accomplishment of the tasks at allowable period of time.

**Resources/ materials**

| Tools and materials | |
|---|---|
| ○ TTLM( Laboratory Log Book Learning module ,Laboratory Manual Checklists<br>○ Reporting format | ○ Cleaning materials |
| ○ Sample | ○ Microscope |
| ○ SOP, | ○ Slide |
| ○ Stationary (pen, pencil, marker, paper ...) | ○ Cover slides |
| ○ Applicator sticks | ○ Sample container |

Precaution: Operating with Microscope requires special care, because microscopy is Expensive material, and all universal precaution-in the medical laboratory should be followed.

Procedure-Learning guide/Checklist:

Rate the performance of each step or task observed using the following rating scale:

**1. Needs Improvement:** Step or task not performed correctly, out of sequence (if necessary), or is omitted

**2. Competently Performed:** Step or task performed correctly in proper sequence (if necessary) but participant/student does not progress from step to step efficiently

**3. Proficiently Performed:** Step or task performed efficiently and precisely in the proper sequence (if necessary)

| Ser.No | Steps/Tasks | Needs improvement | Competently performed | . Proficiently Performed | Remark |
|---|---|---|---|---|---|
| | **Get ready** | | | | |
| 1 | Wearing gown | | | | |
| 2 | Washing your hand with soap and water | | | | |
| 3 | Wearing glove | | | | |
| 4 | Cleaning the working area | | | | |
| 5 | Confirming the working area fit for purpose(i.e. safe to work) | | | | |
| 5 | Arrange necessary materials& microscopy in appropriate place | | | | |
| 6 | Operate Support component of Microscope | | | | |
| 7 | Operate Magnification part of Microscope | | | | |
| 8 | Operate Illumination part of Microscope | | | | |
| 9 | Operate Adjustment part of Microscope | | | | |
| 10 | Practice switching on/ of Microscope | | | | |
| 11 | Practice focusing Object under Microscopy | | | | |
| 12 | Practice placing Microscope At safe protected place at the end of day work | | | | |
| 13 | Review SOP for operating Microscope | | | | |
| 14 | Operates all parts, set up,a adjustment or focus maintainance, with form | | | | |

**Quality criteria** :

**During accomplishment Operating parts of Microscope the trainees should be** :

- Able to Operate support parts of Microscope
- Able to Operate magnification part Microscope
- Able to Operate Illumination part of microscope
- Able to Operate Adjustment part of microscope
- Able to Focus Objects under Microscopy

| Operation Sheet 3 | Focus Objects under Microscope |
|---|---|

Purpose

The purpose of this activity is to enable you to practice those skills necessary to Focus Objects under Microscope , and to achieve competency in these skills.

INSTRUCTIONS: - This activity should be conducted in a training institution skills laboratory. Trainee's should review Learning Guide/check list for focus Objects Under Microscope before beginning the activity,the trainer should demonstrate the steps/tasks in each learning guide one at a time. Under the guidance of the trainer, trainee's should then practice the steps/tasks in the Learning Guide/check for Focusing Objects Under Microscope.

Trainee's should be able to perform the steps/tasks before skills competency is assessed using the Checklist for.

Focusing Objects under Microscope.

Conditions or situation for the operations:

This task should be performed in a well organized skills laboratory which has an electric light source and water supply for accomplishment of the tasks at allowable period of time.

Teaching/learning materials

**Resource/ materials**

| Tools and materials | |
|---|---|
| ○ TTLM( Laboratory Log Book Learning module ,Laboratory Manual Checklists<br>○ Reporting format | ○ Cleaning materials |
| ○ Sample | ○ Microscope |
| ○ SOP, | ○ Slide |
| ○ Stationary (pen, pencil, marker, paper ...) | ○ Cover slides |
| ○ Applicator sticks | ○ Sample container |

Precaution: Operating with Microscope requires special care, because microscopy is Expensive material, and all universal precaution-in the medical laboratory should be followed. all specimen should be considered as potential source of pathogens ,hence wearing PPE, and care handling of specimen should be considered.

Rate the performance of each step or task observed using the following rating scale:

**1. Needs Improvement:** Step or task not performed correctly, out of sequence (if necessary), or is omitted

**2. Competently Performed:** Step or task performed correctly in proper sequence (if necessary) but participant/student does not progress from step to step efficiently

