Assist. Prof. Sinai Najy Muhsin

Pract.Bio. First class

Dentistry college

Entamoeba histolytica

E. histolytica is worldwide in distribution but more common in tropical and subtropical countries. Humans are the principal hosts and source of infection.

Morphology

E. histolytica has three stages—(1) tropho zoite: the habitat of trophozoites is th wall and lumen of the colon especially the cecum and sigmoido-rectal region (2) precyst and (3) cyst: is formed in the lumen of the colon and with feces and is immediately infctive.

Mode of infection

1-Autoinfectio :fecal-oral route
2-Hetroinfection: throught:
A-Eating raw vegetable feritilized by human faeces
B-Open source of watr contaminated with human excreta
C-Flies and cockroaches carrying cysts to food or drink
D-Food handlers especially chronic asymptomatic cyst carriers.

Pathogenesis

A-Intestinal amoebiasis including **1-**Amoebic ulcer The classical ulcer is **flask-shaped (broad base with a narrow neck). 2- Amoebic dysentery:** Trophozoites of *E. histolytica* secrete proteolytic enzymes that cause destruction and necrosis of tissue, and produces flask shaped ulcers on the intestinal mucosa.
B-Extraintestinal amoebiasis including **1-**hepatic amoebiasis **2-** pulmonary amoebiasis.

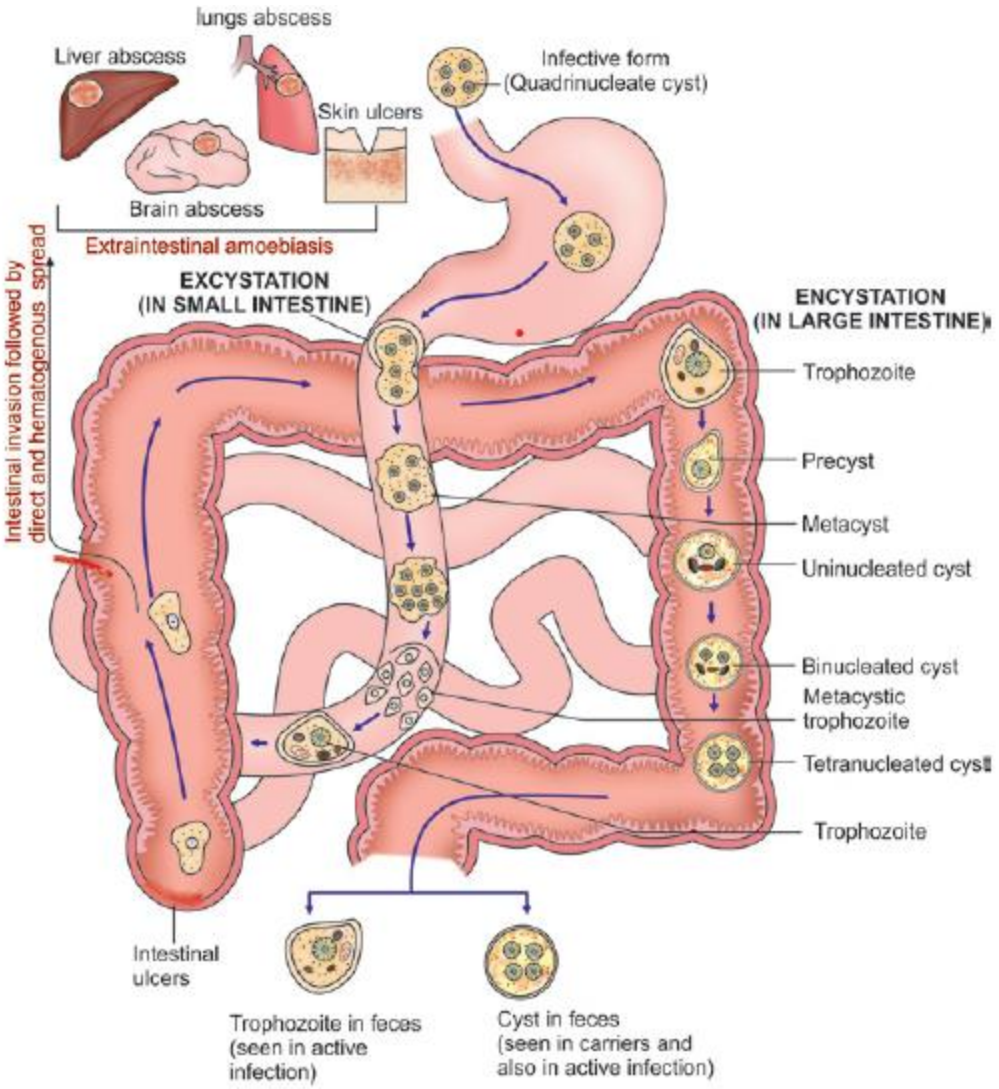


Fig. 3.2: Life cycle of *Entamoeba histolytica*

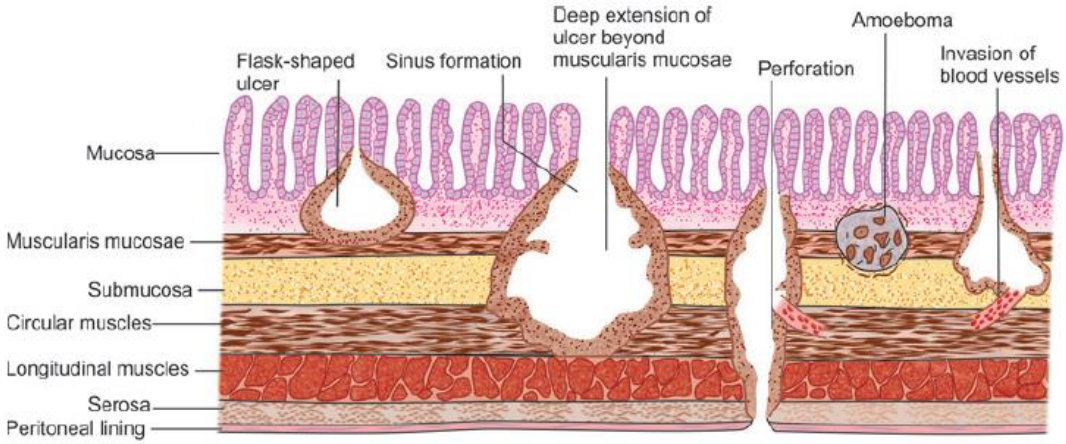
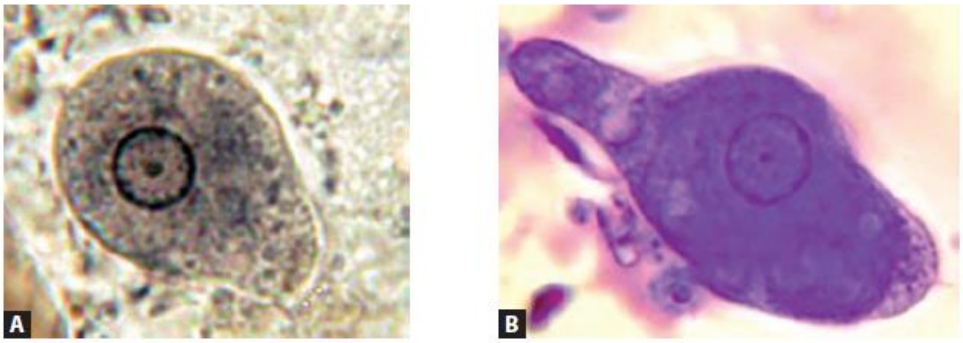


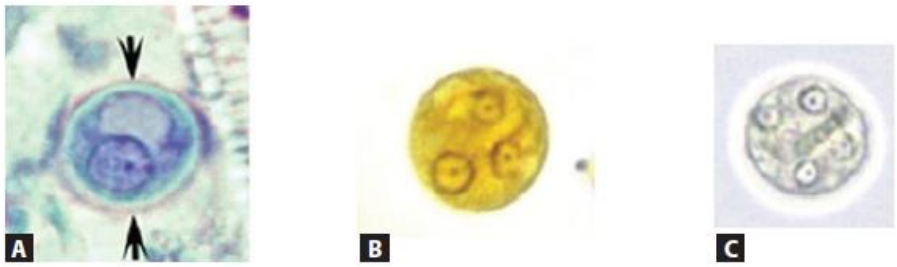
Fig. 3.3: Complications of intestinal amoebiasis (cross section of intestinal wall)

Laboratory Diagnosis

1-Stool microscopy by wet mount, permanent
stains, etc—detects cysts and trophozoites
2- Stool culture
A- Polyxenic and axenic culture
3- Stool antigen detection: ELISA(The enzyme-linked immunosorbent assay)
4- Serology
1- Amoebic antigen—ELISA
2- Amoeboic antibody— ELISA and IFA (Immunofluorescence assay: which uses fluorescent microscopy to detect antibodies to specific antigenic material)
3- Isoenzyme (zymodene) analysis
5- Molecular diagnosis
A-Nested multiplex PCR (Polymerase chain reaction).



Figs 3.5 A and B: Trophozoite of *Entamoeba histolytica* shows finger like psuedopodia and visible fine chromatin granule lining the nuclear membrane (A) hematoxylin stain; (B) trichrome stain
Source: Giovanni Swierczynski, Bruno Milanesi."Atlas of human intestinal protozoa Microscopic diagnosis" (*with permission*)



Figs 3.6 A to C: Cyst of *Entamoeba histolytica* (A) Giemsa stain shows uninucleated cyst (with glycogen vacuole); (B) iodine mount shows immature cyst (three nuclei); (C) saline mount shows mature cyst (with four nuclei)
Source: Giovanni Swierczynski, Bruno Milanesi. "Atlas of human intestinal protozoa Microscopic diagnosis" (*with permission*)

Entamoeba coli

E. coli is a nonpathogenic amoeba that colonizes the large intestine.
Th e life cycle is similar to *E. histolytica*
* It has also three forms—trophozoites, precyst
and cyst .
* It is frequently found in the stool samples of healthy individuals and should be differentiated from that of *E. histolytica*

Table 3.6: Differences between *Entamoeba histolytica* and *Entamoeba coli*

	Entamoeba histolytica	***Entamoeba coli***
Trophozoite		
Size	15–20 μm	20–25 μm
Motility	• Very active and unidirectional purposeful motility • Pseudopodia with finger like projection	• Sluggish, nonpurposeful and aimless motility in any direction • Blunt pseudopodia
Cytoplasm	Clearly differentiated to ectoplasm and endoplasm	Not differentiated
Cytoplasmic inclusions	RBC, leucocytes, tissue debris and bacteria	Same except it doesn't contain RBC
Nucleus	• Karyosome is small and central • Nuclear membrane is thin and lined by fine chromatin granules	• Karyosome is large and eccentric • Nuclear membrane is thick and lined by coarse chromatin granules
Precyst		
	10–20 μm size, oval with blunt pseudopodium, no food vacuoles and nucleus same as trophozoite	Same as *E. histolytica* except size is 20 μm
Cyst		
Size	12–15 μm	15–25 μm
Nucleus	Same as trophozoite	Same as trophozoite
Number of nuclei	1–4	1–8
Chromatoid body	Thick bars with rounded ends	Filamentous and thread like ends

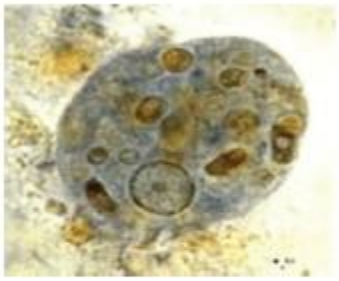


Fig. 3.8: Trophozoite of *Entamoeba coli* (Iron hematoxylin stain) shows nucleus with coarse peripheral chromatin and abundant food vacuoles in the cytoplasm containing fecal debris

Fig. 3.9: Cyst of *Entamoeba coli* (Iodine mount) shows seven nuclei

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Dentistry college

Flagellates (Intestinal and Genital)

