Ministry of Higher Education and Scientific Research

Al-Israa University College

Dept. of Medical Lab. Technology

Manual of Human Biology

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First Stage

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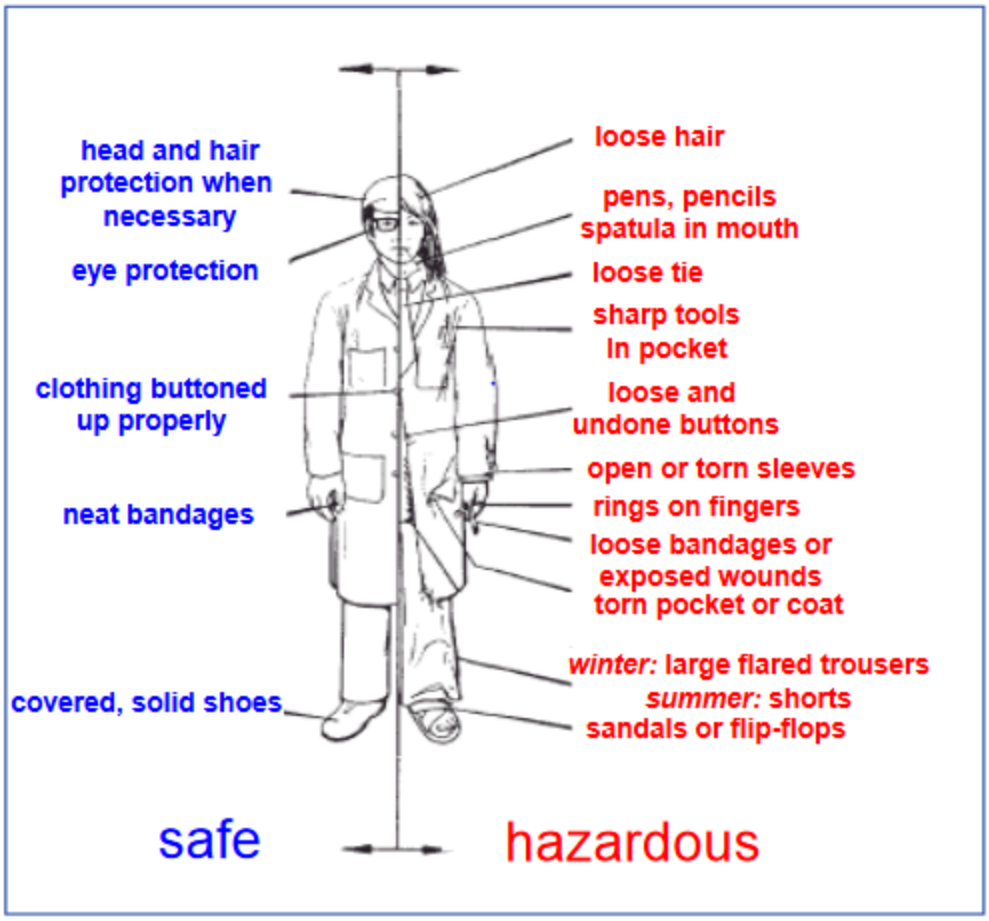
Lab 1 Laboratory Safety

General Laboratory Rules:

1- Do not eat, drink, chew, or apply cosmetics in the work area
2- Wear a buttoned laboratory coat and closed-toe shoes

3- Pin long hair up to prevent contact with chemicals, equipment, or flames
4- Do not wear chains, bracelets, rings, other loose jewelry
5- Use gloves when handling hazardous chemicals and biological specimens
6- Clean and disinfect the work area before and after laboratory work
7- Wash hands before and after any laboratory procedures, after removing drugs,
8- Wear safety glasses, goggles, or a face shield, masks when working with strong chemicals and whenever splashes are possible
9- Wipe up spills using the appropriate procedure whenever it occurs
10- Follow manufacturers' instructions for operating all equipment. Handle all equipment with care and store properly
11- Report any broken slants and glass petri dishes of culture media or damaged equipment
12- Do not use hands to pick up broken glass. Use a broom or brush and discard into special containers for broken glass
13- Report any accident (spills, breakage or injury like cuts, burn..) immediately to the supervisor
14- Never work without the presence of the instructor in the lab
15- Keep hands away from face, eyes, mouth and body while you're working in the lab
16- Laboratory should be supplied with first aid kit and fire extinguisher
17- Contact lenses maybe not worn in the lab
18- All chemicals are to be considered dangerous. DO NOT TASTE OR SMELL ANY CHEMICALS.
19- Label all chemical containers with name of material, its origin.
20- Do not throw any solid substances in the sink
21- Use fume cupboard to remove toxic vapors
22- Avoid contact of toxic liquids with skin
23- Do not open centrifuge cover until machine stops completely. Use only tubes especially designed for centrifuge.



Labs 2&3 **Microscope**

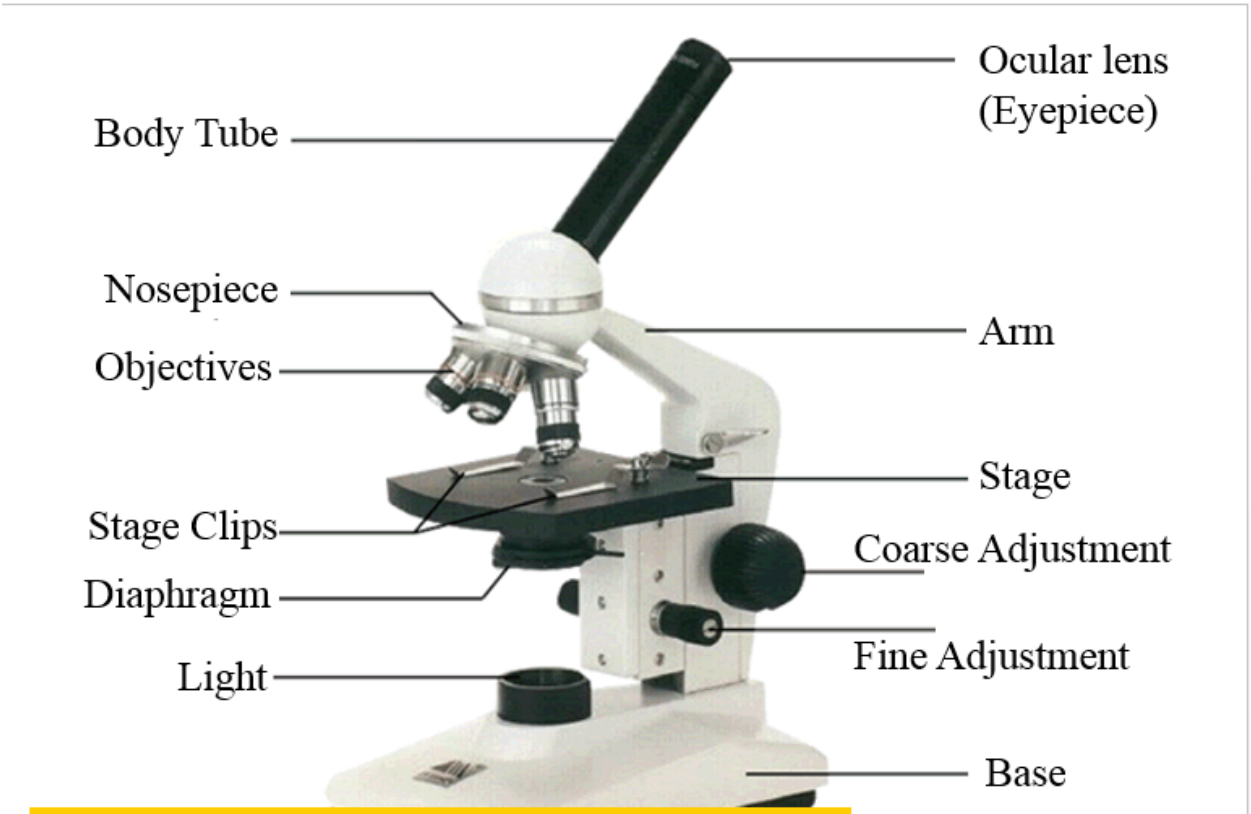
What is a microscope? A microscope is a high precision optical instrument that uses a lens or a combination of lenses to produce highly magnified images of small specimens or objects especially when they are too small to be seen by the naked (unaided) eye. A light source is used (either by mirrors or lamps) to make it easier to see the subject matter.

What is microscopy? Microscopy is the use of a microscope or investigation by a microscope.

Types of microscopes

1- Compound light microscope (Light microscope)
2- Phase Contrast Microscope
3- Fluorescence Microscope
4- Electron Microscope : a) Transmission Electron Microscope
b) Scanning Electron Microscope

Parts of microscope



Always carry a microscope with one hand holding the **arm** and one hand under the **base**.