**3. Proficiently Performed:** Step or task performed efficiently and precisely in the proper sequence (if necessary)

| Ser.No | Steps/Tasks | Needs improvement | Competently performed | . Proficiently Performed | Remark |
|---|---|---|---|---|---|
| | **Get ready** | | | | |
| 1 | Wearing gown | | | | |
| 2 | Washing your hand with soap and water | | | | |
| 3 | Wearing glove | | | | |
| 4 | Cleaning the working area | | | | |
| 5 | Confirming the working area fit for purpose(i.e. safe to work) | | | | |
| 5 | Arrange necessary materials& microscopy in appropriate place | | | | |
| 6 | Turn the rotary lamp brightness control anti-clockwise to its lowest setting and then switch on the microscope. | | | | |
| 7 | Turn up the brightness control to about three quarters of its full power(final adjustment will be made at a later stage). | | | | |
| 8 | Carefully revolve the nosepiece until the 10objectiveislocatedverticallyabove the stage. *Make suret here is no danger of the objective* | | | | |
| 9 | Prepare a specimen slide such as amounted stained thin blood film .A temporary mounted preparation can be made by adding a drop of oil to the lower third of the blood film and covering it with a cover glass. Make sure the underside | | | | |

| | | | | | |
|---|---|---|---|---|---|
| | of the slide is dry ,clean ,and free of stain marks. | | | | |
| 10 | Place the specimen slide, cover glass upper-most, on the front of the stage .Gently holding back the spring arm of the mechanical stage, push the slide back into the slide holder and release the arm slowly. The specimen will be held firmly. | | | | |
| 11 | While looking from the side(not downtheeye-pieces), turn the coarse focusing control to bring the specimen close to the objective i.e. about 5mm from the objective. | | | | |
| 12 | Looking down through the eyepieces, bring the specimen into focus by slowly turning the coarse focusing control in the opposite direction to increase the distance between specimen and objective. The specimen will come into focus ,first as a blurred image and then a sa clear image. | | | | |
| 13 | Use the fine focusing control to obtain a sharp image (this will not be the best image | | | | |
| 14 | Using the iris lever, open the iris fully. | | | | |
| 15 | Focus the condenser as follows:<br>● Using the condenser focusing knob located on the left, raise the condenser to it stop- most position.<br>● Using the iris lever, open the iris fully.<br>● Check that the filter holder is located against its stop and not out of position and blocking the light.<br>● Looking down the eye pieces and with the specimen in focus, slowly lower the con-denser until the mottled image of the ground glass light diffusing screen(located below the lens of the illuminator) is seen in the background.<br>● Slowly raise the condenser until the mottled image of the diffusing screen just disap-pears(thisisusuallyabout1mmbelow the condenser's topmost position).The con-denser is no in focus and should be left in | | | | |

| | | | | | |
|---|---|---|---|---|---|
| | this position. | | | | |
| 16 | Check the centering of the condenser unless the microscopes fitted with precentred condenser (if precentred there will be n countering screws, only a single screw holding the condenser in its mount).To check the centering of a condenser that is not precentred: | | | | |
| 17 | Looking down the eye pieces with the specimen in focus, obtain the best possible image by adjusting the condenser aperture and lamp brightness control. For the 10 objective, the condenser will need to be closed about two thirds to provide a good image .Adjust the lamp brightness control to a level which provides good illumination without glare. | | | | |
| 18 | Examine the specimen with the x40 objective .Carefully revolve the nosepiece to bring the40 objective into place .It will locate every close to the specimen. Providing the objectives are parfocal (in focus one with a another),only slight focusing with the fine focusing control should be necessary to bring the specimen into sharp focus. | | | | |
| 19 | Examine the specimen with the 100 oil immersion objective. Revolve the nosepiece to move 40x objective one side before bringing 100x in position place one drop of immersion oil on the specimen. Carefullylocatethe100 objective. The lens of this objective should just dip into the drop of oil (providing the objectives are parfocal).<br><br>Use the fine focusing control to focus the specimen. Open the condenser iris fully and increase the illumination to give a bright clear image. | | | | |

| | | | | | |
|---|---|---|---|---|---|
| | | | | | |
| 20 | Before removing the specimen from under the oil immersion objective, revolve the nosepiece so that the objective moves to one side. Only then remove the slide from the slide holder. | | | | |
| 21 | Clean microscope, and place in appropriate way of caring | | | | |

**Quality criteria** :

**During accomplishment Operating parts of Microscope the trainees should be :**

- Able to Focus Object under Microscope under diff objectives

| LAP Test | Practical Demonstration |
|---|---|

Name: _____ Date: _____

Time started: _____ Time finished: _____

*Instructions:* Given necessary materials, reagents and color plates you are required to perform the following tasks within --- hours.