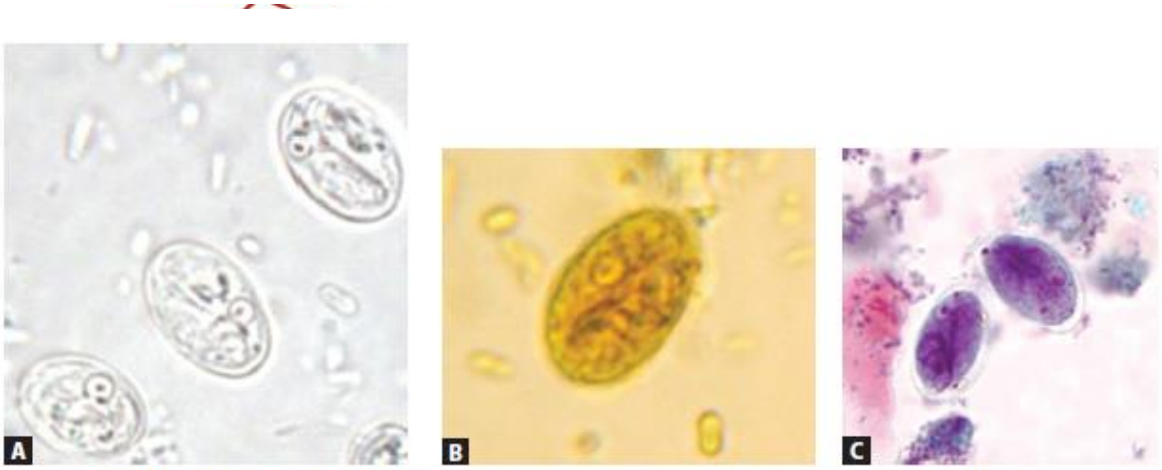
Giardia lamblia

Habitat and Morphology

Duodenum and upper part of jejunum. *Giaridia* completes its life cycle in one host It occurs in two forms—(1) trophozoite: The trophozoite has a falling leaf-like motility, usually measures 10–20 μm in length and 5–15 μm in width. And (2) cyst: *Giardia* cyst is oval shaped, measures 11–14μm in length and 7–10 μm in width. zz It contains four nuclei. Mature cyst acts **infective form.**

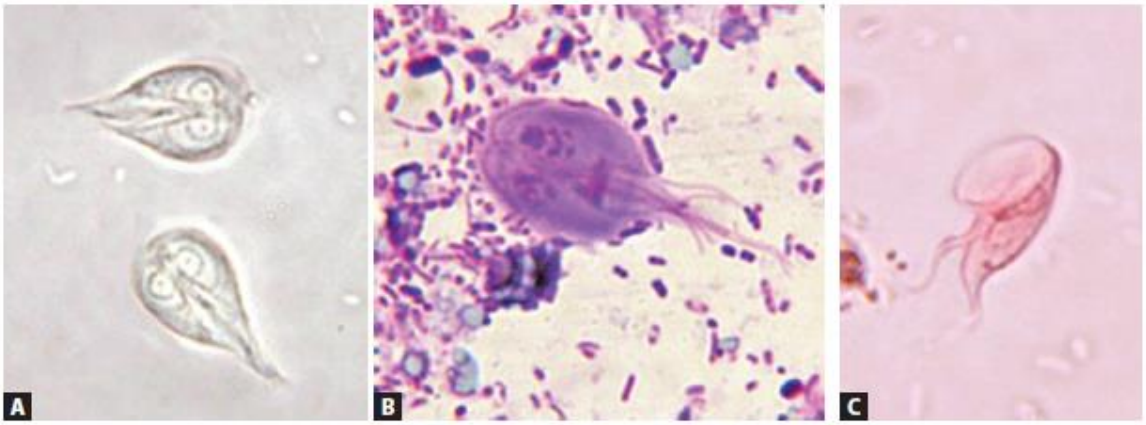
Mode of transmission: Man acquires infection by ingestion of food and water contaminated with mature cysts or rarely by sexual route.

Pathogenicity: Steatorrhea (fatty diarrhea)



Figs 4.3A and B: Cysts of *Giardia lamblia* (A) saline mount (B) iodine mount (C) trichrome stain

Source: A- and B- Giovanni Swierczynski, Bruno Milanesi "Atlas of human intestinal protozoa Microscopic diagnosis" (*with permission*); C- DPDx Image Library, Centre for Disease Control and prevention (CDC), Atlanta (*with permission*)



Figs 4.4A to C: Trophozoites of *Giardia lamblia* (A) saline mount front view; (B) Giemsa stain front view; (C) merthiolate iodine formalin (MIF) stain lateral view (spoon shaped)

Source: Giovanni Swierczynski, Bruno Milanesi. "Atlas of human intestinal protozoa Microscopic diagnosis" (*with permission*)

Laboratory diagnosis

1-*Giardia* cysts can be demonstrated by iodine and saline wet mount

2-Concentration techniques like zinc sulfate floatation or formalin ether sedimentation methods.

3-Duodenal sampling: If stool examination is negative, then direct duodenal samples like aspirates (obtained by entero-test) or biopsy (done by endoscopy) should be processed.

4-Entero-test:It uses a gelatin capsule attached to a thread.

*One end of the thread is attached to the inner aspect of the patient's cheek, and then, the capsule is swallowed * Capsule gets dissolved in the intestine releasing the thread which is kept there for 4–6 hours to take the duodenal fluid * Later, the thread is withdrawn and shaken in saline to release trophozoites which can be detected microscopically * Th e entero-test is also useful in the search for other upper intestinal parasites.

5-Antigen Detection in Stool: Th e enzyme linked inmunosorbent assay (ELISA) the tests are highly sensitive (90–100%) and specific (99–100%).

6-Antibody detection: Both indirect fluorescent antibody (IFA) and ELISA formats are developed to detect antibodies in serum.

7-Culture: Giardia can be cultivated in **axenic media like Diamond's media** used for *E. histolytica.*

8-Molecular methods: by polymerase chain reaction (PCR).

9-Radiological finding: X-ray is generally nonspecific and may be positive in 20% of cases

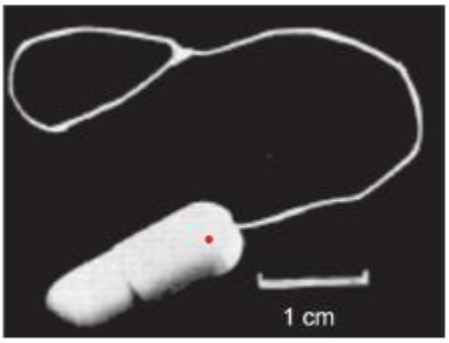
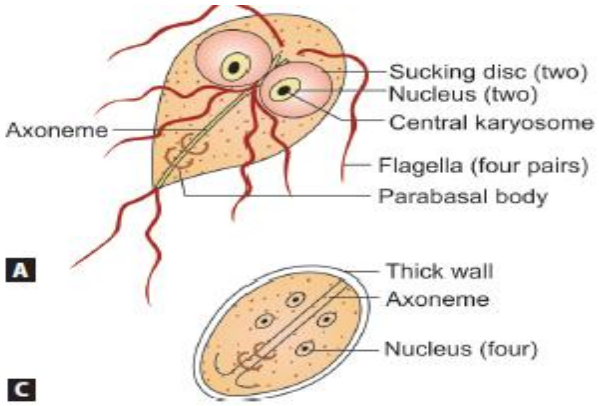


Fig. 4.5: Entero-test equipment showing duodenal capsule attached with thread at other end



Figs 4.1 A to C: *Giardia lamblia* (schematic diagram) (A) trophozoite

Trichomonas vaginalis

Habitat and Morphology

It is the most common parasitic cause of sexually transmitted diseases (STDs). Females are commonly affected than males Trophozoites are the only stage, there is no cystic stage. Trophozoite is pear (pyriform) shaped, measures 7–23 μm and 5–15 μm wide, resides in vagina and urethra of women and urethra, seminal vesicle and prostate of men.

Pathogenicity: Trichomoniasis is the most common parasitic cause of STDs.

Laboratory diagnosis

1-Direct microscopy

Samples: Vaginal, urethral discharge, urine sediment and prostatic secretions can be examined

A- **Wet (saline) mounting** of fresh samples (within 10–20 minutes of collection) should be done to demonstrate the jerky motile trophozoites and pus cells.

B-Permanent stain: Giemsa stain is routinely performed to demonstrate the morphology trophozoites

C- Direct fluorescent antibody test (DFA): Trophozoites are detected by staining with fluorescent

labeled monoclonal antibodies. DAF test is more sensitive (70–90%) than wet-mount examination.

2- Culture

A-Lash's cysteine hydrolysate serum media

B-Diamond's trypticase yeast maltose media

3- Antigen detection in Vaginal Secretion

4- Antibody detection

5- Molecular methods

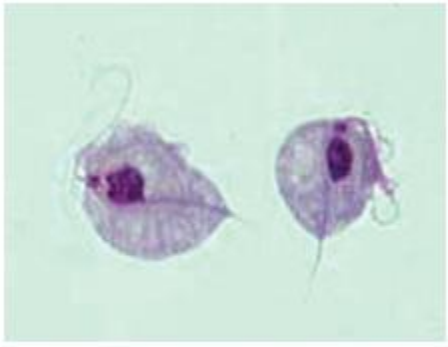


Fig. 4.7: *Trichomonas vaginalis* trophozoite (Giemsa stain)
Source: DPDx Image Library, Centre for Disease Control and prevention (CDC), Atlanta (*with permission*)

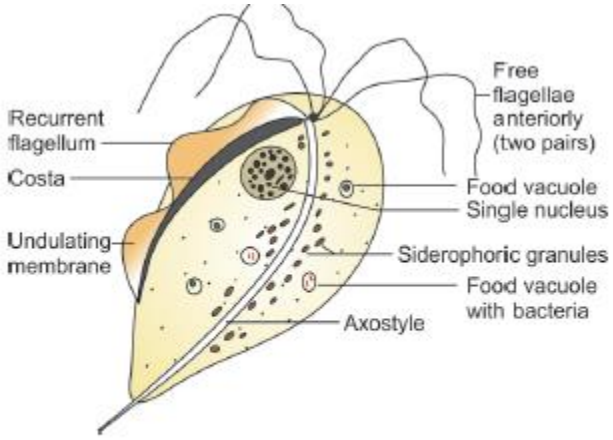


Fig. 4.6: Trophozoite of *Trichomonas vaginalis* (schematic diagram)

Trichomonas tenax

T. tenax is a harmless commensal in the mouth (gum and tartar of the teeth).
Trophozoite is pyriform shaped, measures (5–12 μm long and 5–10 μm wide)

Figure 22.11 *Trichomonas tenax*, specimen from the mouth, Giemsa stain. Note the undulating membrane extends almost the entire length of the body (arrow). Also note the axostyle protruding from the posterior end (oval). In this example, the flagella are clearly visible. doi:10.1128/9781555819002.ch22.f11

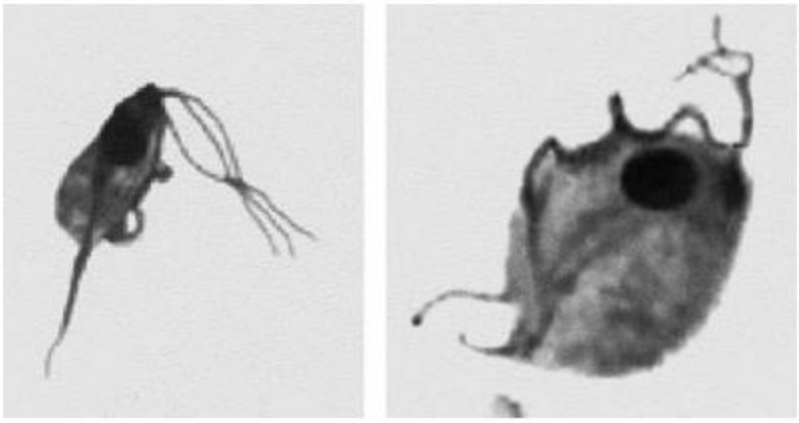
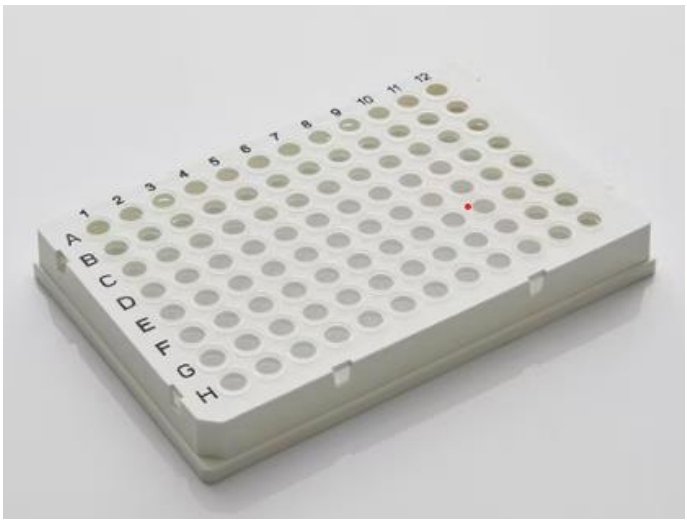
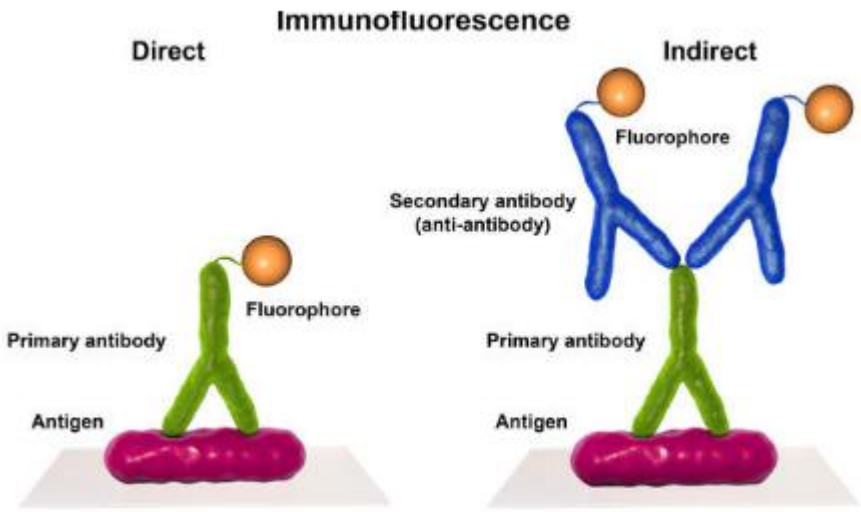


Figure 30.3 (Left) *Trichomonas tenax* from the mouth (stained with Giemsa stain). (Right) *Trichomonas vaginalis* from a genital specimen (stained with Giemsa stain). Note the large nucleus in *T. tenax* and the fact that this flagellate is somewhat smaller than *T. vaginalis*.