Working with the microscope

- Take out your microscope. Grasp the arm with one hand while placing your other hand under the base (bottom of the microscope). ALWAYS use two hands.
- Set your microscope on a tabletop
- Plug the microscope's power cord into an outlet.

- Switch on your microscope's light source and then adjust the diaphragm, allowing the greatest amount of light through.
- Rotate the nosepiece to the lowest-power objective usually 4x.
- Place a microscope slide on the stage, either under the stage clips
- Adjust the large coarse focus knob until the specimen is in focus. Slowly move the slide to center the specimen under the lens, if necessary.
- Rotate the nosepiece to the lowest-power objective usually 10x
- Adjust the large coarse focus knob until the specimen is in focus
- Rotate the nosepiece to the lowest-power objective usually 40x
- Adjust the large coarse focus knob until the specimen is in focus
- Turn the nosepiece so that no objective lens is directly over the slide. Then place a small drop of immersion oil on top of the coverslip.
- Turn the nosepiece so that the oil immersion objective is locked into place over the specimen, making sure that the lens is immersed into the oil.
- Adjust the small fine focus knob until the specimen is clearly in focus. Then adjust the diaphragm to get the best lighting. With the 100x lens, you will be able to see additional cell detail, but you will need to take extra care with focus and contrast for a clear image
- When you are done using the slide, clean the oil off of the slide and the lens with lens cleaning paper and solution.
- Turn the nosepiece of the microscope until the low-power objective locks into place.
  Carefully lower the objective to its lowest position by turning the coarse adjustment knob.
- Turn off the light source.
- Remove your slide.
- Unplug the microscope and store it under a dustcloth.

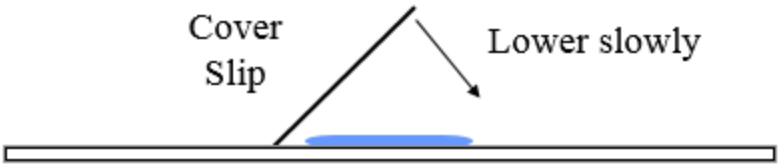
Labs 3&4 **Making a wet mount slide**

1 – Get a clean slide and coverslip from your teacher.

2 – Place ONE drop of water in the middle of the slide. Don't use too much or the water will run off the edge and make a mess!

3 – Place the edge of the cover slip on one side of the water drop.

4 - Slowly lower the cover slip on top of the drop.



– Place the slide on the stage and view it first with the red-banded objective. Once you see the image, you can rotate the nosepiece to view the slide with the different objectives.

Labs 5&6 **Eukaryotic Cell Structure**

Differences between Prokaryotes and Eukaryotes

S.No.	Prokaryotes	Eukaryotes
(*i*)	Most prokaryotes are unicellular.	Most eukaryotes are multicellular.
(*ii*)	The nucleus is poorly defined due to the absence of a nuclear membrane.	The nucleus is well defined and is surrounded by a nuclear membrane.
(*iii*)	Nucleolus is absent.	Nucleolus is present.
(*iv*)	Cell organelles such as plastids, mitochondria, golgi bodies, etc. are absent.	Cell organelles such as plastids, mitochondria, golgi bodies, etc. are present.
(*v*)	Bacteria and blue-green algae are prokaryotic cells.	Fungi, plant, and animal cells are eukaryotic cells.

Eukaryotic cells have a membrane-bound nucleus and organelles.

Cell Membrane

The cell membrane surrounds each cell and regulates which materials enter and leave the cell. It consists of a double layer of phospholipid molecules, and may appear as a thin double line on electron micrographs

Nucleus: The nucleus is a membrane-bound structure that contains the genetic material (deoxyribonucleic acid, or DNA) of eukaryotic cells. Also present in the nucleus are one or more nucleoli (singular, nucleolus) which are where the molecular components of ribosomes are manufactured.

Ribosomes

Ribosomes are the sites of protein synthesis in both prokaryotic and eukaryotic cells. They are sometimes free in the interior of the cell, but most are bound to internal cellular membranes. The membrane most commonly bound to ribosomes is called the endoplasmic reticulum. Areas of the endoplasmic reticulum that have

many associated ribosomes are called “rough” endoplasmic reticulum. Smooth endoplasmic reticulum lacks attached ribosomes. The vesicles formed by the endoplasmic reticulum contain enzymes and other proteins produced by the ribosomes. The endoplasmic reticulum then transports the proteins to another structure, the Golgi Apparatus.

Golgi Apparatus

The Golgi apparatus (or complex) is also a system of membranes that form small vesicles or cisternae. It is in these vesicles that the proteins are modified and transported.

Vacuoles

Some organelles consist of little more than a membranous sac. These are vacuoles. In plant cells, vacuoles are numerous, and occupy most of the cell’s interior. **Chloroplasts** The organelles that perform photosynthesis in plants are called chloroplasts. Chloroplasts take the energy from the sun and store it in organic molecules (carbohydrates) with the help of a pigment called chlorophyll. The chlorophyll is found in membranous thylakoids (remember the cyanobacteria?) inside each chloroplast.

Mitochondria Although animals lack chloroplasts, both animals and plants have mitochondria. Mitochondria are the organelles that use the carbohydrates (produced during photosynthesis, and ingested by the animals) to release energy.

Cytoplasm

The cytoplasm of the cell is everything enclosed by the cell membrane, except the nucleus. The fluid portion of the cytoplasm (everything outside of the membrane bounded organelles) is called the cytosol. The cytosol contains different types of fibers called the cytoskeleton. The cytoskeleton contributes to the cell’s shape, helps to move the organelles around within the cytoplasm (this movement is called cytoplasmic streaming, or cyclosis), and plays an important role during cell division.

Preparing human cheek cells

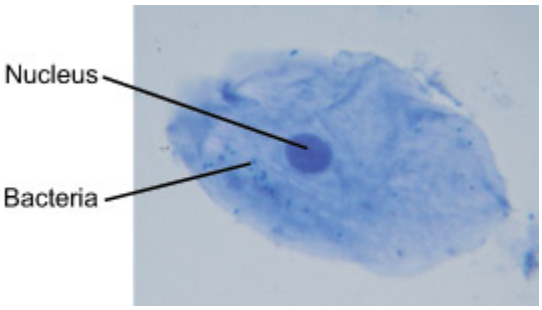
Materials

- Glass microscope slides
- Plastic cover slips
- Paper towels or tissue
- Methylene Blue solution (0.5% to 1)
- Plastic pipette or dropper

- Sterile, individually packed cotton swabs

Methods

1. Take a clean cotton swab and gently scrape the inside of your mouth.
2. Smear the cotton swab on the centre of the microscope slide for 2 to 3 seconds.
3. Add a drop of methylene blue solution and place a coverslip on top. Concentrated methylene blue is toxic if ingested. Wear gloves and do NOT allow children to handle methylene blue solution or have access to the bottle of solution.
4. Remove any excess solution by allowing a paper towel to touch one side of the coverslip.
5. Place the slide on the microscope, with 4 x or 10 x objective in position and find a cell. Then view at higher magnification.



Methylene blue stains negatively charged molecules in the cell, including DNA and RNA. This dye is toxic when ingested and it causes irritation when in contact with the skin and eyes.

The cells seen are squamous epithelial cells from the outer epithelial layer of the mouth. The small blue dots are **bacteria** from our teeth and mouth.

Labs7&8 Observing Prokaryotic Cells

Prokaryotic cells are cells that lack a nucleus and membrane-bound organelles. Bacteria and related microorganisms are prokaryotes.