Task 1:Identify parts of a microscope

Task 2: Operate Parts of Microscope

Task 3: performing Focusing objects under Microscope.

| Instruction Sheet | LG46.Performparasitological tests |
|---|---|

This learning guide is developed to provide you the necessary information regarding the following content coverage and topics –

**Process parasitological tests**

4. Diagnostic Techniques in Medical Parasitology
    - 4.1. Authorizing the requested test
    - 4.2. Performing quality control procedures
    - 4.3. Recording of individual results
    - 4.4. Result verification before release
    - 4.5. Sample storage for further use

This guide will also assist you to attain the learning outcome stated in the cover page. Specifically, upon completion of this Learning Guide, you will be able to –

- select authorized tests that are indicated for the requested investigations
- perform quality control *procedures*
- conduct individual tests according to documented methodologies
- record all results by noting any phenomena that may be relevant to the interpretation of results
- verify results before releasing for clinician/client
- discuss with colleague when result interpretation is outside parameters of authorized approval
- store unused sample or sample components for possible future reference, under conditions suitable to maintain viability
- store tested sample or sample components according to organizational sample retention policy for retesting when requested

**Learning Instructions:**

1. Read the specific objectives of this Learning Guide.
2. Follow the instructions described in number 3 to 19.
3. Read the information written in the “Information Sheets 1”. Try to understand what are being discussed. Ask your trainer for assistance if you have hard time understanding them.

4. Accomplish the “Self-check 1” in page 6.
5. Ask from your trainer the key to correction (key answers) or you can request your trainer to correct your work. (You are to get the key answer only after you finished answering the Self-check 1).
6. If you earned a satisfactory evaluation proceed to “Information Sheet 2”. However, if your rating is unsatisfactory, see your trainer for further instructions..
7. Submit your accomplished Self-check. This will form part of your training portfolio.
8. Read the information written in the “Information Sheet 2”. Try to understand what are being discussed. Ask your trainer for assistance if you have hard time understanding them.
9. Accomplish the “Self-check 2” in page 9.
10. Ask from your trainer the key to correction (key answers) or you can request your trainer to correct your work. (You are to get the key answer only after you finished answering the Self-check 2).
11. Read the information written in the “Information Sheets 3 and 4”. Try to understand what are being discussed. Ask your trainer for assistance if you have hard time understanding them.
12. Accomplish the “Self-check 3” in page 10.
13. Ask from your trainer the key to correction (key answers) or you can request your trainer to correct your work. (You are to get the key answer only after you finished answering the Self-check 3).
14. If you earned a satisfactory evaluation accomplish the “Self-check 4” in page 12
15. Ask from your trainer the key to correction (key answers) or you can request your trainer to correct your work. (You are to get the key answer only after you finished answering the Self-check 4).

| Information Sheet-1 | Diagnostic Techniques in Medical Parasitology |
|---|---|

4. Diagnostic Techniques in Medical Parasitology

4.1. Authorizing the requested test

Introduction

The reasons for performing laboratory tests and follow-up investigations must be clear. The tests performed in laboratories must reflect the common and emergency health needs of the area and provide information that can be easily interpreted. The tests must also be efficient, i.e. provide sufficient benefit to justify their cost and any risks involved in their performance. Medical officers should encourage qualified experienced laboratory staff to provide maximum information from laboratory tests and to proceed to further testing when this is obviously indicated and will lead to better and earlier treatment for a patient.

In deciding which tests and test methods are appropriate it is important to consider:

- the clinical and public health needs of the laboratory,
- wellbeing of patients,
- laboratory technical aspects,
- costs involved

| Self-check 1 | Written test |
|---|---|

**Write True if the statement is correct and False if it is incorrect**

1. Medical Parasitology deals with parasites that cause disease. (2 points)
2. A parasite is an organism that can survive without help of other organism.( 2 points )
3. A vector can transmit an infective stage of a parasite.( 2 points)

**Note: satisfactory rating is 4 points, unsatisfactory 2 points. You can ask your instructor for copy of correct answer.**

4.2. Performing quality control procedures

- **Stool Sample Collection**

- Provide the patient with a suitable wide-mouthed, container with a lid.
- Ask the patient to collect a walnut size piece or about 10ml of a watery specimen.
- It is not necessary to fill the whole container.
- Ask the patient to keep the outside of the container clean, health hazard!

- This is especially true of protozoa

- Amebic trophozoites will begin to degenerate 1- 2 hours after passage and alterations in appearance may result in erroneous identification
- Flagellate trophozoites may also undergo changes that would make differentiation difficult
- Cysts will deteriorate if fecal specimens are left standing for many hours or overnight, especially if the temperature is high

- Helminth eggs and larvae are less affected by the age of the specimen than are protozoa

- Nevertheless, changes may occur that would affect identification
- Hookworm eggs, for example, may become embryonated and larvae amy hatch from the eggs
- Even Ascaris eggs may develop to multicellular stages
- In addition, larvae may degenerate in old stools making it impossible to identify the species

- To ensure that good specimens are provided for examination, pay attention to the following points:

- Use clean, dry containers for collecting feces
- Dirt will interfere with examinations and may introduce free – living organisms from the soil.
- Urine and water will destroy trophozoites, if present
- Have the specimen brought to the laboratory as soon as it is passed to prevent deterioration of protozoa and alterations in the morphology of protozoa and helminths.
- Note the patient's name and the date and time of passage on the specimen

• Some reagents will last indefinitely if kept properly stoppered and out of direct sunlight.