ELISA kit



Wells board of ELISA kit



principle of direct & indirect fluorescent antibody test

Assist. Prof. Sinai N.Muhsin AL-Doury

Pract. Bio.First class\Dentistry collage

Leishmania sp.

1-*Leishmania tropica* causes oriental sore, cutaneous leishmaniasis and Delhi boil.

2-*Leishmania donovani*. causes (or visceral leishmaniasis) Dum-Dum fever or kala-azar.

***Sand flies of genus *Phlebotomus sp* are the intermediate hosts and vectors.**

***Parasite morphology: Two developmental stages are formed: amastigotes and promastigotes.**

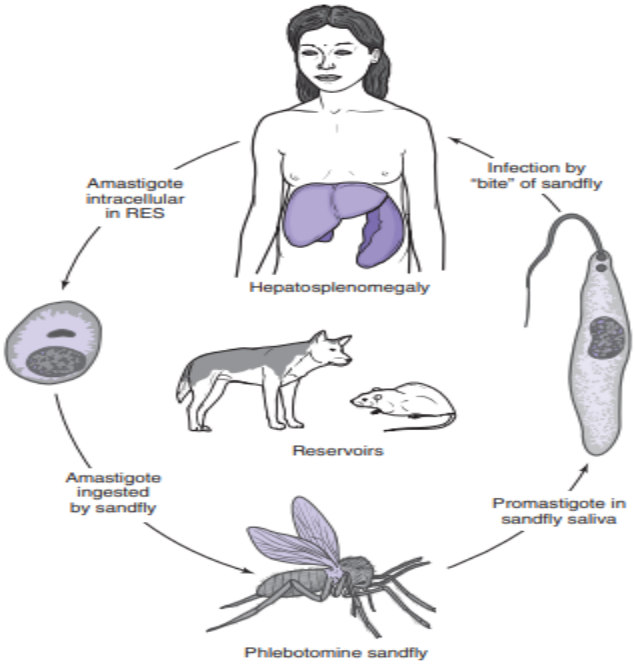


FIGURE 5-20 ■ Life cycle of *Leishmania donovani* complex.

(For viewing)

Laboratory Diagnosis…… *Leishmania tropica*

1- Microscopy—detects amastigotes
2- Culture—NNN medium
3- Montenegro test

Laboratory Diagnosis….*Leishmania donovani*

1-Microscopy (detects LD bodies)

A-Bone marrow aspiration: Most commonly Preferred
B-Lymph node aspirates (in African patients)

C-Liver biopsy

D-Peripheral blood smear(in HIV infected people)
E-Biopsy of various organs (in HIV infected people)
2-Culture (detects promastigotes)

A-NNN medium (Novy-MacNeal-Nicolle medium)

B-Schneider's liquid medium

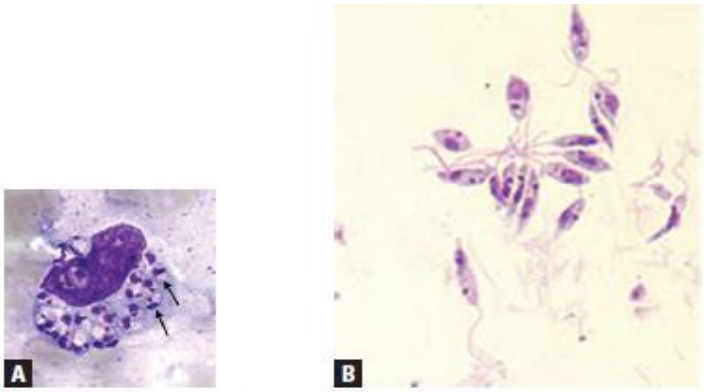
3-Antidbody detection in serum

A-ELISA

B-Direct agglutination test

4-Molecular method—PCR

5-Animal inoculation—golden hamster
6- Pancytopenia.
7- Leishmanin test (montenegro test)



Figs 5.5A and B: (A) Amastigote form [arrows shows inside a macrophage (Giemsa stain)]; (B) smear shows promastigote form (Giemsa stain) from culture
Source: DPDx Image Library, Centre for Disease Control and prevention (CDC), Atlanta (*with permission*)

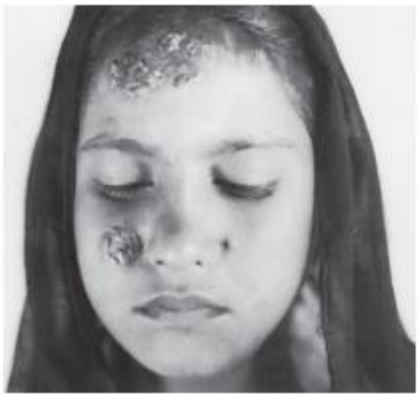


FIGURE 5-15 ■ Oriental sores on face and forehead. (Armed Forces Institute of Pathol
#80,717.)

Figure 5.17 The sand fly *Phlebotomus* sp., a vector of *Leishmania* spp.

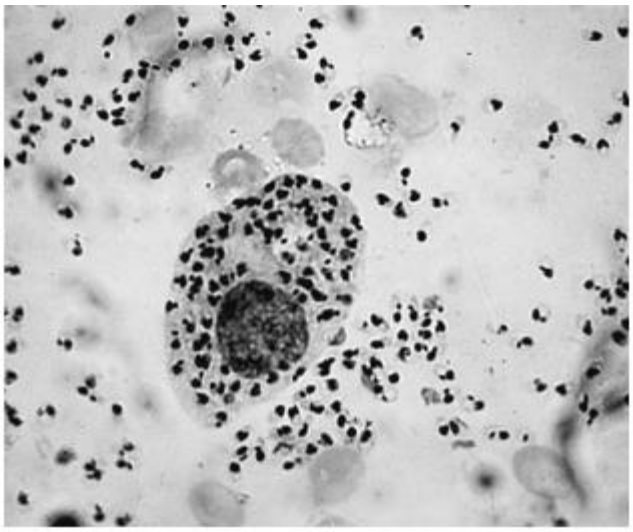


Figure 5.18 Spleen smear showing numerous intracellular and extracellular amastigotes of *Leishmania*

Kala azar

Lab (1)

Lecturer Assistant

Marwa Malik Khalaf Klara Majeed Shukur

Biology:

Laboratory instrumentation

Laboratory instrumentation is the use or application of instruments for observation, measurement, or control. the use of one or more instruments in carrying out laboratory tests. The use of UV spectrophotometry (to measure light intensity)

Laboratory Instrument is any implement, tool, or utensil used for laboratory test. An instrument is a device that measures a physical quantity, such as flow, concentration, temperature, level, distance, angle, or pressure. Instruments may be as simple as direct reading hand-held thermometers or as complex as multi-variable process analyzers.

Medical instrument is a device used to diagnose or treat diseases. A tool or device used for a particular purpose; especially: a tool or device designed to do careful and exact work. A device that measures something.

Laboratory equipment. Laboratory equipment refers to the various tools and equipment used by scientists working in a laboratory. Laboratory equipment is generally used to either perform an experiment or to take measurements and gather data. Larger or more sophisticated equipment is generally called a scientific instrument.

The classical equipment. includes tools such as Bunsen burners and microscopes as well as specialty equipment such as spectrophotometers and calorimeters.

Laboratory techniques. are the sum of procedures used on pure and applied sciences in order to conduct an experiment, all of them follow scientific method; while some of them involves the use of complex laboratory equipment from laboratory glassware to electrical devices others require such specific or expensive supplies.

Laboratory apparatus is a set of equipment or tools or a machine that is used for a particular purpose. **Laboratory apparatus** is the individual instruments or pieces of equipment, or the entire set of equipment to conduct projects and experiments. The laboratory apparatus depends upon the type of laboratory you are in and the experiment you are going to perform.

Laboratory tool. is any physical item that can be used to achieve a goal, especially if the item is not consumed in the process. Tools that are used in particular fields or activities may have different designations such as "instrument", "utensil", "implement", "machine", "device," or "apparatus". The set of tools needed to achieve a goal is "equipment". The knowledge of constructing, obtaining and using tools is technology

Lab (2) Biology

Lecturer Assistant

Marwa Malik Khalaf **Klara Majeed Shukur**

Biology:

Laboratory Apparatus

1. A microscope is one of the most common apparatus used in biology laboratories. It is mainly used to magnify small objects. To observe a specimen at the cellular level, a sample is taken and studied at the micro-level with the help of a microscope. It also helps to observe the shape and structure of a cell, distinguish various parts of a cell from each other, identify their particular functions, and determine the prime characteristics of microorganisms. A microscope is helpful to study almost all types of pathogens, bacteria, and viruses.

2. Beaker

Beaker is a cylindrical container that has a small spout and a flat base. The small spout helps to pour solutions with minimum spillage. Beakers come in a variety of shapes. Mostly, beakers are used to contain and store solutions. They are also used in combination with a burette to perform the titration process. Some of the beakers also contain a graduated scale attached to its exterior that helps to note the quantity of solution.

3. Crucible

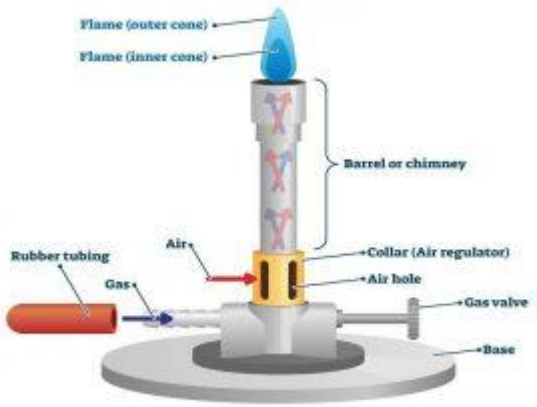
Crucible is a small container made up of ceramic or metal. A crucible is able to withstand high temperatures, and therefore, it is generally used to melt elements. One of the most common applications of crucibles lies in gravimetric chemical analysis.

4. Test Tubes

A test tube is a glass or plastic container that has a hemispherical base. The shape of a test tube is analogous to the shape of a human finger. The main purpose of a test tube is to hold, mix, and heat chemical substances and solutions. A test tube is also known as a sample tube or a culture tube.

5. Bunsen Burner

Bunsen burner is one of the most important laboratory instruments. It is a gas burner that produces a single open gas flame. A number of chemical reactions take place in the presence of fire. Bunsen burner acts as a source of heat to perform such experiments and reactions. It is also used to perform physical phenomena such as heating, boiling, and sterilization.

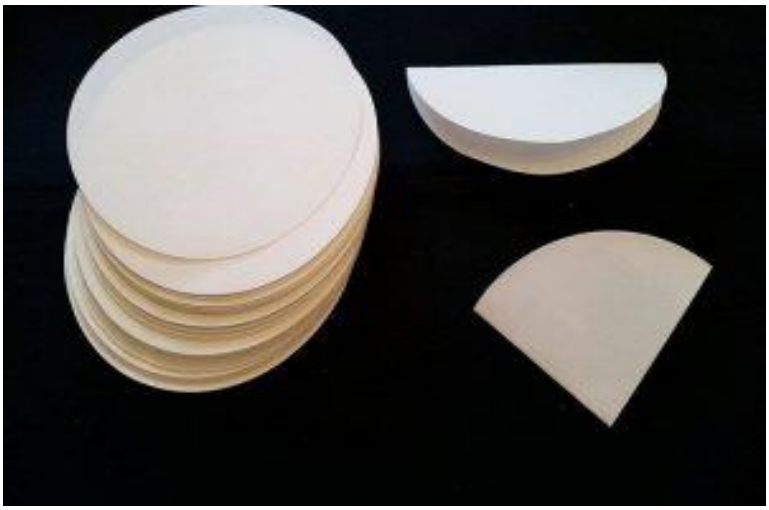


6. Flask

The flasks used in laboratories are available in a variety of shapes and sizes. They can have a curved, conical, or flat. Flasks are generally made up of glass or plastic and are used to store the solutions. Other uses of a flask include mixing of fluids, titration, etc. A flask may also be attached with a graduated scale to keep a record of the amount of solution poured into it.