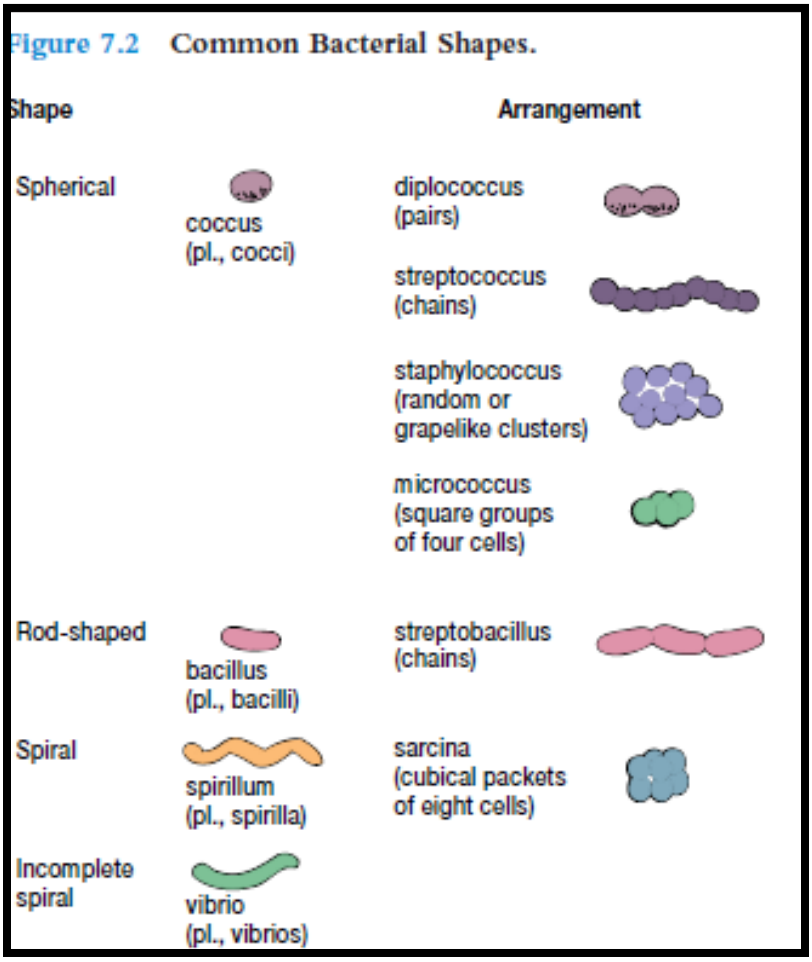
With the light microscope, the size, shape and cells arrangement of the various bacteria are easily described.

* The shape of bacteria: **1- Spherical or coccoid [singular: coccus]**

2- Rod or bacilli [= : bacillus]

3- Spiral or spirilla [= : spirillum]

4- Curved or vibrios [= : vibrio]



Smear Preparation

-Mark the slide in the left corner of slide

-For the broth culture: shake the tube and with an inoculating loop, transfer 1-2 loopfuls of bacteria to center of the slide and spread.

-While when preparing a smear from a slant or plate, place a loopful of water in the center of the slide. With the needle, pick up a very small amount of culture and mix with water drop and spread.

-Allow the slide to air dry.

-Pass the slide through a Bunsen burner flame three times to heat fix and kill bacteria.

Simple Stain

-Place the slide on a staining rack and flood smear with simple stain (Crystal violet or Safranin)

-After staining 1min. wash the slide with tap water gently and drain off excess water

-Air dry

-Examine with microscope under oil-immersion lens

Lab 9 Human Blood Groups

Blood groups

A **blood type** (also called a **blood group**) is a classification of **blood**, based on the presence and absence of **antibodies** and **inherited antigenic substances** on the surface of red blood cells (**RBCs**).

A total of **36 human blood group systems** are now recognized by the **International Society of Blood Transfusion** (**ISBT**).

The two most important blood group systems are:

1- **The ABO blood group system.**

2- The Rh blood group system.

They determine someone's **blood type** (**A, B, AB** and **O**, with **+, −** or **null** denoting **RhD status**) for **suitability** in **blood transfusion**.

1-The ABO blood group system:

The ABO blood group system involves:

1- **Two** antigens (**antigen A and antigen B**) present on the **surface of red blood cells**.

2- **Two** antibodies (**antibody A and antibody B**) present in the **serum**.

All **human beings** can be classified into **4 groups**:

1- **Humans** with antigen **A** (**group A**).

2- **Humans** with antigen **B** (**group B**).

3- **Humans** with both antigen **A** and **B** (**group AB**).

4- **Humans** with **neither** antigen (**group O**).

The **antibodies** present in the **plasma** together with the **antigens** (on the surface of red blood cells) are found as follows:

1. Antigen **A** with antibody **B.**
2. Antigen **B** with antibody **A.**
3. Antigen **AB** has **no** antibodies**.**
4. Antigen **nil** (**group O**) with antibody **A** and **B.**

There is an **agglutination reaction** between **similar antigen and antibody.** **[**For example: **antigen A (**agglutinates) the **antibody A / antigen B** (agglutinates) the **antibody B]**. Thus, **transfusion** can be considered safe as long as the **serum** of the **recipient** does **not** contain **antibodies** for the blood cell **antigens** of the **donor**.

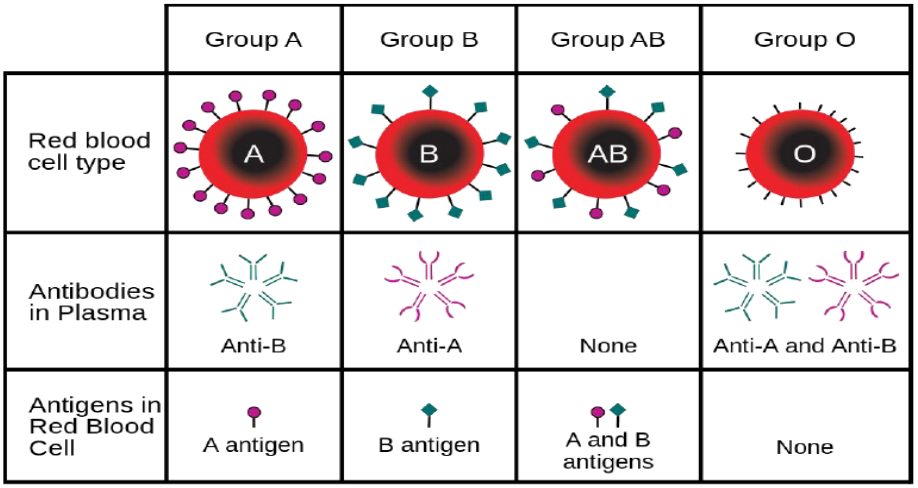


Figure (1): The ABO blood group system.

Red blood cell compatibility

Blood group AB individuals have both **A and B antigens** on the surface of their **RBCs**, and their blood **plasma** does not contain any antibodies against either A or B antigen.

Blood group A individuals have the **A antigen** on the surface of their **RBCs**, and blood **serum** containing **IgM antibodies** against the **B antigen**.

Blood group B individuals have the **B antigen** on the surface of their **RBCs**, and blood **serum** containing **IgM antibodies** against the **A antigen**.

Blood group O individuals do **not** have either **A or B antigens** on the surface of their **RBCs**, and their blood **serum** contains **IgM antibodies** against the **A antigen** and the **B antigen**.
Individuals with type **AB** blood **can receive** blood from **any group** (with AB being preferable), but **cannot donate** blood to **any group** other than **AB**. They are known as **universal recipients**.
Individuals with type **O** blood **can receive** blood only from a **group O** individuals, but **can donate** blood to individuals of **any ABO** blood group. They are known as **universal donors**.

2-The Rh blood group system:
The **Rh** system (**Rh** meaning ***Rhesus***) is the **second** most significant blood-group system in human-blood transfusion with currently **50 antigens**.
The most significant **Rh** antigen is the **D antigen**, because it is the most likely to **provoke** an **immune system response** of the **five** main **Rh antigens**.
The **presence** or **absence** of the **Rh(D) antigen** is signified by the (+ or – **sign**).
[For example: the **A− group** is **ABO type A** and does **not** have the **Rh (D) antigen**].

Sample Required

- This can be done on whole blood or even on clotted blood.
- The sample can be stored at 4 °C. and stable for 5 days.
- Sometimes weak subgroup may result in mistyping where Coombs test may be helpful.

Blood Grouping reagent

Reagents	Color	Volumes
Anti A Sera	Blue	5 ml
Anti B Sera	Yellow	5 ml
anti-D	Clear	5 ml

Procedure

- Inform the patient or individual about the procedure to be carry out
- Dangle the hand down to increase the flow of blood in the fingers.
- Clean the fingertip to be pierced with spirit or 70% alcohol (usually ring or middle finger) and gently massage the finger to increase blood flow

- With the help of the sterile lancet or pricker, pierce the fingertip and place one drop of blood in each of the four cavities
- Now add one drop of each antiserum into each cavity respectively

ABO and Rhesus Blood Grouping Tiles

- Mix each blood drop with the antiserum using a fresh mixing stick or applicator stick
- Now you can observe agglutination in the form of fine red granules within 30 seconds. Anti RhD takes slightly longer time to agglutinate compared to Anti A and Anti B.

Blood grouping result interpretation

- If agglutination is observed when individual's blood is mixed with **Anti A reagent**, then the individual is said to have a blood group **"A"**.
- If agglutination is observed when individual's blood is mixed with **Anti B reagent**, then the individual is said to have a blood group **"B"**
- If agglutination is observed when individual's blood is mixed with **Anti A and Anti B reagent**, then the individual is said to have a blood group **"AB"**
- If no agglutination is observed when individual's blood is mixed with **Anti A and Anti B reagent**, then the individual is said to have a blood group **"O"**
- If agglutination is observed when individual's blood is mixed with **Anti RhD reagent**, then the individual is said to have a **"+ve" Rh factor**.
- If no agglutination is observed when individual's blood is mixed with **Anti RhD reagent**, then the individual is said to a have **"-ve" Rh factor.**

Lab 10 Mitosis

What is mitosis?