– Examples are formalin solutions, isotonic saline, fixatives, and alcohol solutions (unless evaporation occurs)

- The “life” of each solution is indicated in the direction for preparing it

- Label all reagents with the date of preparation. Keep records for each solution. Review these every week and discard outdated solutions

- Many of the solutions used in the method for trichrome stain need to be changed at regular intervals.

- No procedure used for examining fecal specimens is 100% effective – that is the procedures will not always recover all the species present and, if a particular species is present in only very low numbers, they may fail to demonstrate them when used on a single specimen.

- Because the techniques are not perfect, you should perform them as carefully as possible for optimum results. Also, be sure to use techniques that are appropriate for the material you are examining.

- **BLOOD PARASITES**

- **Sample collection time:**

- The number of certain parasites in the blood depends on the time of collection.

- **Malaria**

- ⇒ Highest number of parasites is found during fever attacks and before the start of treatment

- **Microfilaria**

- ⇒W.bancrofti and Brugiamalayi – take specimen at night between 10 p.m. – 2 a.m.
- Smear for Malaria Parasites
- Follow proper collection procedures.

- Glass slides must be clean and free from grease.

- Thick films and thin films must be prepared properly.

- While drying protect blood films from dust, flies and insects.

- Do not dry exposed to direct sun light

- When fixing the thin film, be careful not to let methanol touch the tick film.
- Performance of a blood film examination on a poor quality film achieves inaccurate results.
    - A poor quality film caused by:
- Poor spreading technique
- Poor leukocytes distribution
- Too small a working area

- Red cell morphology altered by the spreading process.

- Use of the wrong anticoagulant, resulting in altered blood cell size and/or morphology

- Patient sample mix up.

- Transcriptional error.

- Inadequate mixing of the sample with the anticoagulant, resulting in the formation of small fibrin strands and a decrease in the platelets count.

- Poor stain quality etc

- Smear for Microfilaria

- Filaria are seldom found in the early and in the late stage of the disease.

- The proper time of collection is important (10 p.m. to 2 a.m. )

- Un sheated non-pathogenic filaria can be found any time of the day.

- Patients with filaria in the blood show also eosinophilia in the blood.

| Self-check 2 | Written test |
|---|---|

**Write True if the statement is correct and False if it is incorrect**

1. Providing a quality service to patients and those requesting tests is part of total laboratory quality management.
2. A quality test result of clinical laboratory should be from right patient with right procedure for testing
3. Quality assurance is a onetime action which can evaluate the performance of the laboratory.

**Note: satisfactory rating is 4 points, unsatisfactory 2 points. You can ask your instructor for copy of correct answer.**

Score = _____

Rating: _____

4.3. Recording of individual results

In clinical laboratories, records of test results can be kept by retaining carbon copies of reports, using work sheets, or recording test results in registers (exercise books). Whichever system is used it must be reliable and enable patients' results to be found quickly. Test records are also required when preparing work reports and estimating the workload of the laboratory. If carbon copies or work sheets are used these must be dated and filed systematically each day. If registers are used, backing cards which are headed and ruled can be placed behind pages to avoid having to rule and head each page separately. The cards must be heavily ruled with a marker pen so that the lines can be seen clearly. Separate registers, each with its own cards, can be prepared to record the results of parasitological tests.

| Self-check 3 | Written test |
|---|---|

**Write True if the statement is correct and False if it is incorrect**

1. A laboratory record book can be used as a carbon copy for the test results reported.
2. The laboratory record should be with components that specify the patient demographic informations, type of the test and test results.

**Note: satisfactory rating is 4 points, unsatisfactory 2 points. You can ask your instructor for copy of correct answer.**

Score = _____

Rating: _____

Answers:

1. _____
2. _____
3. _____

4.4. Result verification before release

Laboratory staff should provide as much relevant information as possible to assist those requesting tests to interpret the results of tests correctly and use the information in the best possible way to benefit patients and the community. Reports should be clearly and neatly written (particularly figures).A patient's notes must contain the signed reports issued by the laboratory. In the use and interpretation of laboratory test results it is important to understand the limitations of tests, e.g. the ability of tests to indicate when disease is present or absent or whether the value in a report is normal or abnormal for a patient.

| Self-check 4 | Written test |
|---|---|

**Write True if the statement is correct and False if it is incorrect**

**1.** The test results should be properly verified before the release of the test. (2 points)

**2.** Results should be clearly written and verified by the lab supervisor.( points)

**3.** Always Lab tests should provide reliable test results with clinical examinations. (2 points)

**Note: satisfactory rating is 4 points, unsatisfactory 2 points. You can ask your instructor for copy of correct answer.**

Score = _____

Rating: _____

Answers:

1. _____
2. _____
3. _____

## 4.5. Sample storage for further use

| Preservative | Advantages | Disadvantages |
|---|---|---|
| **10% Formalin** | **All-purpose fixative**<br>**Easy to prepare**<br>**Long shelf life**<br>**Good preservation of morphology of helminth eggs, larvae, protozoan cysts, and coccidia**<br>**Suitable for concentration procedures and UV fluorescence microscopy**<br>**Suitable for acid-fast, safranin, and chromotrope stains**<br>**Compatible with immunoassay kits and UV fluorescence microscopy** | **Not suitable for some permanent smears stained with trichrome**<br>**Inadequate preservation of morphology of protozoan trophozoites**<br>**Can interfere with PCR, especially after extended fixation time** |
| **MIF merthiolate-iodine-formaldehyde)** | **Components both fix and stain organisms**<br>**Easy to prepare**<br>**Long shelf life**<br>**Useful for field surveys**<br>**Suitable for concentration procedures** | **Not suitable for some permanent smears stained with trichrome**<br>**Inadequate preservation of morphology of protozoan trophozoites**<br>**Iodine interferes with other stains and fluorescence**<br>**Iodine may cause distortion of protozoa** |
| **LV-PVA (low viscosity polyvinyl-alcohol)** | **Good preservation of morphology of protozoan trophozoites and cysts**<br>**Easy preparation of permanent smears stained with such as trichrome (solution both preserves organisms and makes them adhere to slides)**<br>**Preserved samples remain stable for several months** | **Inadequate preservation of morphology of helminth eggs and larvae, coccidia, and microsporidia**<br>**Contains mercuric chloride**<br>**Difficult and expensive to dispose of**<br>**Difficult to prepare in the laboratory**<br>**Not suitable for concentration procedures**<br>**Cannot be used with immunoassay kits**<br>**Not suitable for acid-fast, safranin and chromotrope stains** |
| **SAF (sodium acetate-acetic acid-formalin)** | **Suitable for both concentration procedures and preparation of permanent stained smears**<br>**Easy to prepare**<br>**Long shelf life**<br>**Suitable for acid-fast, safranin, and chromotrope stains**<br>**Compatible with immunoassay kits** | **Requires additive (e.g., albumin-glycerin) for adhesion of specimens to slides**<br>**Permanent stains not as good as with PVA or Schaudinn's fixative** |
| **Schaudinn's Fixative** | **Good preservation of morphology of protozoan trophozoites and cysts**<br>**Easy preparation of permanent stained smears** | **Less suitable for concentration procedures**<br>**Contains mercuric chloride**<br>**Inadequate preservation of morphology of helminth eggs and larvae, coccidia, and microsporidia**<br>**Poor adhesion of liquid or mucoid specimens to slides** |
| **Modified PVA copper or zinc** | **Permanent smears can be made and stained with trichrome**<br>**Zinc is preferred over copper**<br>**No mercuric chloride** | **Staining not consistent**<br>**Organism morphology may be poor**<br>**Copper-morphology of cysts and trophozoites is poor**<br>**Zinc-better morphology but not comparable to LV-PVA** |
| **One-Vial Fixatives (such as Ecofix, Parasafe, Unifix, Proto-fix, STF, and others that may be available)** | **Concentrate and permanent smear can be made out of one vial**<br>**Immunoassays can be done on most**<br>**No mercuric chloride** | **Certain one-vial fixatives must use certain stains**<br>**Color difference of stain**<br>**Staining not always consistent**<br>**Sometimes more expensive than formalin and LV-PVA** |

**Preservation of specimens** is necessary when stool specimens cannot be examined within the prescribed time interval. Various preservatives are available (see table), with the two most commonly used being 10% aqueous formalin and PVA (polyvinyl-alcohol).

Because 10% formalin and PVA have complementary advantages (see table 4.1. below), it is recommended that the specimen be divided and preserved in both types of preservatives (add one volume of stool to three volumes of the preservative). Preserved specimens can be stored for several months.

| Self-check 5 | Written test |
|---|---|

**Write**

**True if the statement is correct and False if it is incorrect**

1. Stool specimen should be preserved always.(2 points)
2. Formol ether is all purpose preservative but not suitable for PCR technique. .(2 points)
3. SAF (sodium acetate-acetic acid-formalin) can be used both for direct and concentration techniques. ( 2 points)
4. Merthiolate-iodine-formaldehyde is Suitable for acid-fast, safranin, and chromotrope stains. (2 points).
5. Preserved specimens can be stored for several months and can be used for teaching purpose. ( 2 points).