7. Filter Paper

Filter papers are usually made up of cotton fibres. The key component used in the manufacturing of filter paper is cellulose. The main purpose of filter paper is to separate fine particles of substances from liquids or gases. The pores of the filter paper are fine enough to allow the liquid and gas molecules to pass through it easily but act as a barrier to solid particles, thereby blocking and capturing them.

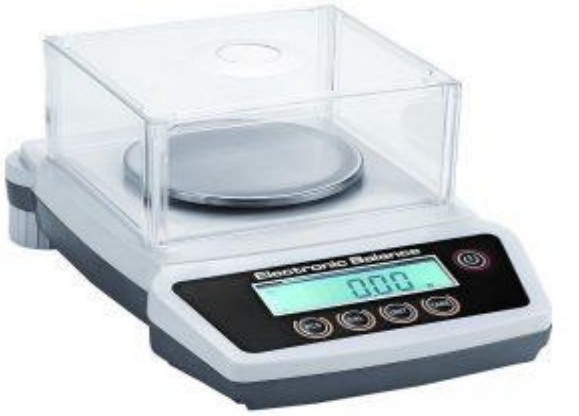


8. Dropper

A dropper consists of a glass tube that has a small opening at one end and is attached to a vacuum rubber bulb at the other end. A dropper is used when it is required to control the amount of solution being added to a reaction. To fill the dropper with a solution, the vacuum bulb is pressed and the open tip of the dropper is dipped into the container that contains the solution. When the vacuum bulb is released, the solution gets sucked into the glass tube. On pressing the bulb, the solution can be poured drop by drop. A dropper is also used as a medicine dropper.

9. Weighing Machine

A weighing balance is used to determine the mass or weight of certain objects. Most of the weighing instruments used in laboratories are electronically powered. These machines come in a variety of shapes and sizes. The weighing machines used in laboratories are compact and portable. The weighing capacity of such machines can be selected according to need.



10. Brush

The main task of a brush is to clean objects. Laboratory brushes are specifically designed to clean instruments that have a narrow opening such as test tubes, flasks, etc.

11. Spatula

A spatula is a laboratory utensil mainly used for mixing substances into a solution, stirring the solution, and scrapping objects. It is shaped like a spoon and is generally made up of carbon steel, stainless steel, porcelain, etc. It also consists of an insulator handle that allows the user to have a firm grip and avoid injuries.

12. Wash Bottles

Wash bottles are bottles attached with a nozzle typically made up of LDPE material. These bottles are mainly used to rinse various laboratory glassware.

13. Pipette

A pipette is a glass tube commonly used to transport a measured amount of liquid to a container. A pipette is designed in such a way that it is broad in the middle and consists of narrow ends. A mark present at the top portion of the pipette indicates the amount of liquid contained by it. A pipette is available in a number of sizes; therefore, different pipettes are used to transfer different volumes of liquid.

14. Funnel

The main purpose of a funnel is to channel or direct the flow of liquid in a particular direction. In the absence of a funnel, there may be chances of spilling the solution in the surroundings. The materials generally used to construct a funnel include glass, porcelain, plastic, etc.

15. Safety Apparatus Kit

Various safety equipment present in a safety apparatus kit, includes safety goggles, gloves, lab coats, etc. They protect the scientists working in the laboratory from severe injuries and helps to prevent mishaps and accidents.

16. Hot Plate

A hot plate is a device that consists of a flat plate attached to an electronic heating mechanism. The main aim of a hot plate is to evenly heat a substance or sample placed on the top of it. The advantage of using a hot plate in place of a bunsen burner is that a hot plate does not make use of flammable fuel, thereby minimizing the chances of accidents. Also, the temperature of a bunsen burner cannot be determined easily, but the temperature of a hot plate gets recorded and

displayed in digital format on the indicator attached to the device and can be controlled easily.

17. Forceps

Forceps are the tweezers, typically made up of metals, which are used to hold or pick up small objects. They are available in a variety of shapes and sizes. It consists of two tapered strips of metals attached to each other at one end. The angle between the two strips is maintained in such a way that when a force is applied to the middle portion of forceps, it gets squeezed and grips the object present in the middle of the open edges. The edges of the forceps can either be pointed or flat.

18. Measuring Cylinders

A measuring cylinder is a common laboratory instrument that is used to measure the amount of solution poured into it. As the name suggests, a measuring cylinder is a hollow glass cylinder with a flat base and a graduated scale attached to its curved boundary.

19. Dissecting Pans

Dissecting pans or trays are one of the most essential equipment required at a biology laboratory. While analyzing the internal structure of an organism or a specimen, a dissecting pan is used to hold the sample and allows the scientist study the characteristics of the sample with clarity. Dissecting trays are typically made up of aluminium and consist of a layer of paraffin wax. They also include odor absorbent pads to lock the foul smell.

20. Coverslips

Coverslips are the small square or circle shaped thin glass sheets that are used to cover the specimens that are under observation. It is also used to protect the microscope and prevent the slide from drying by locking the moisture. The placement of coverslips on the sample should be done with utmost care in such a way that air bubbles do not get trapped under the glass sheet. To properly cover the specimen with a coverslip, a few drops of water are poured on the sample to prevent it from drying and sticking to the base of the glass sheet. The edge of

the coverslip is then placed on the sample and is gently lowered with the help of a pointed tool.

21. Inoculating Loops

Inoculating loops are made up of platinum or nichrome wire. The tip of such a wire is shaped like a small loop that is about 5 mm in diameter. The main purpose of the inoculating loops is to pick up, separate, and transfer small pieces of a sample from a culture of microorganisms. An inoculating loop is also known as a smear loop, inoculation wand, or microstreaker. They are available in both disposable or reusable forms.

22. Petri Dishes

A petri dish is a shallow, transparent, cylinder-shaped lidded dish. A petri dish is mainly used to culture different types of cells including bacteria, fungi, moulds, etc. It is mainly made up of glass or plastic and consists of a thin layer of agar that provides a nutritional medium in which the cells can grow.

23. Centrifuge Machine

A centrifuge machine is a laboratory device that is mainly used to separate fluids (gases and liquids) on the basis of their density. A centrifuge machine mainly works on the basis of spinning. It consists of a vessel that spins at a high speed. The material poured into the spinning vessel experiences a significant amount of centrifugal force that pushes heavy substances to the outer side, leaving the light particles in the middle of the vessel. The heavy and light substances, therefore, get separated.

Lab (3)

Lecturer Assistant

Marwa Malik Khalaf **Klara Majeed Shukur**

Biology:

Introduction to the Microscope

Microscope History

• **1000AD** – The first vision aid was invented (inventor unknown) called a reading stone. It was ;a glass sphere that magnified when laid on top of reading materials.

• **1284 -** Italian, Salvino D'Armate is credited with inventing the first wearable eye glasses.
• **1590** – Two Dutch eye glass makers, Zaccharias Janssen and son Hans Janssen experimented with multiple lenses placed in a tube. The Janssens observed that viewed objects in front of the tube appeared greatly enlarged, creating both the forerunner of the compound microscope and the telescope.

• **1665** – English physicist, Robert Hooke looked at a sliver of cork through a microscope lens and noticed some "pores" or "cells" in it.

• **1674** – Anton van Leeuwenhoek built a simple microscope with only one lens to examine blood, yeast, insects and many other tiny objects. Leeuwenhoek was the first person to describe bacteria.

• **18th century** – Technical innovations improved microscopes, leading to microscopy becoming popular among scientists. Lenses combining two types of glass reduced the "chromatic effect" the disturbing halos resulting from differences in refraction of light.

• **1830** – Joseph Jackson Lister: reduces spherical aberration or the "chromatic effect" by showing that several weak lenses used together at certain distances gave good magnification without blurring the image. This was the prototype for the compound microscope.

• **1872** – Ernst Abbe wrote a mathematical formula called the "Abbe Sine Condition". His formula provided calculations that allowed for the maximum resolution in microscopes possible.

• **1903** – Richard Zsigmondy developed the ultra-microscope that could study objects below the wavelength of light. He won the Nobel Prize in Chemistry in 1925.

• **1931** – Ernst Ruska co-invented the electron microscope for which he won the Nobel Prize in Physics in 1986. An electron microscope depends on electrons rather than light to view an object, electrons are speeded up in a vacuum until their wavelength is extremely

short, only one hundred thousandth that of white light. Electron microscopes make itpossible to view objects as small as the diameter of an atom.

• **1932** – Frits Zernike invented the phase-contrast microscope that allowed for the study of colorless and transparent biological materials for which he won the Nobel Prize in Physics in 1953.

• **1981** – Gerd Binnig and Heinrich Rohrer invented the scanning tunneling microscope that gives three-dimensional images of objects down to the atomic level. Binnig and Rohrer won the Nobel Prize in Physics in 1986.

Microscope Care:

 Always carry with 2 hands

 Never touch the lenses with your fingers.

 Only use lens paper for cleaning

 Keep objects clear of desk and cords

 When you are finished with your "scope", rotate the nosepiece so that it's on the low power objective, roll the stage down to lowest level, rubber band the cord, then replace the dust cover.

Types of microscopes:

Light Microscope - the models found in most schools, use compound lenses and light to magnify objects. The lenses bend or refract the light, which makes the object beneath them appear closer.

Scanning Electron Microscope - allow scientists to view a universe too small to be seen with a light microscope. SEMs don’t use light waves; they use electrons (negatively charged electrical particles) to magnify objects up to two million times.

Transmission Electron Microscope - also uses electrons, but instead of scanning the surface (as with SEM's) electrons are passed through very thin specimens.

You will first learn to properly use the **Compound Light Microscope.**

Parts of the Microscope:

1. OCULAR LENS or EYEPIECE — On a binocular scope there are two ocular lenses, one for each eye. These lenses magnify the image at 10X power. The power of the ocular lens multiplied by the objective lens gives the total magnification of the microscope.

2. ARM — A support for the upper portion of the scope. It also serves as a convenient carrying handle.

3. MECHANICAL STAGE CONTROLS — A geared device to move the slide (placed in the slide clamp) precisely.

4. COARSE ADJUSTMENT KNOB — A rapid control which allows for quick focusing by moving the objective lens or stage up and down. It is used for initial focusing.

5. FINE ADJUSTMENT KNOB — A slow but precise control used to fine focus the image when viewing at the higher magnifications.

6. BASE — The part of your microscope that sits on a level, stable support.

7. OCULAR ADJUSTMENT — An adjustment for differences in the focusing abilities of your eyes.

8. DIOPTIC ADJUSTMENT — A horizontal adjustment of the oculars. Adjust for your eyes so you see only one field of view with both eyes open.

9. NOSEPIECE — A circular plate with 4 objective lenses that can be rotated into position for different magnifications.

10. OBJECTIVE LENS — Four separate lenses that magnify the image (4X, 10X, 40X and 100X) depending on the objective in use. The lens is positioned just above the object being viewed.

OBJECTIVE POWER OBJECTIVE NAME

4X SCANNING

10X LOW POWER

40X HIGH POWER

100X OIL IMMERSION

11. SLIDE CLAMP — A clamp to hold the slide on the stage.

12. STAGE — A platform for placement of the microscope slide.

13. CONDENSER — A lens that concentrates or directs the light onto the slide.

14. IRIS DIAPHRAGM CONTROL — A lever (or rotating disk) that adjusts the amount of light illuminating the slide. Use just enough light to illuminate the object on the slide and give good contrast.