Mitosis is a type of cell division in which one cell (the **mother**) divides to produce two new cells (the **daughters**) that are genetically identical to itself. In the context of the cell cycle, mitosis is the part of the division process in which the DNA of the cell's nucleus is split into two equal sets of chromosomes.

Phases of mitosis

Mitosis consists of four basic phases: prophase, metaphase, anaphase, and telophase. Some textbooks list five, breaking prophase into an early phase (called prophase) and a late phase (called prometaphase). These phases occur in strict sequential order, and cytokinesis - the process of dividing the cell contents to make two new cells - starts in anaphase or telophase.

n early **prophase**, the cell starts to break down some structures and build others up, setting the stage for division of the chromosomes.

- The chromosomes start to condense (making them easier to pull apart later on).
- The **mitotic spindle** begins to form. The spindle is a structure made of microtubules, strong fibers that are part of the cell's "skeleton." Its job is to organize the chromosomes and move them around during mitosis. The spindle grows between the centrosomes as they move apart.
- The **nucleolus** (or nucleoli, plural), a part of the nucleus where ribosomes are made, disappears. This is a sign that the nucleus is getting ready to break down.

In **metaphase**, the spindle has captured all the chromosomes and lined them up at the middle of the cell, ready to divide.

- All the chromosomes align at the **metaphase plate** (not a physical structure, just a term for the plane where the chromosomes line up).
- At this stage, the two kinetochores of each chromosome should be attached to microtubules from opposite spindle poles.

Before proceeding to anaphase, the cell will check to make sure that all the chromosomes are at the metaphase plate with their kinetochores correctly attached to microtubules. This is called the **spindle checkpoint** and helps ensure that the sister chromatids will split evenly between the two daughter cells when they separate in the next step. If a chromosome is not properly aligned or attached, the cell will halt division until the problem is fixed.

In **anaphase**, the sister chromatids separate from each other and are pulled towards opposite ends of the cell.

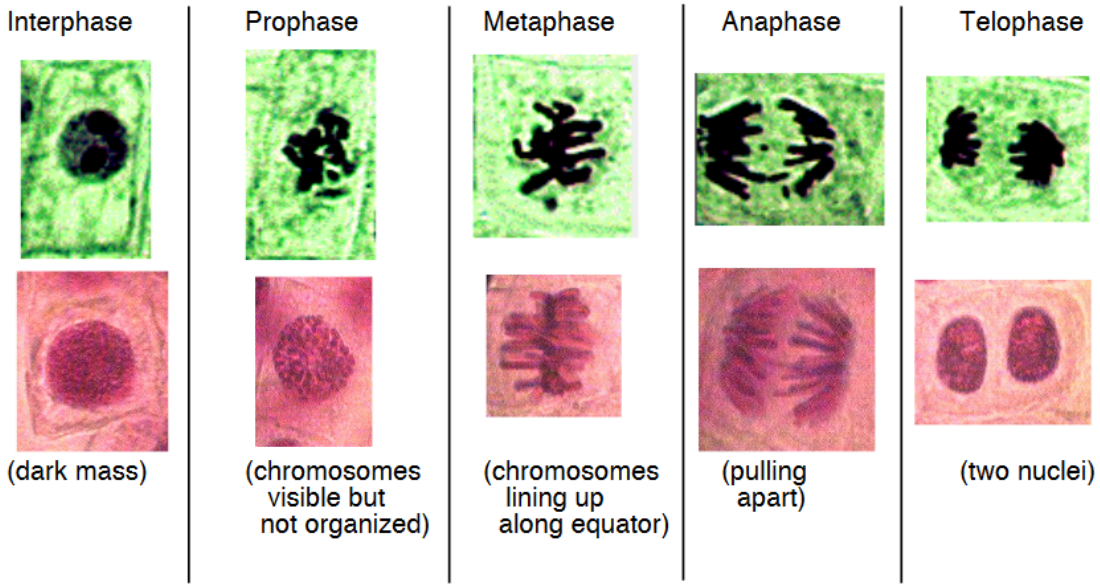
- The protein "glue" that holds the sister chromatids together is broken down, allowing them to separate. Each is now its own chromosome. The chromosomes of each pair are pulled towards opposite ends of the cell.

- Microtubules not attached to chromosomes elongate and push apart, separating the poles and making the cell longer.

In **telophase**, the cell is nearly done dividing, and it starts to re-establish its normal structures as cytokinesis (division of the cell contents) takes place.

- The mitotic spindle is broken down into its building blocks.
- Two new nuclei form, one for each set of chromosomes. Nuclear membranes and nucleoli reappear.
- The chromosomes begin to decondense and return to their "stringy" form.
- **Cytokinesis**, the division of the cytoplasm to form two new cells, overlaps with the final stages of mitosis. It may start in either anaphase or telophase, depending on the cell, and finishes shortly after telophase.

When cytokinesis finishes, we end up with two new cells, each with a complete set of chromosomes identical to those of the mother cell. The daughter cells can now begin their own cellular "lives," and – depending on what they decide to be when they grow up – may undergo mitosis themselves, repeating the cycle



Labs 11&12 **Meiosis**

Meiosis, on the other hand, is used for just one purpose in the human body: the production of **gametes**—sex cells, or sperm and eggs. Its goal is to make daughter cells with exactly half as many chromosomes as the starting cell.

To put that another way, **meiosis** in humans is a division process that takes us from a diploid cell—one with two sets of chromosomes—to haploid cells—ones with a single set of chromosomes. In humans, the haploid cells made in meiosis are sperm and eggs. When a sperm and an egg join in fertilization, the two haploid sets of chromosomes form a complete diploid set: a new genome.

Phases of meiosis

In many ways, meiosis is a lot like mitosis. The cell goes through similar stages and uses similar strategies to organize and separate chromosomes. In meiosis, however, the cell has a more complex task. It still needs to separate **sister chromatids** (the two halves of a duplicated chromosome), as in mitosis. But it must also separate **homologous chromosomes**, the similar but nonidentical chromosome pairs an organism receives from its two parents.

These goals are accomplished in meiosis using a two-step division process. Homologue pairs separate during a first round of cell division, called **meiosis I**. Sister chromatids separate during a second round, called **meiosis II**.

Since cell division occurs twice during meiosis, one starting cell can produce four gametes (eggs or sperm). In each round of division, cells go through four stages: prophase, metaphase, anaphase, and telophase.

Meiosis I

Before entering meiosis I, a cell must first go through interphase. As in mitosis, the cell grows during G1_11start subscript, 1, end subscript phase, copies all of its chromosomes during S phase, and prepares for division during G2_22start subscript, 2, end subscript phase.

During **prophase I**, differences from mitosis begin to appear. As in mitosis, the chromosomes begin to condense, but in meiosis I, they also pair up. Each chromosome carefully aligns with its homologue partner so that the two match up at corresponding positions along their full length.

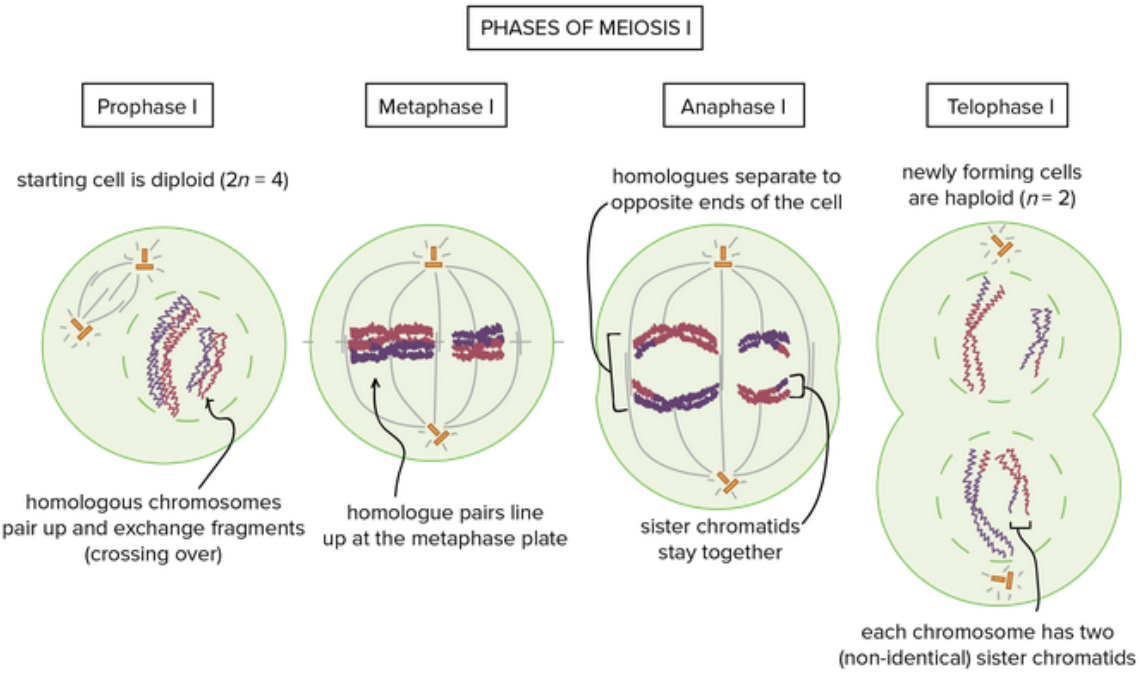
For instance, in the image below, the letters A, B, and C represent genes found at particular spots on the chromosome, with capital and lowercase letters for different forms, or alleles, of each gene. The DNA is broken at the same spot on each homologue—here, between genes B and C—and reconnected in a criss-cross pattern so that the homologues exchange part of their DNA.