**Note: satisfactory rating is 4 points, unsatisfactory 2 points. You can ask your instructor for copy of correct answer.**

Score = _____

Rating: _____

Answers:

1. _____
2. _____
3. _____
4. _____
5. _____

| Instruction Sheet 5 | LG47: Maintain a safe environment |
|---|---|

This learning guide is developed to provide you the necessary information regarding the following content coverage and topics –

**5. Maintaining safe work environment**

5.1. Safe work practice in parasitology

5.1.1. Using of PPE

5.1.2. Ensuring safety of self and others

5.2. Cleaning of splashes

5.3. Waste minimization

5.4. Laboratory waste disposal

This guide will also assist you to attain the learning outcome stated in the cover page. Specifically, upon completion of this Learning Guide, you will be able to –

- Ensure personal safety and that ofother laboratory personnel by using safe work practices and PPE.
- Clean up spills using appropriate techniques to protectpersonnel, work area and environment from contamination
- Minimize generation of wastes
- Ensure safe disposal of biohazardous materials andother laboratory wastes are in accordance with enterpriseprocedures

**Learning Instructions:**

1. Read the specific objectives of this Learning Guide.
2. Follow the instructions described in number 3 to 17.
3. Read the information written in the “Information Sheets 1”. Try to understand what are being discussed. Ask your trainer for assistance if you have hard time understanding them.
4. Accomplish the “Self-check 1” in page 7.
5. Ask from your trainer the key to correction (key answers) or you can request your trainer to correct your work. (You are to get the key answer only after you finished answering the Self-check 1).
6. If you earned a satisfactory evaluation proceed to “Information Sheet 8”. However, if your rating is unsatisfactory, see your trainer for further instructions..
7. Submit your accomplished Self-check. This will form part of your training portfolio.
8. Read the information written in the “Information Sheet 2”. Try to understand what are being discussed. Ask your trainer for assistance if you have hard time understanding them.
9. Accomplish the “Self-check 2” in page 8.

| Information Sheet-1 | Safe work practice in parasitology |
|---|---|

5.1. Safe work practice in parasitology

**Introduction**

**In parasitology laboratory the laboratory personnel shold consider all the following conditions**

- Consider all stool samples as highly infectious.
- Avoid contact with bare fingers, wear gloves if possible or handle with care.
- Do not reuse stool containers, burn stool sample containers and wooden applicators.
- Soak glass slides in 5 % Phenol solution (e.g. Lysol) at least overnight.
- Cover slips break easily and may cause injuries, therefore soak in a separate container in 5 % Phenol solution (e.g. Lysol) at least overnight.
- Chemical waste disposal
- Pour old and used chemicals into the sink and flush with water if the sink is connected to a soak pit.
- Otherwise, pour chemicals directly into the soak pit. Ensure that the soak pit is not near a natural water source.
- Formalin is irritating to the skin and the vapor should not be inhaled.

5.1.1. Using of PPE

Recommended activities to improve Infection in the parasitology laboratory are:

- Using appropriate hand hygiene techniques.
- Wearing Personal Protective Equipment (PPE): some of the personal protective equipment that should be used in parasitology lab are:
  - Gown
  - Gloves
  - Eye google
  - Apron specially if you wash slides for reusing purpose
  - Waste disposal container and etc…..

Table 5.1. types of PPE

| Type of PPE | Must be used for | Primarily protects |
|---|---|---|
| Caps, Gowns/scrub suits, masks, aprons, drapes | Invasive procedure where tissue beneath the skin is exposed | Service provider and client |
| Closed boots or shoes (open sandals are not acceptable) | Situation involving sharp instruments or contact with blood and/or body fluids is likely | Service provider |
| Goggles or glasses, Masks, Apron or Mackintosh | Situation were splashing or blood, body fluids, secretions or excretions is likely | Service provider |
| Apron or Mackintosh | Situation were splashing or spillage of blood, body fluids, secretions or excretions is likely | Service provider |
| Masks | Situation which call for air borne or droplet transmission precaution | Service providers |

5.1.2. Ensuring safety of self and others

Laboratory facilities are ideal settings for the transmission of infections because;

- procedures during sample receiving and processing are the potential to introduce microorganism as the service providers and support staff are constantly performing these proceduresor other activities
- And Clients receiving services and their parents could be infected.
- The community:Members of the community are also at risk of infections, particularly from inappropriate disposal of medical waste.

So, laboratory personnel have responsibility to follow standard precautions during specimen receiving, processing and waste disposal.

| Self-check 1 | Written test |
|---|---|

**Write True if the statement is correct and False if it is incorrect**

6. Laboratory personnel have no any responsibility during specimen reception. (2 points)
7. Laboratory personnel should use appropriate PPE during laboratory working hours. (2 points)
8. The community could be easily infected by improper waste disposal( 2 points)

**Note: satisfactory rating is 4 points, unsatisfactory <4 points. You can ask your instructor for copy of correct answer.**

**Answer Sheet**

**1.**_____

**2.** _____

**3.** _____

Score = _____

Rating: _____

Name: _____ Date: _____

| Information sheet 2 | Cleaning splashes |
|---|---|

5.2. Cleaning of splashes

A splash is a tiny amount of a liquid that is formed during specimen processing in laboratory. It could be a potential source of infection whenever it get in to our body .Splashes could contaminate working area the table, floor or the other equipment duringpreparation of stool smear, blood film and urine or other body fluids for parasitological examination. So laboratory personnel should properly use eye protections such as eye glasses and goggles always during these procedures. In some cases there must be eye wash stations.