15. FILTER HOLDER — A blue filter rests in this holder below the substage condenser.

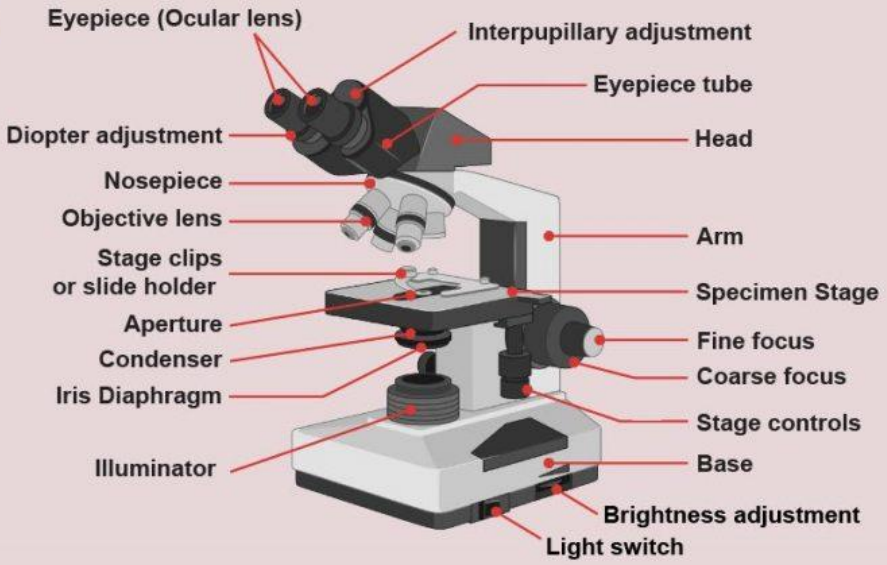
16. CONDENSER HEIGHT CONTROL — A knob that controls the height of the condenser.

17. LAMP — The light source.

18. LAMP SWITCH — Turns the lamp “on” and “off”.

Compound microscope

their parts and function



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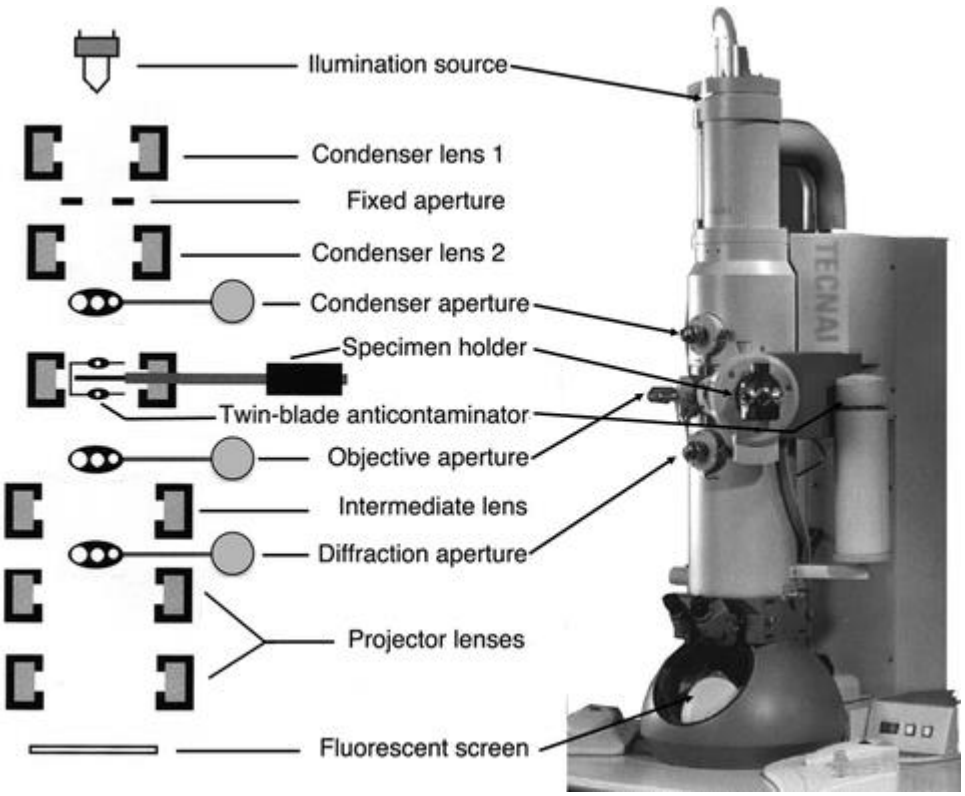


Microscope Accessories:

Prepared Slides – for those who do not wish to make their own slides or to supplement their collections, prepared slides are readily available. These slides can open up the world of microorganisms that lead to learning, discovery, and enjoyment. For best results use only glass slides that are 1" x 3" in size.

Filters – they can be useful in providing enhanced contrast and color correction for observing but not for photography. In some cases, colored filters can be a simple substitute for staining, which would kill live specimens. They usually lay over the top of illumination systems or on more expensive microscopes they sometimes have a special holder under the condenser. Blue is the most used filter since it absorbs some of the yellow to red light from of the illumination bulbs, used in many microscopes, resulting in a more natural coloration of the specimen. Green, yellow, and frosted filters all give varying effects and all filters should be experimented with to see the actual changes they make in observations of specimens.

Blank Slides – these are offered for those who want to make their own slides to observe. The higher quality ones are made of glass. Some may have a small depression or well to hold a few drops of liquid.

Cover Glass (Cover Slip) – these are extremely thin, flat glass or plastic covers that go over a specimen on a glass slide that has been made by you to protect the specimen during observation and storage. They come in different thicknesses which are usually matched to a number engraved on an objective lens for best performance.

Slide Making Kits – kits can contain blank slides, cover glasses, various types of stains to color objects or specimens, dissecting tools, labels, etc. which are all useful when making specimen slides

Imagers and Photo Adapters – you can do photomicrography (documenting images) through your microscope. The most common form to image (and view) through a microscope is to use digital or CCD cameras. Most imagers are used as an accessory on the microscope and use specific adapters to attach them to the eyepiece tubes of both compound and stereo microscopes .Various adapters are available to attach digital or film cameras to certain microscopes.

BASIC UNITS FOR MICROSCOPE

1 meter = 1000 millimeter
1 millimeter = 1000 micrometer (□m) = 10-6 meter
1 micrometer = 1000 nanometer (nm) = 10-9 meter
1 Angstrom (1 A) = 10-10 meter
1 nanometer = 10 Angstrom

Relative size of the microorganisms and their visibility. Man can see about 0.5 mm sized object whereas the light microscopes can be used to visualize up to 1 mm and EM (electron microscopes) can be used to view 1 nm objects.

Lab (4)

Lecturer Assistant

Marwa Malik Khalaf **Klara Majeed Shukur**

Biology:

Lab Safety and Sterilization:

Sterilization: means any process, either chemical or physical methods, which kills or removes **all forms of life** of pathogenic microorganism including vegetative cells, bacterial spores or viruses.

Disinfection: any process that destroy pathogenic microbes by disinfectants to **reducing the number** of them to point where they no longer cause disease.

Disinfectants are chemicals agents that are used for disinfection. Disinfectants should be used only on inanimate objects.

Antiseptics are mild forms of disinfectants that are used externally on living tissues to kill microorganisms, e.g. on the surface of skin and mucous membranes.

Uses of Sterilization:.

1- Sterilized of Surgical Procedures : Gloves, aprons, surgical instruments, syringes *etc*. are to be sterilized.

2- Sterilization in Microbiological works like preparation of culture media, reagents and equipment where a sterile condition is to be maintained.

Factors affecting of Disinfection and Sterilization :-

* Resistance of microorganism
* Concentration and potency of Disinfectants
* Physical and chemical factors
* Organic and inorganic matter structure
* Duration of exposure
* Number and Location of microorganism .

Types of sterilization:-

Two main types of sterilization are used :-

Chemical sterilization:

The chemicals have two types of effects either bacteriostatic or bactericidal. The chemical substances, when applied on inanimate objects such as surface of bench; are called **disinfectants**.Whereas the chemicals when applied on living tissues (such as skin) are called **Antiseptic agents**.

Chemical agents can kill or inhibit the microorganisms by:

(1) disruption of the lipid-containing cell membrane, Example: 70% Ethanol (widely used to clean the skin).

(2) Denaturation of proteins, Example: Iodine (used prior to obtaining a blood culture).

- **Phenol and Phenolic**
- **Alcohols**
- **Halogens**
- **Heavy metals**
- **Gaseous agents**
- **Soap and detergents**

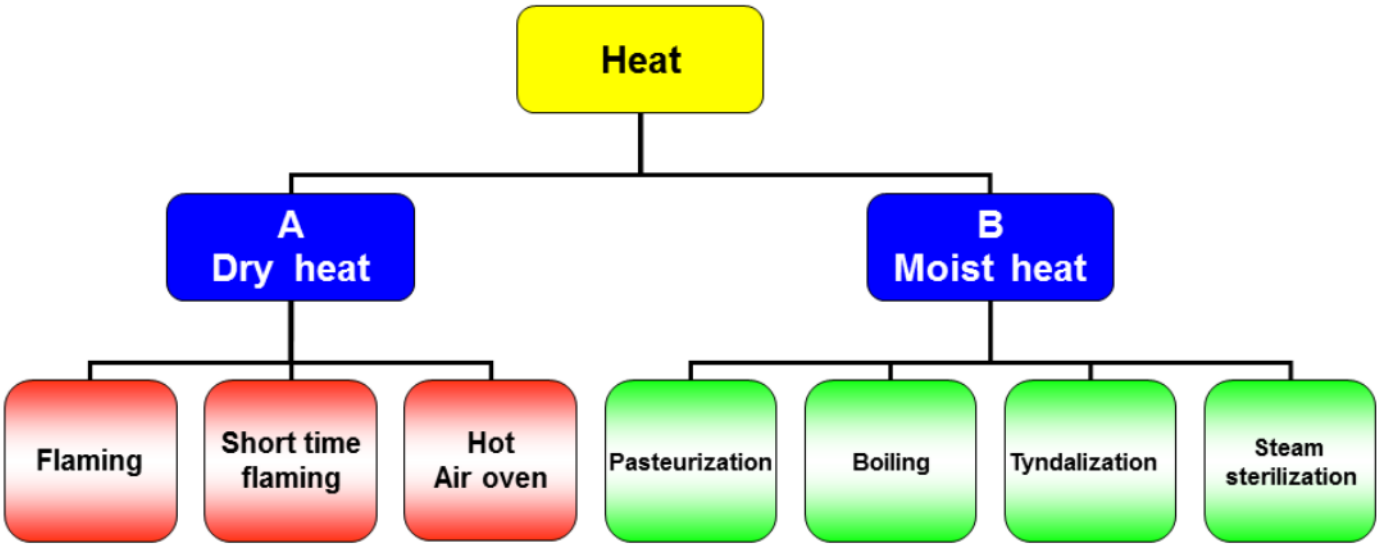


Physical sterilization:

The physical agents act by Heating, Radiation, or Filtration

Heating (high temperature):

The heating cause killing of organisms by denaturation of proteins and degradation of nucleic acid, membrane damage and enzymatic cleavage of DNA or oxidative damage.



The heating sterilization using high temperature involve two types:

1. Moist Heat Sterilization:

- Autoclaving (steam under pressure) is the most effective and most frequently used method of sterilization. Autoclave chamber is used in which steam, at a pressure of 15 pound / inch2, reaches a temperature of 121°C and is held at that temperature for 15 to 20 minutes. This heating kills even the highly heat-resistant spores of bacteria.

This method is used for sterilization of culture media, glassware, cotton swabs, white coat, any liquid.

- Boiling: The temperature employed is 100°C for 15 min. This method should be used only when is not alternative methods of sterilization. The method is sufficient to kill most of pathogenic organisms but not all or bacterial spores. This method used to sterilize metallic articles and glassware by boiling in **water bath**.

- Pasteurization, which is used primarily for milk, consists of heating the milk to 62°C for 30 minutes followed by rapid cooling.“ Flash” pasteurization is often used in very short time at 72°C for 15 seconds. This is sufficient to kill the bacterial pathogens in the milk (e.g. *Mycobacterium bovis* and *Brucella*).

2. Dry Heat Sterilization:

- Hot–air oven: is requiring temperature 170°C for 30 minutes. The sterilizer is used to sterilize all glassware (tubes, flask, beaker, pipette, Petri dish…etc),.

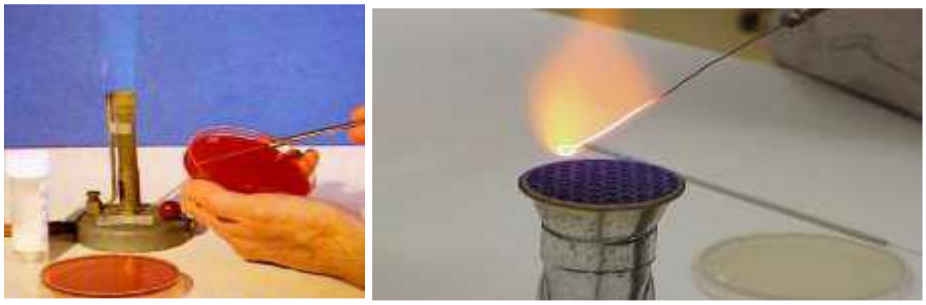
- Incineration is excellent method for rapidly destroying materials such as contaminated dressing, animal carcasses, risk wastes or pathological materials ….etc.