This process, in which homologous chromosomes trade parts, is called **crossing over**. It's helped along by a protein structure called the **synaptonemal complex** that holds the homologues together. The chromosomes would actually be positioned one on top of the other—as in the image below—throughout crossing over; they're only shown side-by-side in the image above so that it's easier to see the exchange of genetic material.

You can see crossovers under a microscope as **chiasmata**, cross-shaped structures where homologues are linked together. Chiasmata keep the homologues connected to each other after the synaptonemal complex breaks down, so each homologous pair needs at least one. It's common for multiple crossovers to take place for each homologue pair.

The spots where crossovers happen are more or less random, leading to the formation of new, "remixed" chromosomes with unique combinations of alleles.

After crossing over, the spindle begins to capture chromosomes and move them towards the center of the cell (metaphase plate). This may seem familiar from mitosis, but there is a twist. Each chromosome attaches to microtubules from just one pole of the spindle, and the two homologues of a pair bind to microtubules from opposite poles. So, during **metaphase I**, homologue pairs—not individual chromosomes—line up at the metaphase plate for separation.



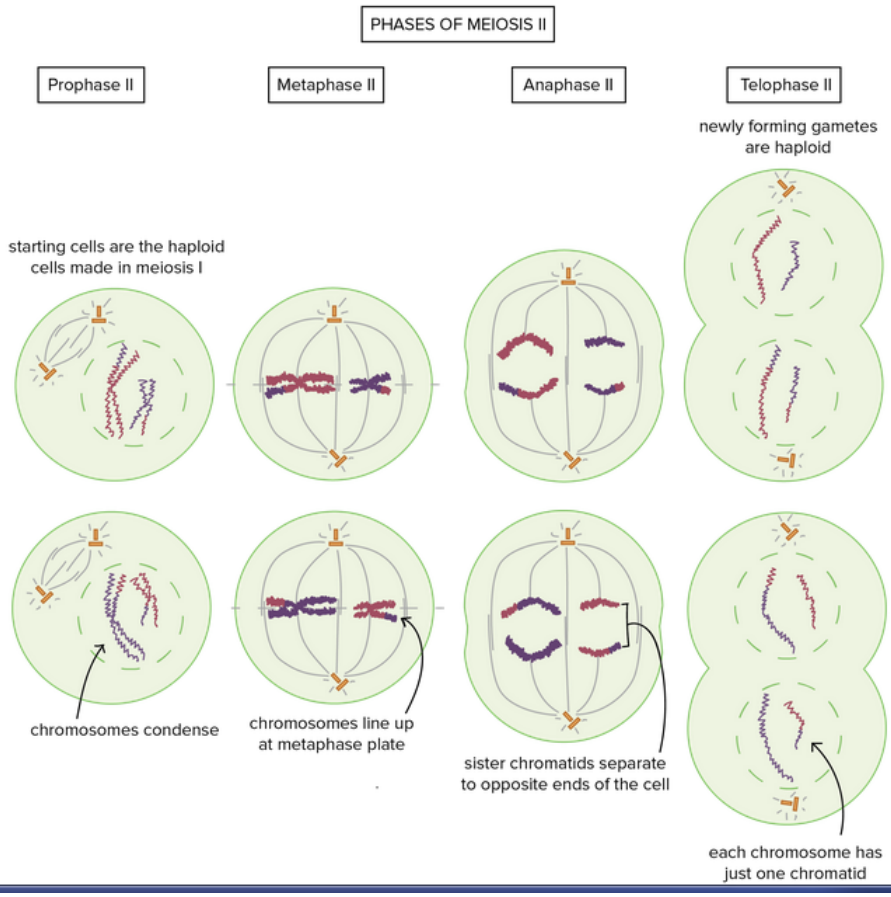
In **anaphase I**, the homologues are pulled apart and move apart to opposite ends of the cell. The sister chromatids of each chromosome, however, remain attached to one another and don't come apart.

Finally, in **telophase I**, the chromosomes arrive at opposite poles of the cell. In some organisms, the nuclear membrane re-forms and the chromosomes decondense, although in others, this step is skipped—since cells will soon go through another round of division, meiosis II2. Cytokinesis usually occurs at the same time as telophase I, forming two haploid daughter cells.

Meiosis II

Cells move from meiosis I to meiosis II without copying their DNA. Meiosis II is a shorter and simpler process than meiosis I, and you may find it helpful to think of meiosis II as “mitosis for haploid cells."

The cells that enter meiosis II are the ones made in meiosis I. These cells are haploid—have just one chromosome from each homologue pair—but their chromosomes still consist of two sister chromatids. In meiosis II, the sister chromatids separate, making haploid cells with non-duplicated chromosomes.



During **prophase II**, chromosomes condense and the nuclear envelope breaks down, if needed. The centrosomes move apart, the spindle forms between them, and the spindle microtubules begin to capture chromosomes.

The two sister chromatids of each chromosome are captured by microtubules from opposite spindle poles. In **metaphase II**, the chromosomes line up individually along the metaphase plate. In **anaphase II**, the sister chromatids separate and are pulled towards opposite poles of the cell.
In **telophase II**, nuclear membranes form around each set of chromosomes, and the chromosomes decondense. Cytokinesis splits the chromosome sets into new cells, forming the final products of meiosis: four haploid cells in which each chromosome has just one chromatid. In humans, the products of meiosis are sperm or egg cells.

Lab 13 Extraction of DNA

All living things, bananas and people included, pass on information from one generation to the next using the same basic material, DNA. Within every living

organism, most cells contain a complete set of DNA instructions. The information in DNA tells our bodies how to develop, grow, and work. It also controls many of the features that make an organism unique.

DNA or deoxyribonucleic acid is found in all living things. Its natural shape is called a double helix and when seen under extremely high-powered microscopes, it looks kind of like a ladder twisted into a spiral shape.

These instructions are in segments of DNA called genes. Genes, along with other parts of our DNA that turn genes on and off, hold information for how our body develops and functions. They produce molecules called proteins that do most of the work in the body. Variants of genes, called alleles, are responsible for differences in hair color, eye color, and earlobe shape.

Materials

- 1/2 peeled ripe banana (you can also use strawberries or other fruit)
- 1/2 cup hot water
- 1 tsp salt
- 1/2 tsp liquid dishwashing soap
- resealable zip-top bag (quart size)

- very cold rubbing alcohol (isopropyl alcohol) placed in freezer ahead of time
- coffee filter
- narrow glass
- wooden stirrer

Extracting DNA

1. Mush the banana in the resealable bag for about a minute until all the lumps are gone and it almost looks like pudding.
2. Fill a cup with the hot water and salt.
3. Pour the saltwater mix into the bag. Close the bag and very gently squeeze and move the saltwater and banana mush together. Do this for 30 to 45 seconds.
4. Add the dishwashing soap into the bag and gently mix the contents. Try to avoid making too much foam.
5. Place the coffee filter in a clear glass cup, securing the top of the filter around the lip of the cup.
6. Pour the mix into the filter and let it sit until all of the liquid drips down into the cup.

7. Remove and discard the used coffee filter.
8. Tilt the glass and **slowly** add cold alcohol down the side of the cup. You want the alcohol to form a layer on top of the banana mix, staying separated, so be careful not to pour it too fast. Make a layer of alcohol that is 2.5-5cm (1-2in) thick.
9. After the alcohol layer is set up, wait for eight minutes. You may see some bubbles and cloudy material moving around in the alcohol. This is the DNA pieces clumping together.
10. Use the wooden stirrer to start poking the cloudy stuff in the alcohol layer. Spin the stirrer it in place to start gathering the cloudy stuff. When you are done, take a closer look at the stuff on the stirrer. You are looking at DNA!