The splashes must be cleaned properly by decontaminating the area with 1% hypochlorite.

| Self-check 1 | Written test |
|---|---|

**Answer the following questions**

1. What is a chemical that could be used to clean and disinfect laboratory splashes?

**Note: satisfactory rating is 4 points, unsatisfactory <4 points. You can ask your instructor for copy of correct answer.**

**Answer Sheet**

**1.**_____

Score = _____

Rating: _____

Name: _____ Date: _____

| Information sheet 3 | Waste minimization |
|---|---|

5.3. Waste minimization

The total volume of medical waste generated can vary depending on the volume of the tests generated. Waste minimization is a set of processes and practices intended to reduce the amount of waste produced. By reducing or eliminating the generation of harmful and persistent wastes, waste minimization supports efforts to promote a more sustainable society. Waste minimization creates benefits. Smaller quantities of waste mean less compromise of the environment at disposal sites. Waste minimization leads to safer laboratory conditions and lessens employee exposure to hazardous chemicals. Waste minimization also promotes safer waste handling and transporting. It also reduces disposal costs which benefits the whole community.

Waste minimization activities include:

- Treatment to reduce hazards.
- Substitutions of less hazardous materials.
- Procedural changes to minimize generation.
- Improved laboratory management practices.

| Self-check 3 | Written test |
|---|---|

**Answer the following questions**

1. List waste minimization activities. (4 points)

**Note: satisfactory rating is 4 points, unsatisfactory <4 points. You can ask your instructor for copy of correct answer.**

Score = _____

Rating: _____

**Answer Sheet**

**1.**_____

_____

_____

_____

Name: _____ Date: _____

5.4. Laboratory waste disposal

Disposal is a process of eliminating health care wastes without posing any risk to health facility workers and the general public.

The following Infectious wastes may be produced in parasitology laboratory.

- Blood, blood products & other body fluids or items contaminated with similar fluids & other contaminated material infected with human pathogens are classified as high risk wastes and laboratory personnel should dispose them properly.

Infectious wastes can be disposed as follows:

- On-site burial
- On site incineration provided that the incinerator is standard and capable of destroying such wastes
- Transport of offsite treatment/disposal site, if there is the service

***g. 5.4. Yellow waste bean for infectious waste***

| Self-check 4 | Written test |
|---|---|

Answer the following questions.

1. List infectious wastes produced in parasitology lab. (3 points)
2. List three ways of waste disposal.(3 points)

**Note: satisfactory rating is 3points, unsatisfactory <3 points. You can ask your instructor for copy of correct answer.**

Score = _____

Rating: _____

**Answer Sheet**

**1.**_____

_____

_____

**3.** _____

_____

_____

Name: _____ Date: _____

| Instruction Sheet 6 | LG48:Maintain laboratory records |
|---|---|

This learning guide is developed to provide you the necessary information regarding the following content coverage and topics –

**6. Record of parasitological investigations**

6.1. Recording and reporting of parasitological investigations
6.2. Updating instrument maintenance logs
6.3. Ensure confidentiality

This guide will also assist you to attain the learning outcome stated in the cover page. Specifically, upon completion of this Learning Guide, you will be able to –

- make entries on report forms or into computer systems, accurately calculating, recording or transcribing required data as required
- update instrument maintenance logs, as required
- Maintain security and confidentiality of all clinical information, laboratory data and records are

**Learning Instructions:**

1. Read the specific objectives of this Learning Guide.
2. Follow the instructions described in number 3 to 12.
3. Read the information written in the “Information Sheets 1”. Try to understand what are being discussed. Ask your trainer for assistance if you have hard time understanding them.
4. Accomplish the “Self-check 1” in page 7.
5. Ask from your trainer the key to correction (key answers) or you can request your trainer to correct your work. (You are to get the key answer only after you finished answering the Self-check 1).
6. If you earned a satisfactory evaluation proceed to “Information Sheet 8”. However, if your rating is unsatisfactory, see your trainer for further instructions..
7. Submit your accomplished Self-check. This will form part of your training portfolio.
8. Read the information written in the “Information Sheet 2”. Try to understand what are being discussed. Ask your trainer for assistance if you have hard time understanding them.
9. Accomplish the “Self-check 2” in page 8.
10. Ask from your trainer the key to correction (key answers) or you can request your trainer to correct your work. (You are to get the key answer only after you finished answering the Self-check 2).
11. Read the information written in the “Information Sheets 3. Try to understand what are being discussed. Ask your trainer for assistance if you have hard time understanding them.
12. Accomplish the “Self-check 3” in page 10.

| Information Sheet-1 | Recording and reporting of parasitological investigations |
|---|---|

## 6.1. Recording and reporting of parasitological investigations

Record keeping systems, procedures and practices should work reliably to ensure that records are credible and authoritative. ***Records should be made, maintained and managed systematically.*** Record keeping must be managed through an identifiable records management program.