- Flaming .

 This method is commonly used in microbiology labs.

 Used for small metal or glass objects Inoculating loops, needles, forceps and scissors, but not for large objects.

 Inoculating loops and needles should be heated until they are red-hot. Before they are introduced into cultures, they must be allowed to cool down sufficiently to prevent killing organisms that are to be transferred.



- Short time flaming .

 Used for flaming test tube openings, flasks and pipettes in order to prevent contamination.

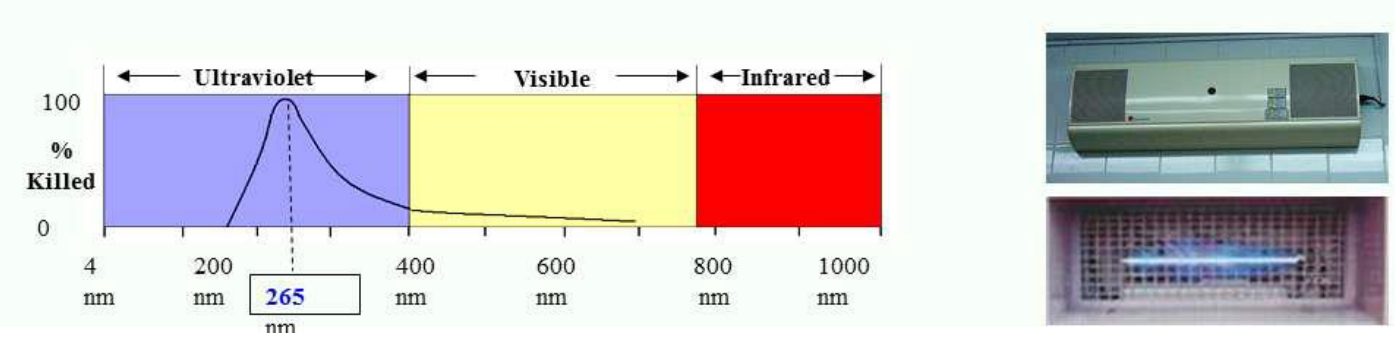
Radiation:

A. **Ionizing radiation** (X-ray and γ-ray):

It is used for sterilization of heat-sensitive items (disposable plastic Petri dishes, plastic tubes, disposable syringes, gloves, sutures…etc).

B. **Non-ionizing** (UV-light and IR-light):

It is used to sterilize any surface in laboratory, air in hospital operating room and preparation of vaccine.



Filtration (mechanical sterilization):

Bacteria can be removed from liquid materials by passing them through filters that have very small pores (millipore filters with pore size of 0.22 μm).

Filtration is used for :

1. Sterilizing substances that sensitive to heat (like serum, urine, sugar solutions, etc).

2. Preparation of antibiotic solutions.

3. Preparation of vaccines.

Types of Filter used :-

1. Membrane filters .

• It is most common used filters in microbiology lab.

• Membrane filters remove microorganisms by screening them out.

• It is made of cellulose acetate, cellulose nitrate, chemically inert and autoclavable.

• Wide vary of pore sizes are available, the most used one is 0.22 μm with a special holder.

2. **Seitz filters :-** Made of asbestos pad layers.
3. **Sintered glass filters : -** Made of glass like plates with pores that materials can pass through.
4. **Chamber land filters :-** Made of kaolin.

Every employee has rights and responsibilities for creating and maintaining as safe and healthy workplace

Rights

- A safe workplace
- To stop work and alert your supervisor when unsafe conditions or actions are recognized
- An obligation not to perform unsafe tasks.
- Information to protect you from hazards Training
- Medical treatment for workplace injuries or illnesses
- Access to your occupational exposure records

Responsibilities

- Work safely and report unsafe conditions to your supervisor
- Follow University policies and procedures
- Properly use appropriate safety equipment
- Participate in required training
- Notify supervisor of accidents and near-misses

‘safety’ means the protection of
people and the environment against radiation risks, and the safety of facilities
and activities that give rise to radiation risks. ‘

Lab (5)

Lecturer Assistant

Marwa Malik Khalaf Klara Majeed Shukur

Biology:

Staining {Gram Stain}

Gram Staining is the common, important, and most used differential staining techniques in microbiology, which was introduced by Danish Bacteriologist Hans Christian Gram in 1884. This test differentiate the bacteria into Gram Positive and Gram Negative Bacteria, which helps in the classification and differentiations of microorganisms.

Bacteria cells are almost colorless and transparent . A staining technique is often applied to the cells to color them → Their shape and size can be easily determined under the microscope**.**

Principle of staining

Stains → combine chemically with the bacterial protoplasm.

Commonly used stains are salts:

- Basic dyes: colored cation + colorless anion

e.g. methylene blue (methylene blue chloride)
MB+ + Cl-

- Acidic dyes: colored anion + colorless cation

e.g. eosin (Na+ + eosin-).

Bacterial cells are slightly negatively charged (rich in nucleic acids bearing negative charges as phosphate groups) → combine with positively charged basic dyes.
Acidic dyes do not stain the bacterial cell → can stain the background material with a contrasting color.

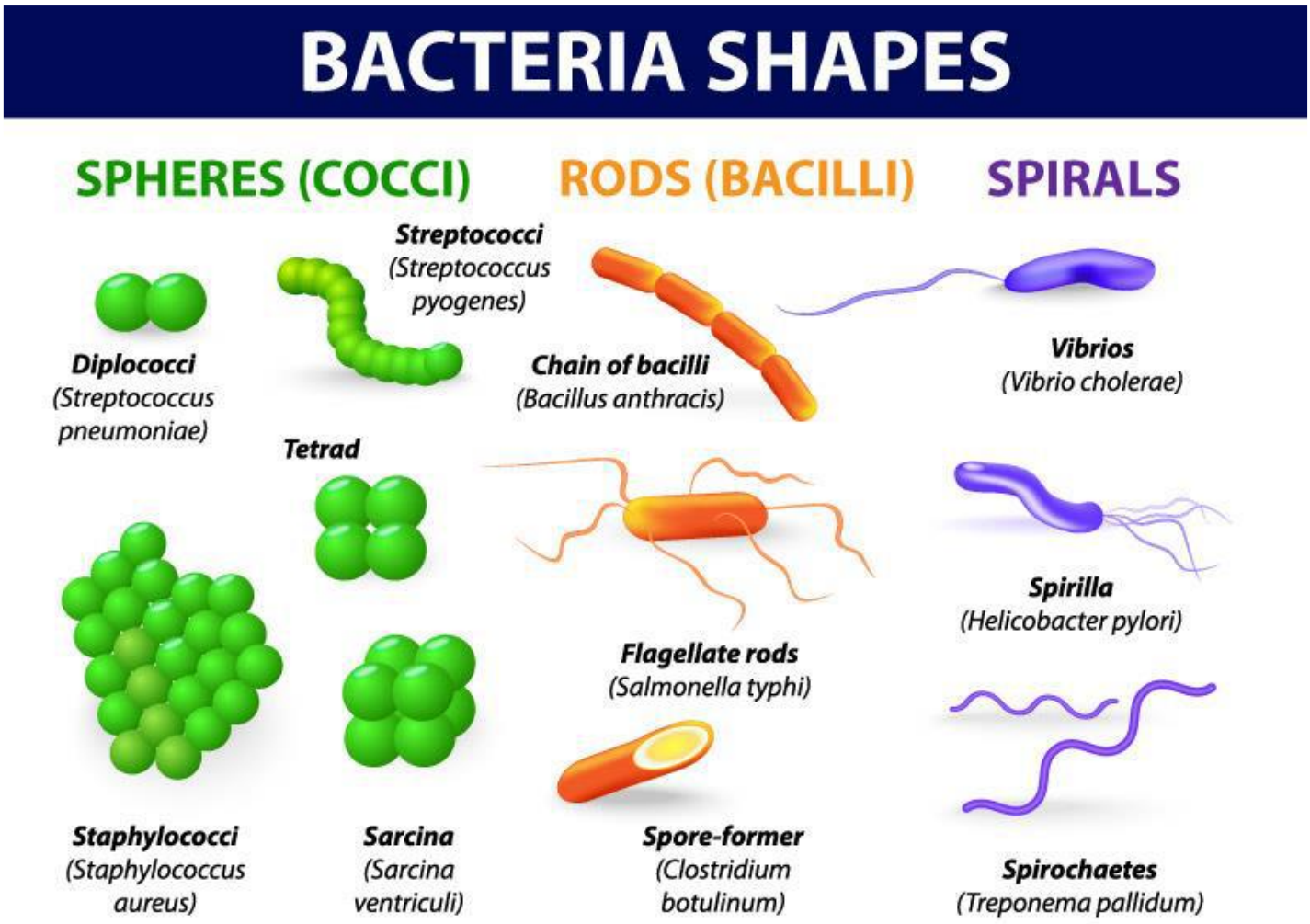
Bacterial Morphology

Bacterial morphology deals with size, shape, and arrangement of bacterial cells

- **Coccus** or **Cocci** are bacterial cells that are spherical, and resemble tiny balls (Streptococcus)
- **Bacillus** or **Bacilli** are bacterial cells that are rod shaped
- **Spiral** bacteria have twisted or helical morphology. They may appear as curved rods, called vibrios, as spirilla or spirochetes having pliant bodies

Arrangement of cells Arrangement of cocci cells
Singly: Bacteria that appear as single cell, is just called as cocci
Diplococci: These cells are found in pairs and they are found attached to each other
(Streptococcus) These bacteria form long chains and remain attached to each other
(Staphylococcus) These bacteria are arranged irregularly in clusters like grapes **Arrangement of Bacilli**

Singly: Bacteria that exists as single cell, called bacilli



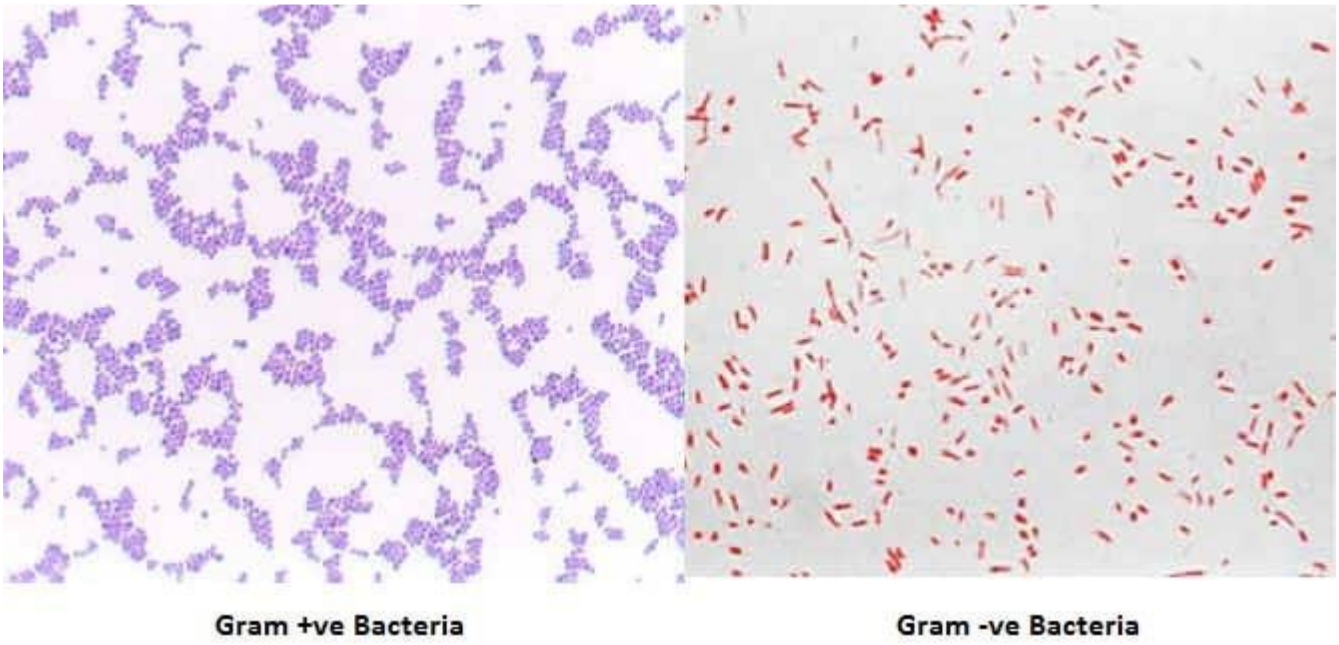
Reagents Used in Gram Staining

- Crystal Violet, the primary stain
- Iodine, the mordant
- A decolorizer made of acetone and alcohol (95%)
- Safranin, the counter stain