Lab 14 Epithelium

Epithelium: is one of the four basic types of animal tissue, along with connective tissue, muscle tissue and nervous tissue.

There are three principal shapes of epithelial cell:

1- Squamous.

2- Columnar.

3- Cuboidal.

Classification

In general, epithelial tissues are classified by:

A- The number of their layers.

B- The shape of the cells.

C- The function of the cells.

A- By layer, epithelium is classed as either :

Simple epithelium: epithelial cells that have been arranged in a single layer of cells.

Stratified epithelium: epithelial cells that have been arranged in layers of two or more cells.

In some tissues, a layer of columnar cells may appear to be stratified due to the placement of the nuclei. This sort of tissue is called Pseudostratified.

Simple epithelial tissues are classified by the shape of their cells to:

1- Simple squamous.

2- Simple cuboidal.

3- Simple columnar.

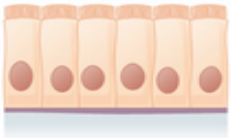
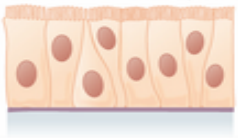
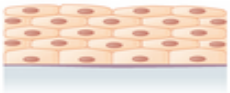
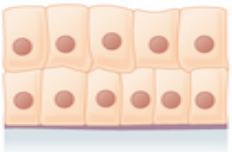
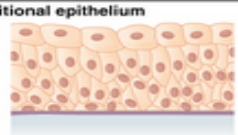
4- Pseudostratified.

Stratified epithelial tissues are classified by the shape of their cells to:

1- Stratified squamous.

2- Stratified cuboidal.

3- Stratified columnar.

Cells	Location	Function
Simple squamous epithelium 	Air sacs of lungs and the lining of the heart, blood vessels, and lymphatic vessels	Allows materials to pass through by diffusion and filtration, and secretes lubricating substance
Simple cuboidal epithelium 	In ducts and secretory portions of small glands and in kidney tubules	Secretes and absorbs
Simple columnar epithelium 	Ciliated tissues are in bronchi, uterine tubes, and uterus; smooth (nonciliated tissues) are in the digestive tract, bladder	Absorbs; it also secretes mucous and enzymes
Pseudostratified columnar epithelium 	Ciliated tissue lines the trachea and much of the upper respiratory tract	Secretes mucus; ciliated tissue moves mucus
Stratified squamous epithelium 	Lines the esophagus, mouth, and vagina	Protects against abrasion
Stratified cuboidal epithelium 	Sweat glands, salivary glands, and the mammary glands	Protective tissue
Stratified columnar epithelium 	The male urethra and the ducts of some glands	Secretes and protects
Transitional epithelium 	Lines the bladder, uretha, and the ureters	Allows the urinary organs to expand and stretch

Lab 14 Human Muscular System

The muscular system is an **organ system** consisting of **skeletal, smooth** and **cardiac muscles**.

Function

The muscular system permits:

1- Movement of the body.
2- Maintains posture and circulates blood throughout the body.

Muscles

There are three types of muscles:

1- Skeletal muscles.
2- Cardiac or heart muscles
3- Smooth (non-striated) muscles.

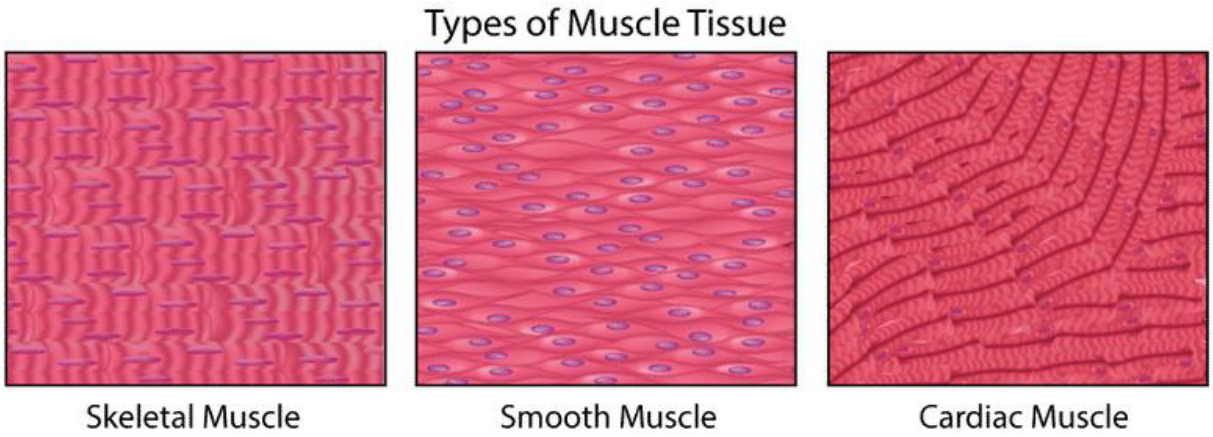


Figure (1): The three types of muscles.

1-Skeletal muscle

They are:
1- Voluntary muscles.
2- Striated muscles.

Function

They are responsible of moving bones and other structures.

2-Cardiac muscle

They are:
1- Involuntary muscles.
2- Striated muscles.

Function

They are responsible of the contraction of the heart to pump blood.

3-Smooth muscle

They are:
1- Involuntary muscles.
2- Non-striated muscles.

Function

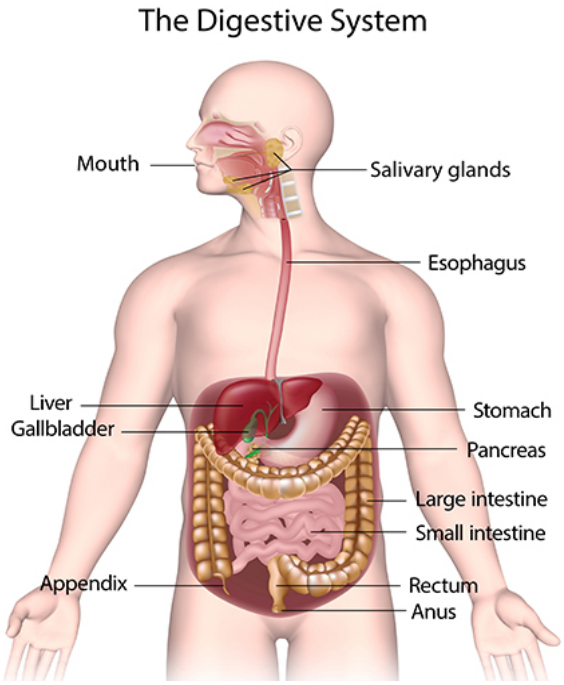
They change the shapes of the organs they form to facilitate bodily functions.

Lab 15 **Digestive System**

What is the Digestive system?

The digestive system is made up of the gastrointestinal tract—also called the GI tract or digestive tract—and the liver, pancreas, and gallbladder. The **GI** tract is a series of hollow organs joined in a long, twisting tube from the mouth to the anus. The hollow organs that make up the GI tract are the mouth, esophagus, stomach, small intestine, large intestine, and anus. The liver, pancreas, and gallbladder are the solid organs of the digestive system.

The small intestine has three parts. The first part is called the duodenum. The jejunum is in the middle and the ileum is at the end. The large intestine includes the appendix, cecum, colon, and rectum. The appendix is a finger-shaped pouch attached to the cecum. The cecum is the first part of the large intestine. The colon is next. The rectum is the end of the large intestine**.**



Bacteria in your GI tract, also called gut flora or microbiome, help with digestion. Parts of your nervous and circulatory NIH external link systems also

help. Working together, nerves, hormones, bacteria, blood, and the organs of your digestive system digest the foods and liquids you eat or drink each day.

Why is digestion important?

Digestion is important because your body needs nutrients from food and drink to work properly and stay healthy. Proteins, fats, carbohydrates, vitamins NIH external link, minerals NIH external link, and water are nutrients. Your digestive system breaks nutrients into parts small enough for your body to absorb and use for energy, growth, and cell repair.

- Proteins break into amino acids
- Fats break into fatty acids and glycerol
- Carbohydrates break into simple sugars

How does my digestive system work?

Each part of your digestive system helps to move food and liquid through your GI tract, break food and liquid into smaller parts, or both. Once foods are broken into small enough parts, your body can absorb and move the nutrients to where they are needed. Your large intestine absorbs water, and the waste products of digestion become stool. Nerves and hormones help control the digestive process.

How does my body control the digestive process?