Recordkeeping systems must have accurately documented policies, assigned responsibilities, and formal methodologies for their management. This applies equally to dedicated recordkeeping systems and to laboratory application systems functioning as recordkeeping systems.

Record keeping systems, procedures and practices should be audited to ensure compliance with regulatory requirements.

Laboratory recordkeeping practices, systems and procedures of public sector bodies operate within a regulatory regime. This regime may consist of standards and requirements to ensure the creation, management and disposal of full and accurate records. It is essential that the recordkeeping practices, systems and procedures are audited on a regular basis. The audits will:

- identify areas of non-compliance within existing regulatory requirements
- identify problem areas for public sector bodies, thus allowing for internal corrective actions
- Improve the quality and reliability of public records.
- A record should contain not only the content, but also the structural and contextual information necessary to document a transaction. It should be possible to understand a record in the context of the organizational processes that produced it and of other, linked records.
- A record comprises content, structure and context. The elements that make up the structural and contextual parts of the record are known as recordkeeping metadata.
- Records should correctly reflect what was communicated, decided or done.
- Recordkeeping procedures and practices must be designed to ensure that a record correctly reflects what occurred. Business processes and systems should be designed to make it easy, or even automatic, to make accurate records of transactions.

| Self-check 1 | Written test |
|---|---|

**Write True if the statement is correct and False if it is incorrect**

9. Laboratory results should maintained with full information and kept confidentially.
10. Laboratory personnel should follow organizational rule and regulation of record keeping. (2 points)
11. The purpose of keeping laboratory record is only to retrieve the information when needed. ( 2 points)

**Note: satisfactory rating is 4 points, unsatisfactory <4 points. You can ask your instructor for copy of correct answer.**

**Answer Sheet**

**1.**_____

**2.** _____

**3.** _____

Score = _____

Rating: _____

Name: _____ Date: _____

## 6.2. Updating instrument maintenance logs

A maintenance log is a document (often relatively simple) that records who did what, when, and why. ...

Equipment logs are basically documents that are used in different purposes and for different functions with regards to equipment handling, maintenance, and usage.

Equipment Maintenance Schedule. It is essential that practices have a schedule for the maintenance and monitoring of all their key clinical equipment. Equipment that requires calibration, or that is electric or battery powered, needs to be serviced regularly to ensure it is, and continues to be, in good working order.


**Fig 6.3. Instrument maintenance template**

## Equipment Maintenance Record

Instrument: _____

Model Number: _____

Serial Number: _____
(or U of A tag number)

Located in Room Number: _____

Lab Supervisor: _____

| Maintenance performed | Date |
|---|---|
| | |
| | |
| | |

| Self-check 2 | Written test |
|---|---|

**Answer the following questions**

1. What is the purpose of equipment maintenance ? (4 points)

**Note: satisfactory rating is 4 points, unsatisfactory < 2points. You can ask your instructor for copy of correct answer.**

**Answer Sheet**

**1.**_____

Score = _____

Rating: _____

Name: _____ Date:

## 6.3. Ensure confidentiality

Professional **ethics** is the moral bond that links a profession, the people it serves, and society. Specific issues that challenge **laboratory** professionals in clinical research are allocation of health-care resources, testing conducted nearer to the patient, confidentiality, screening tests, and molecular biology.

**Definition**. **Confidentiality** is the right of an individual to have personal, identifiable **medical** information kept private. Such information **should** be available only to the physician of record and other health care and insurance personnel as necessary.

One of the most **important** elements of **confidentiality** is that it helps to build and develop trust. It potentially allows for the free flow of information between the client and worker and acknowledges that a client's personal life and all the issues and problems that they have belong to them.

A **breach of confidentiality** occurs when a **patient's** private information is disclosed to a third party without their consent. There are limited exceptions to this, including disclosures to state health officials and court orders requiring medical records to be produced.

| Self-check 3 | Written test |
|---|---|

**Answer the following questions**

1. What is confidentiality? (4 points)

**Note: satisfactory rating is 4 points, unsatisfactory <4 points. You can ask your instructor for copy of correct answer.**

**Answer Sheet**

**1.**_____

Score = _____

Rating: _____

Name: _____ Date:

| Self-check 3 | Written test |
|---|---|

**Answer the following questions**

2. List waste minimization activities. (4 points)

**Note: satisfactory rating is 4 points, unsatisfactory <4 points. You can ask your instructor for copy of correct answer.**

Score = _____

Rating: _____

**Answer Sheet**

**1.**_____

_____

_____

_____

Name: _____ Date: _____

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