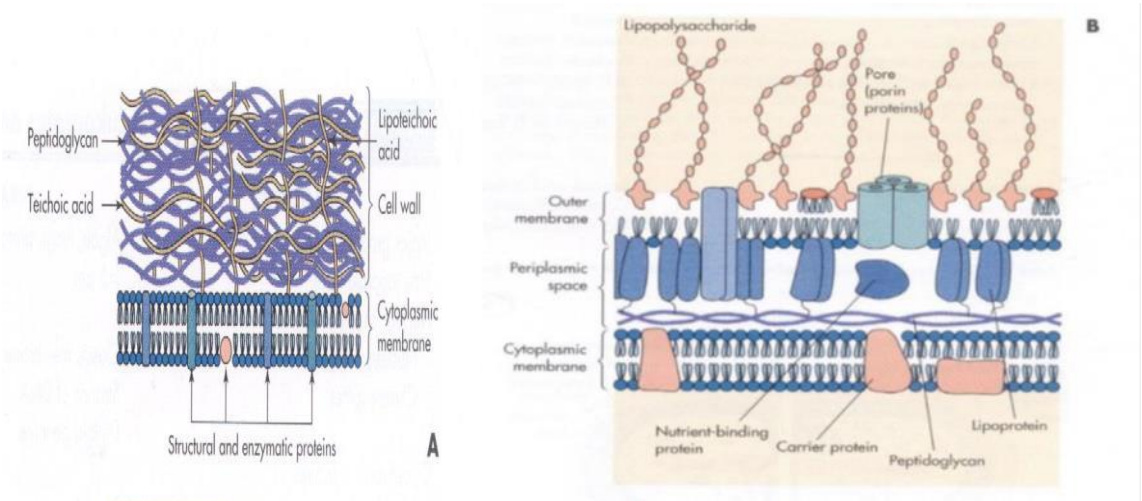
Procedure of Gram Staining

1. Take a clean, grease free slide.
2. Prepare the smear of suspension on the clean slide with a loopful of sample.
3. Air dry and heat fix
4. Crystal Violet was poured and kept for about 30 seconds to 1 minutes and rinse with water.
5. Flood the gram's iodine for 1 minute and wash with water.
6. Then ,wash with 95% alcohol or acetone for about 10-20 seconds and rinse with water.
7. Add safranin for about 1 minute and wash with water.

8. Air dry, Blot dry and Observe under Microscope
Interpretation
Gram Positive: **Purple Color** Gram Negative: **Red Color**.



Gram +ve Bacteria **Gram -ve Bacteria**



Gram-positive bacteria

• Have a thick peptidoglycan layer surrounds the cell.

•The stain gets trapped into this layer and the bacteria turned purple.

•Retain the color of the primary stain (crystal violet) after decolorization with alcohol

Gram-negative bacteria

•Have a thin peptidoglycan layer that does not retain crystal violet stain.

•Instead, it has a thick lipid layer which dissolved easily upon decoulorization with acetone-alcohol.

•Therefore, cells will be counterstained with safranin and turned red.

Lab (6)

Lecturer Assistant
Marwa Malik Khalaf Klara Majeed Shukur

Biology:

Culture Media

Culture media is a gel or liquid that contains nutrients and is used to grow bacteria or microorganisms. They are also termed growth media. Different cell types are grown in various types of medium. Nutrient broths and agar plates are the most typical growth media for microorganisms. Some microorganisms or bacteria need special media for their growth.

The Culture Media are classified in many different ways :

a. Types of Culture Media based On Consistency/Physical Composition :

1. Solid Media.

2. Semi-Solid Media.

3. Liquid Media.

1. Solid Media : It is for the isolation of bacteria as a pure culture on a solid medium. examples of solid media: nutrient agar, macconkey agar, blood agar, chocolate agar.

2. Semi-Solid Media : This media shows the motility of bacteria and the cultivation of microaerophilic bacteria. examples of semi-solid media: stuart's and amies media, hugh and leifson's oxidation fermentation medium, and mannitol motility media.

3. Liquid Media : This media shows the growth of a large number of bacteria. It is called Broth that allows bacteria to grow uniformly with turbidity. examples of liquid media nutrient broth, tryptic soy broth, mr-vp broth, phenol red carbohydrate broth.

b. Types of culture media based on chemical composition /application : There are seven routine laboratory media

1. Basal media.
2. Enriched media.
3. Selective media.
4. Indicator media or differential media.
5. Transport media.
6. Storage media.
7. Synthetic Media.

1. Basal Media: Basal media are those that may be used for growth (culture) of bacteria that do not need enrichment of the media. Examples: Nutrient broth, nutrient agar and peptone water. Staphylococcus and Enterobacteriaceae grow in these media.

2. Enriched Media: The media are enriched usually by adding blood, serum or egg. Examples: Enriched media are blood agar Streptococci grow in blood agar media.

3. Selective Media: These media favors the growth of a particular bacterium by inhibiting the growth of undesired bacteria and allowing growth of desirable bacteria. Examples: MacConkey agar, Lowenstein-Jensen media, tellurite media (Tellurite inhibits the growth of most of the throat organisms except diphtheria bacilli). Antibiotic may be added to a medium for inhibition.

4. Indicator (Differential) Media : An indicator is included in the medium. A particular organism causes change in the indicator, Examples: Blood agar and MacConkey agar are indicator media.

5. Transport Media : These media are used when speciemen cannot be cultured soon after collection. Example: Stuart medium.

6. Storage Media: Media used for storing the bacteria for a long period of time. Examples: Egg saline medium, chalk cooked meat broth.

7. Synthetic Media : These are chemically defined media prepared from pure chemical substances. It is used in research work.

Common Media In Routine Use

Nutrient Broth. Uses: (1) As a basal media for the preparation of other .media, (2) To study soluble products of bacteria

Nutrient Agar. It is solid at 37°C

Peptone Water. It is used as base for sugar media and to test indole formation

Blood Agar. Most commonly used medium. Certain bacteria when grown in blood agar produce haemolysis around their colonies. Certain bacteria produce no haemolysis. **Types of changes : (a) beta** (b) haemolysis. The colony is surrounded by a clear zone of complete haemolysis, e.g. Streptococcus pyogenes is a beta haemolytic streptococci, **(b) Alpha** (a) haemolysis. The colony is surrounded by a zone of greenish discolouration due to formation of biliverdin, e.g. Viridans streptococci, **(c) Gamma** (y) haemolysis, or, No haemolysis. ,There is no change in the medium surrounding the colony.

Chocolate Agar or Heated Blood agar: Prepared by heating blood agar. It is used for culture of pneumococcus, gonococcus, meningo- coccus and Haemophilus. Heating the blood inactivates inhibitor of growths.

MacConkey Agar. Most commonly used for enterobacteriaceae.

Blood agar

chocolate agar

macConkey agar

How to prepare culture media?

1. Weigh the amount of ingredients powder on weighing machine.

2. Dissolve the ingredients in distilled water.

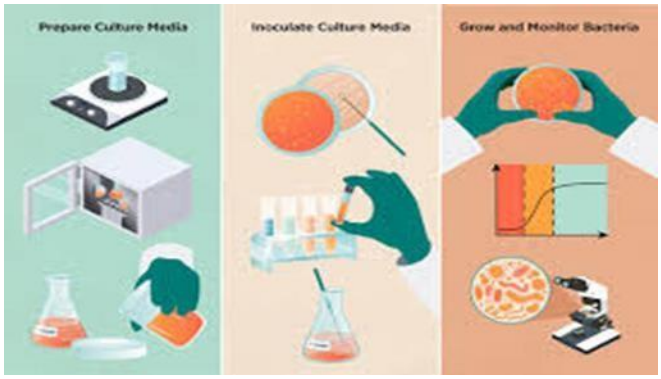
3. Adjust PH of the medium if needed.

4. Add agar and boiled it to dissolve.

5. Pour the media into flask.

6. Autoclave the media when ingredients fully dissolve.

7. Sterilization is done in autoclave to prevent from contamination, at 121ºC for 15 min at 15lbs.



8. After the autoclave place the media flask in laminar air flow.

9. Sterilize the laminar air flow with 70% alcohol.

10. A bit cools down the media and pours into sterile Petri-plates for solidification.

11. Then sample is ready to spread(spreader) / streak

12. (Inoculation loop) on the medium for identification or isolation of microbes.

13. Sealed the Petri plates with paraffin, label them.

14. Keep them inverted in incubator at 37ºC for 24hrs.

15. Observe the result next day colonies formation is visible on the media.

Application of culture media:

1. To culture microbes.
2. To identify the cause of infection.
3. To identify characteristics of microorganisms.
4. To isolate pure culture.
5. To store the culture stock.
6. To observe biochemical reactions.
7. To test microbial contamination in any sample.
8. To check antimicrobial agents and preservatives effect.
9. To observe microbe colony type, its color, shape, cause.
10. To differentiate between different colonies.
11. To create antigens for laboratory use.
12. To estimate viable count.
13. To test antibiotic sensitivity.

Assist. Prof. Sinai N.Muhsin AL-Doury

Pract. Bio.First class

Dentistry collage

Plasmodium vivax

*Transmitted to humans by the bite of female ***Anopheles mosquitoes***.

Plasmodium vivax* is the common cause of benign **tertian malaria

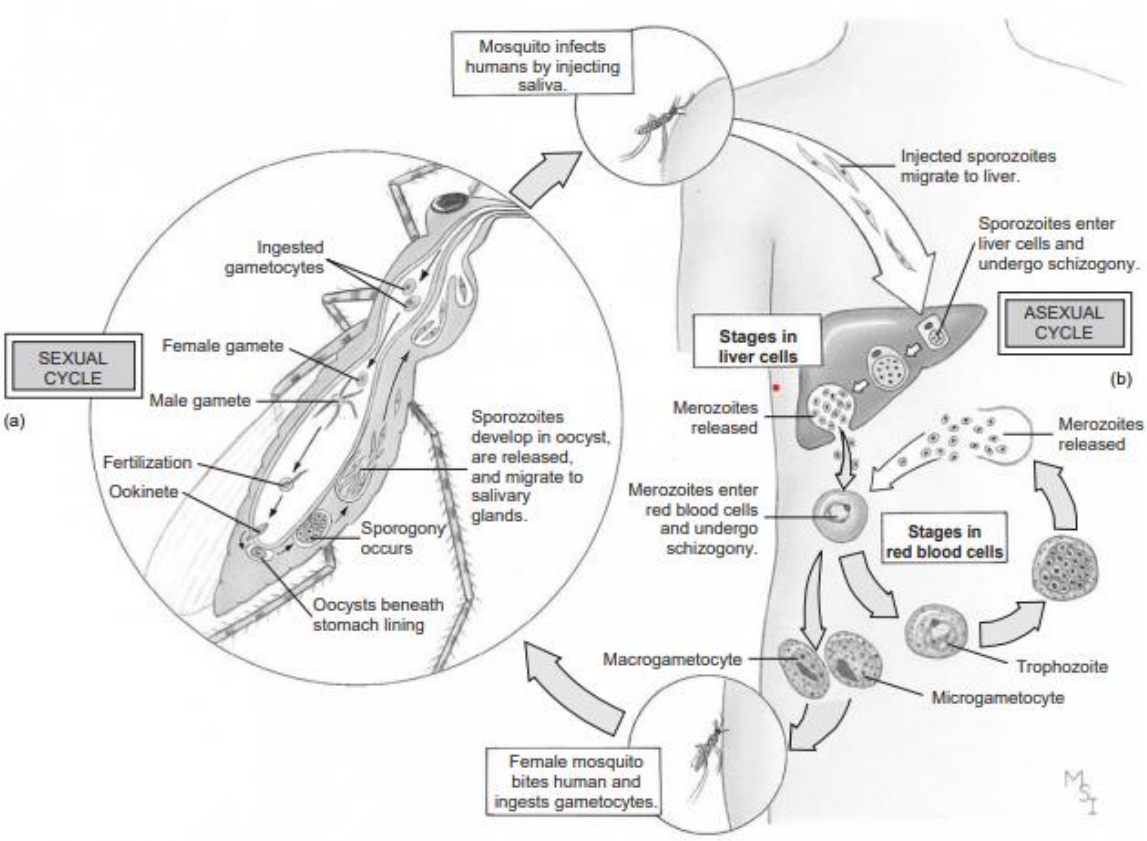


Figure 9.1 **Life cycle of *Plasmodium vivax*.**
(*a*) Sexual cycle produces sporozoites in body of mosquito. Meiosis occurs just after zygote formation (zygotic meiosis). (*b*) Sporozoites infect a human and reproduce asexually, first in liver cells and then in red blood cells.