Your hormones and nerves work together to help control the digestive process. Signals flow within your GI tract and back and forth from your GI tract to your brain.

- **Hormones**
- **Nerves**
- **Clinical Trials**

Lab 16 The Nervous system

The **nervous system** is a complex collection of **nerves** and specialized cells known as **neurons** that transmit signals between different parts of the body.

Cells

The nervous system contains two types of cells:

A- **Neurons** : are specialized cells **transmitting nerve impulses**.

Types of **neurons** include:

- Motor neurons.
- Sensory neurons.
- Interneurons.

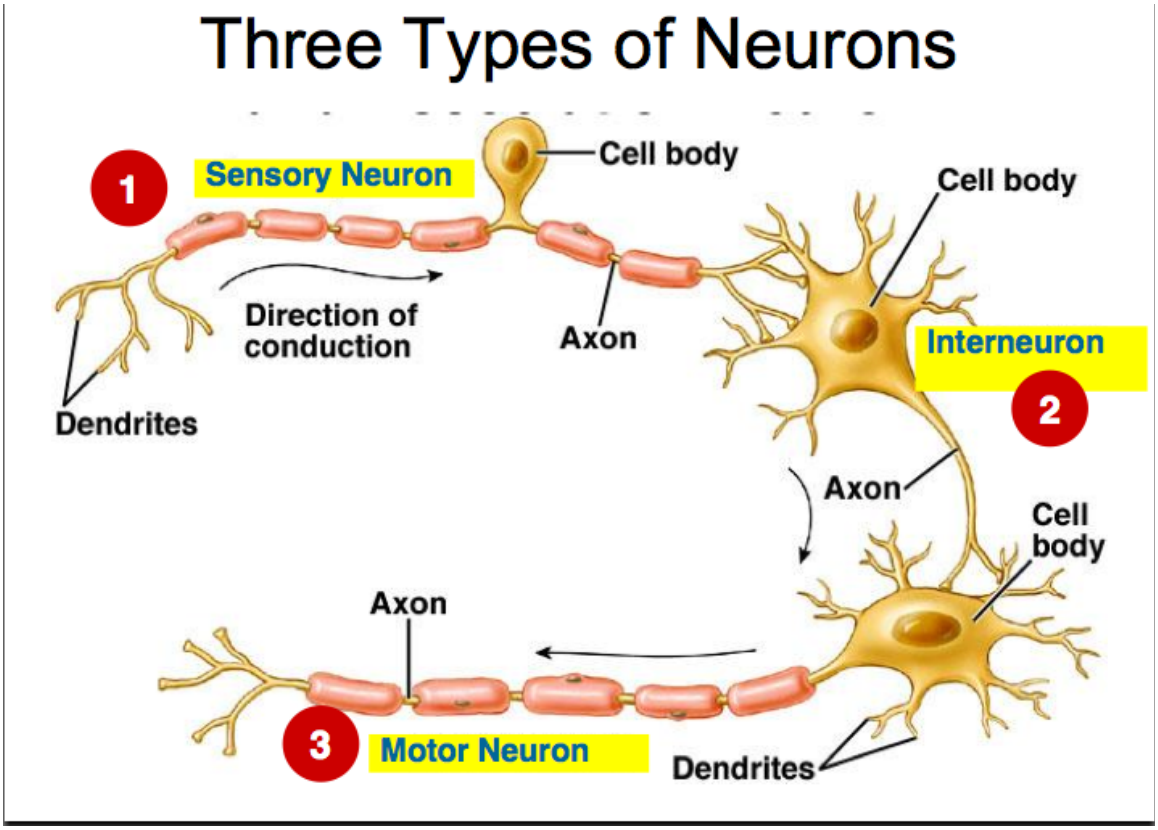


Figure (1): Types of neurons.

B- **Glial cells**: are non-neuronal cells that **provide support, nutrition and form myelin**.

Types of ***glial cells*** include

- Oligodendrocytes.
- Astrocytes.
- Ependymal *cells*.

- Schwann *cells*.
- Microglia.
- Satellite *cells*.

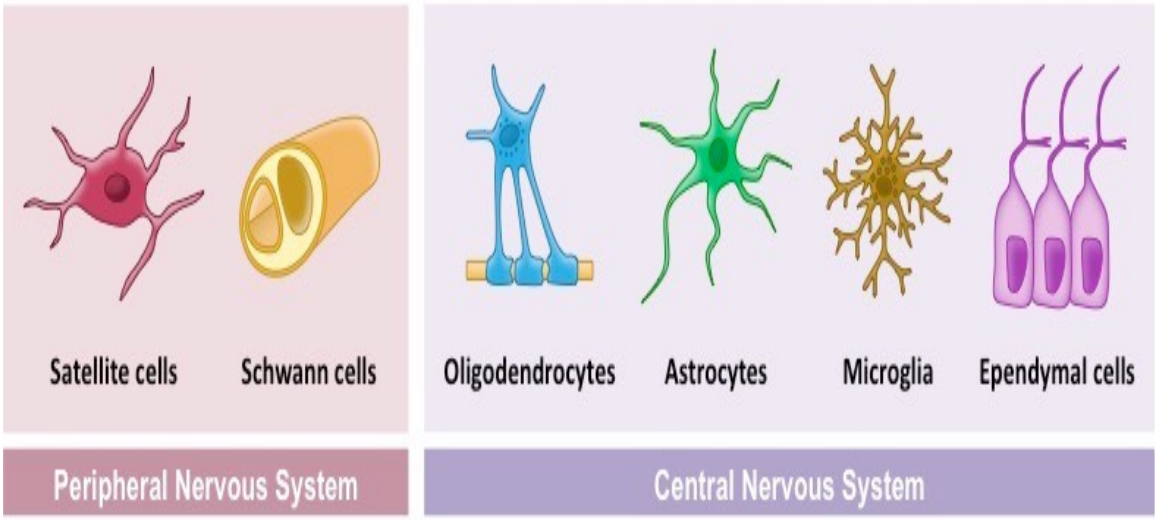


Figure (2): Types of Glial cells.

In **vertebrates** the nervous system consists of two main **divisions**:

1- The central nervous system **(CNS)** consist of:

- Brain.
- Spinal cord.

Contains:

- Interneurons.

Lab 17 **Taxonomy**

Taxonomy: is a branch of biology, which scientifically groups and names organisms based on their characteristics and the evolutionary history.

Linnaean taxonomy: The Swedish botanist **Carl Linnaeus** is regarded as the **father of taxonomy**, as he developed a system known as **Linnaean taxonomy** for categorization of organisms and **binomial nomenclature** for naming organisms.

Organisms are classified according to Linnaeus system into seven ranks:

1. Kingdom.
2. Phylum (in Zoology) or Division (in Botany).
3. Class.
4. Order.
5. Family.
6. Genus.
7. Species.

The Three Domain System

The Three Domain System, groups organisms primarily based on differences in ribosomal RNA (rRNA) structure.

Under this system, organisms are classified into **three domains** and **six kingdoms**.

The domains are **Archaea**, **Bacteria**, and **Eukaryota**.

The kingdoms are **Archaebacteria** (ancient bacteria), **Eubacteria** (true bacteria), **Protista**, **Fungi**, **Plantae**, and **Animalia.**

Archaea Domain

This domain contains single-celled organisms known as **archaea**.

Archaea are **prokaryotic organisms** and do not have a membrane-bound nucleus.

Archaea include one kingdom (**Archaebacteria Kingdom**) and are divided into three main **phyla** (Singular-**Phylum**):

- ***Crenarchaeota*.**
- ***Euryarchaeota*.**
- ***Korarchaeota*.**

Bacteria Domain

Bacteria are **prokaryotic organisms** and do not have a membrane-bound nucleus.

Bacteria include one kingdom (**Eubacteria Kingdom**) and are grouped into five **Phyla** (Singular-**Phylum**):

- **Proteobacteria.**

- **Cyanobacteria.**
- **Firmicutes.**
- **Chlamydiae.**
- **Spirochetes.**

Eukaryota Domain

The Eukaryota domain includes **eukaryotic organisms** that have a membrane-bound nucleus.

This domain is further subdivided into **four kingdoms**:

- **Protista kingdom.**
- **Fungi kingdom.**
- **Plantae kingdom.**
- **Animalia kingdom.**

Organisms are classified according to three domain system into eight ranks:

1. Domain.
2. Kingdom.
3. Phylum (in Zoology) or Division (in Botany).
4. Class.
5. Order.
6. Family.
7. Genus.
8. Species.

Archaea differ from **bacteria** in **cell wall** composition and differ from both **bacteria** and **eukaryotes** in **membrane** composition and **rRNA** type.

The earliest systems recognized only two kingdoms (plant and animal). The current Three Domain System is the best organizational system for now.