Indirect life cycle

Laboratory Diagnosis

1- microscopic tests:

A- Peripheral blood smear

*Thick smear—more sensitive

*Thin smear—speciation can be done

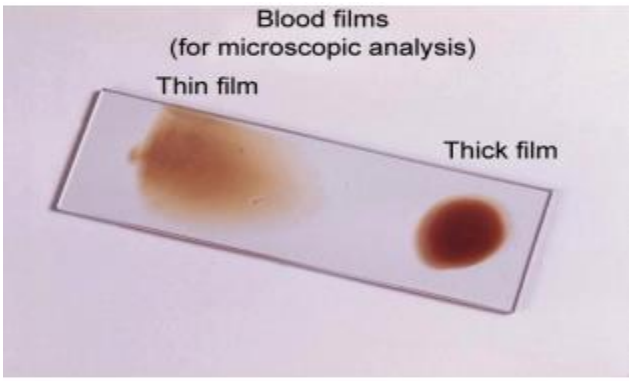
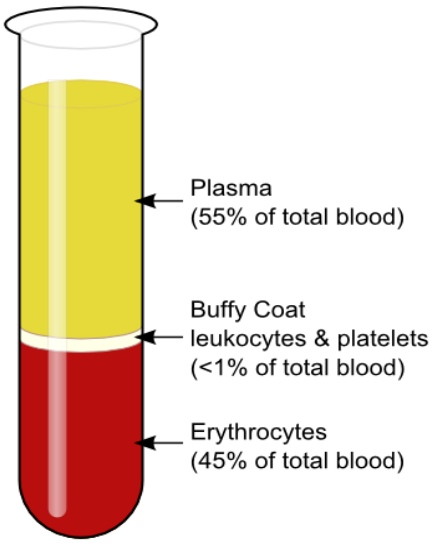


Fig. 6.4: Glass slide showing thin and thick blood smear

B-Fluorescence microscopy (Kawamoto's technique).

C- Quantitative buff y coat examination (QBC).



(QBC)

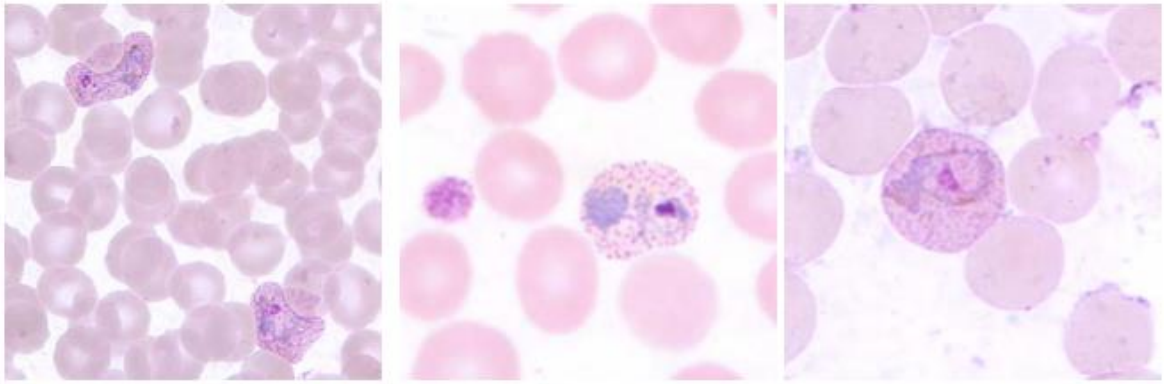
2-Nonmicroscopic tests:

A-Antigen detection tests.

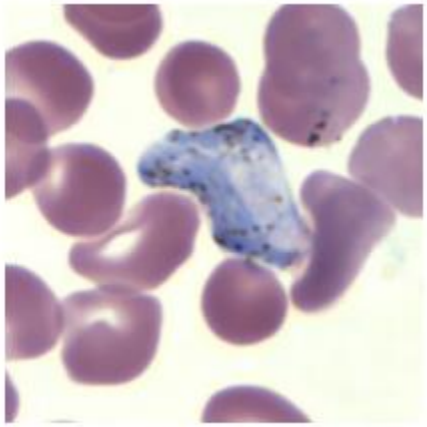
B-Antibody detection—ELISA.

C-Culture— Roswell Park Memorial Institute RPMI 640 medium .

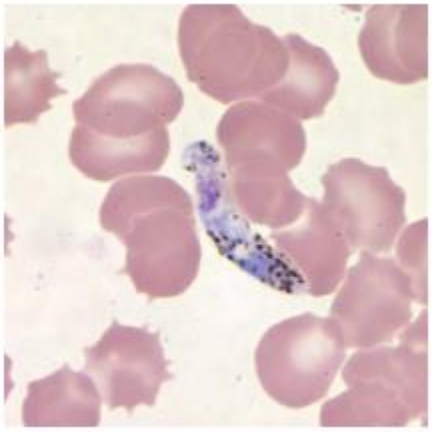
D-Molecular diagnosis—PCR.



Large, ameboid trophozoites in thin blood smears. Note the presence of Schüffner's dots, which are best seen when the blood is stained with Giemsa, and not Wright's stain.



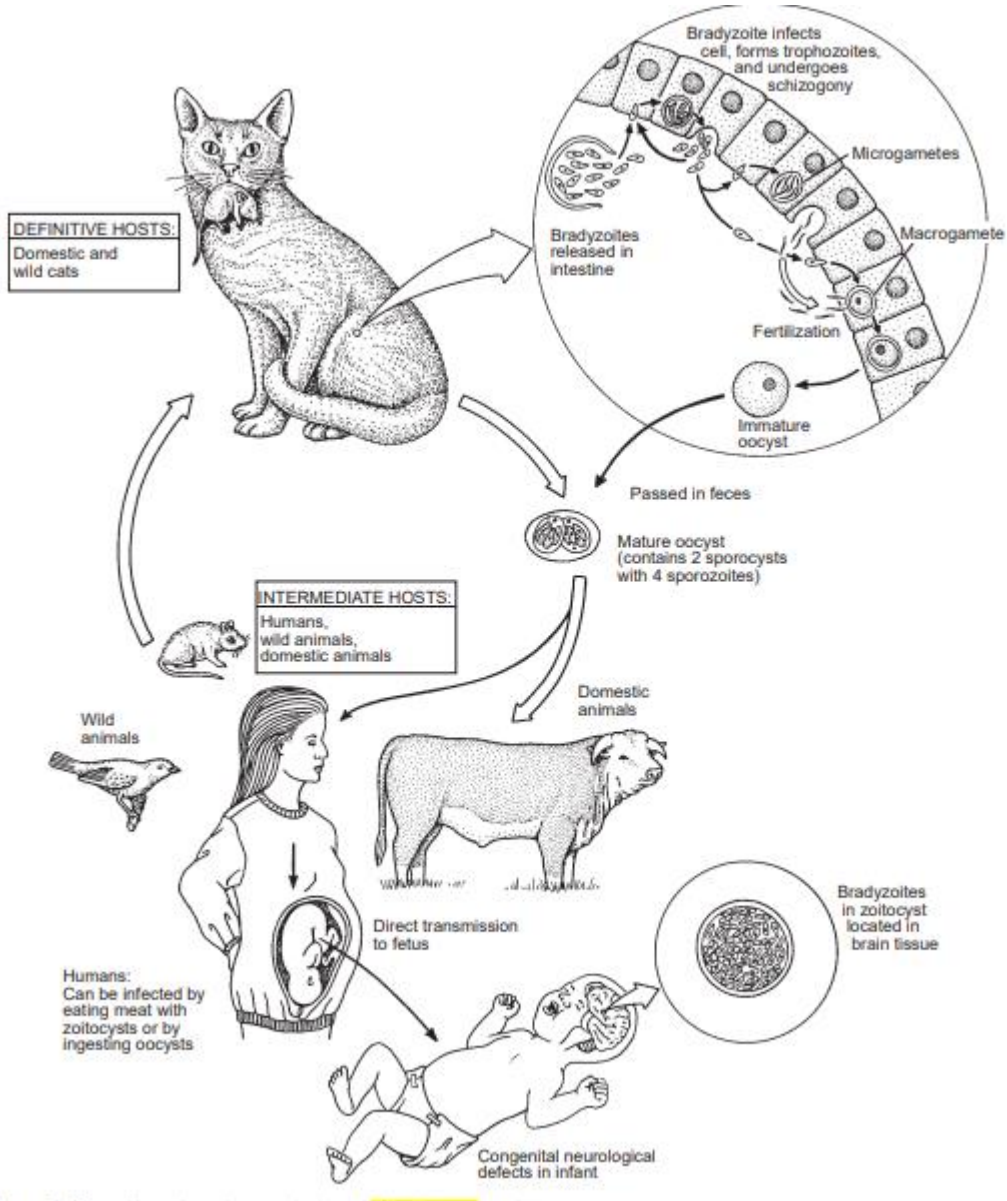
Gametocytes in thin blood smears.



Ookinete

Toxoplasma gondii

Causes **Toxoplasmosis** The life cycle includes intestinal-epithelial (enteroepithelial) and extraintestinal stages in domestic cats and other felines but only extraintestinal stages in Other hosts. Sexual reproduction occurs in cats, and only asexual reproduction is known in other hosts. Extraintestinal stages begin when a cat or other host ingests **bradyzoites**. Ingested **tachyzoites or sporocysts** also sometimes are infective.



Toxoplasma gondii. **Life cycle(indirect)**

Laboratory Diagnosis

1-Direct microscopy (Detect tachyzoites in blood and tissue cyst in tissue biopsy):

A-Giemsa, silver stains, immune-peroxidase stain.

B-Direct fluorescent antibody test.

2-Antibody detection.

A-Detection of IgG in serum—ELISA.

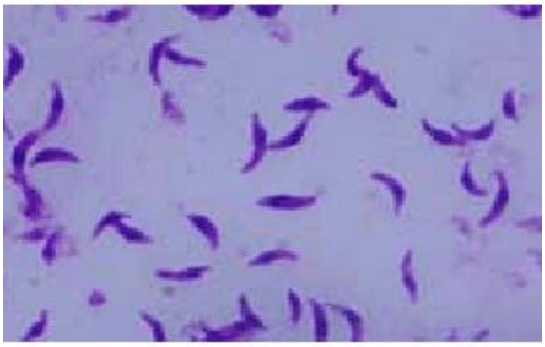
B-Detection of IgM in serum—ELISA.

3-Detection of Toxoplasma antigen—ELISA .

4-Molecular diagnosis—PCR .

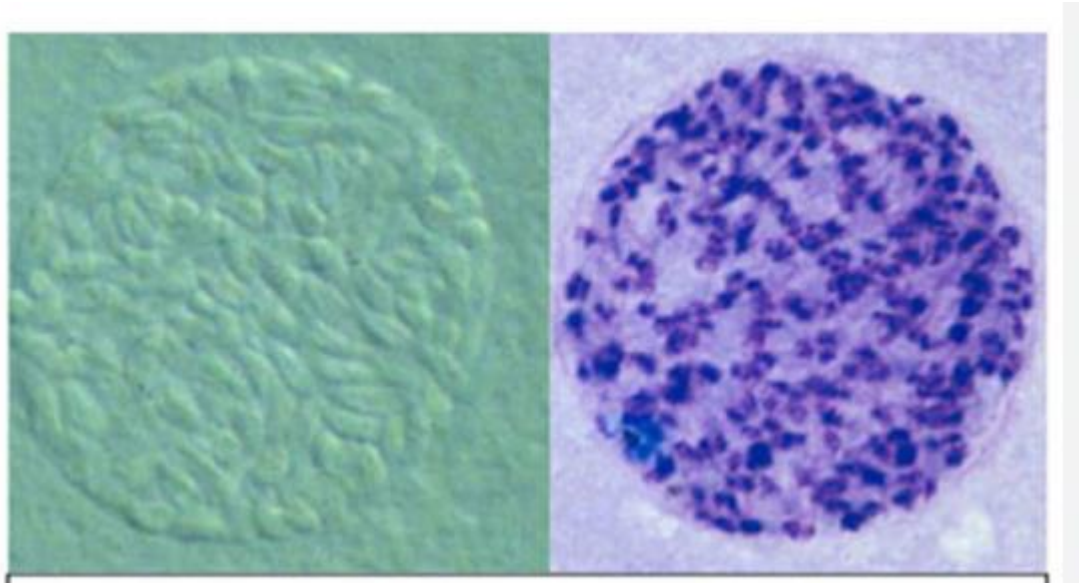
5-Animal inoculation—intraperitoneal inoculation into mice.

6- Imaging methods—Computed tomography CT and MRI magnetic resonance imaging .



Toxoplasma gondii. Tachyzoite parasites

Toxoplasma gondii oocyst



Toxoplasma gondii. **bradyzoite parasites**