Lab 18 Fungi

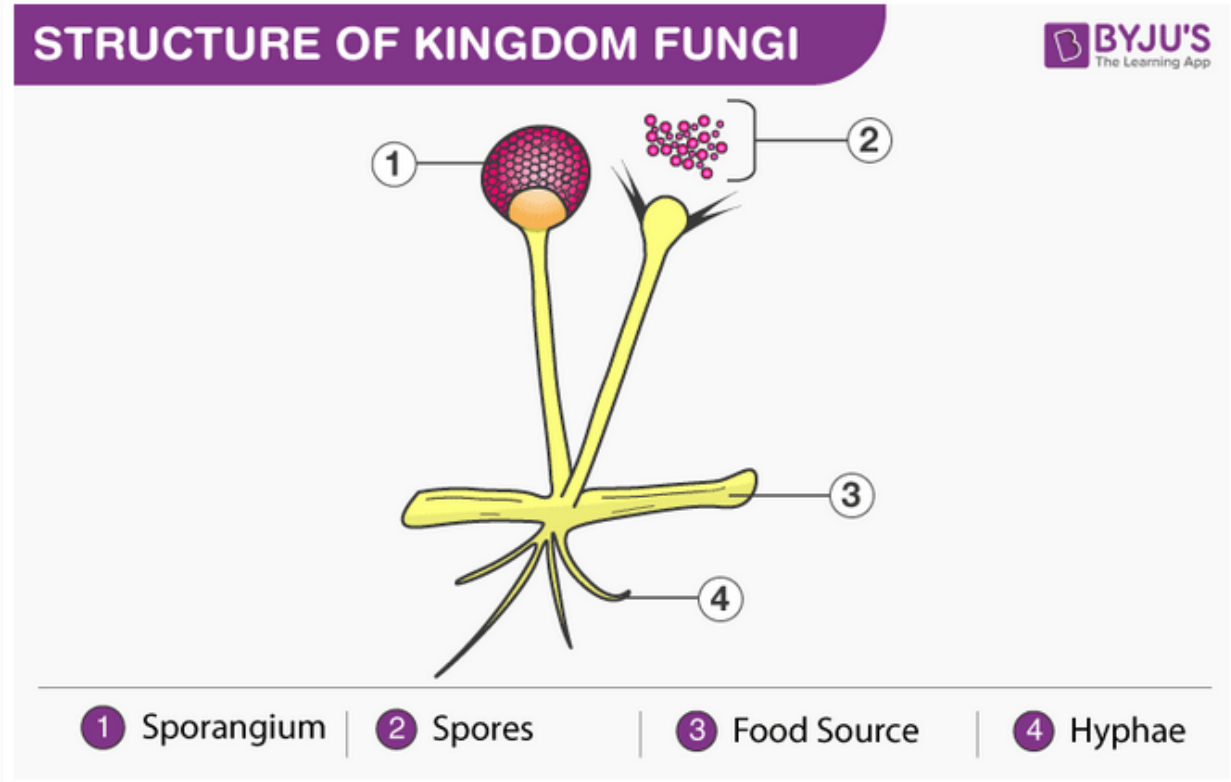
Characteristics of Fungi

Following are the important characteristics of fungi:

1. Fungi are eukaryotic, non-vascular, non-motile and heterotrophic organisms.

2. They may be unicellular or filamentous.
3. They reproduce by means of spores.
4. Fungi exhibit the phenomenon of alternation of generation.
5. Fungi lack chlorophyll and hence cannot perform photosynthesis.
6. Fungi store their food in the form of starch.
7. Biosynthesis of chitin occurs in fungi.
8. The nuclei of the fungi are very small.
9. The fungi have no embryonic stage. They develop from the spores.
10. The mode of reproduction is sexual or asexual.
11. Some fungi are parasitic and can infect the host.
12. Fungi produce a chemical called pheromone which leads to sexual reproduction in fungi.
13. Examples include mushrooms, molds, yeast.

Structure of Fungi



The structure of fungi can be explained in the following points:

1. Almost all the fungi have a filamentous structure except the yeast cells.
2. They can be either single-celled or multicellular organism.

3. Fungi consist of long thread-like structures known as hyphae. These hyphae together form a mesh-like structure called mycelium.
4. Fungi possess a **cell wall** which is made up of chitin and polysaccharides.
5. The cell wall comprises protoplast which is differentiated into other cell parts such as cell membrane, cytoplasm, cell organelles and nuclei.
6. The nucleus is dense, clear, with chromatin threads. The nucleus is surrounded by a nuclear membrane.

Reproduction in Fungi

Reproduction in fungi is both by sexual and asexual means. The sexual mode of reproduction is referred to as teleomorph and the asexual mode of reproduction is referred to as anamorph.

Vegetative reproduction – By budding, fission and fragmentation

Asexual reproduction – This takes place with the help of spores called conidia or zoospores or sporangiospores

Sexual reproduction – ascospores, basidiospores, and oospores

The conventional mode of **sexual reproduction** is not always observed in the kingdom Fungi. In some fungi, the fusion of two haploid hyphae does not result in the formation of a diploid cell. In such cases, there appears an intermediate stage called the dikaryophase. This stage is followed by the formation of diploid cells.

Lab 19 Yeasts

Yeasts are **eukaryotic single-celled microorganisms** classified as members of the **fungi kingdom**. The most known species of yeast is ***Saccharomyces cerevisiae* (*S. cerevisiae*).**

Reproduction

Yeasts have **asexual (budding)** and **sexual** reproductive cycles.

The yeast cell's life cycle:

1. Budding.

2. Conjugation.

3. **Spore.**

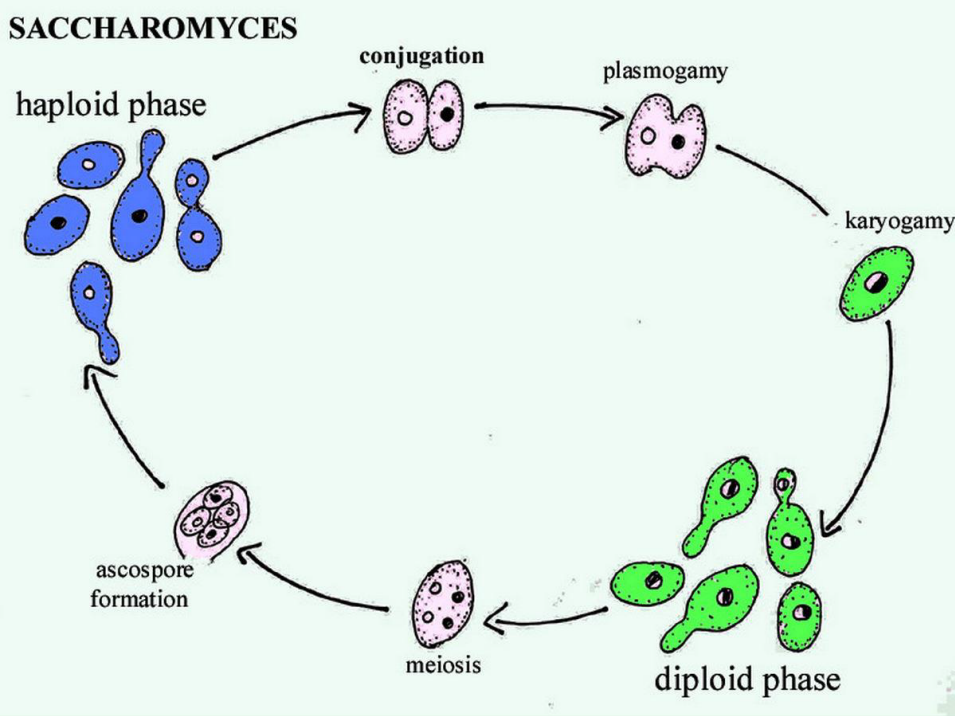


Figure (1): The yeast cell's life cycle.

Fields of Use

The useful physiological properties of yeast have led to their use in the fields of:

1- **Biotechnology.**

2- **Genetics.**

3- **Cell biology.**

Uses:

A- Genetically engineered yeasts

Various yeast species have been genetically engineered to efficiently produce various drugs, a technique called **metabolic engineering**.

A wide variety of chemicals in different classes can be produced by engineered yeast, including:

1- **Phenolics**.

2- **Isoprenoids**.

3- **Alkaloids**.

4- **Polyketides**.

About 20% of biopharmaceuticals are produced in ***S. cerevisiae***, including:

1- Insulin.

2- vaccines for hepatitis.

3- Human serum albumin.

B-Probiotics

Some probiotic supplements use yeasts to maintain and restore the **natural flora** in the **gastrointestinal tract**. by reducing the symptoms of **acute diarrhea**, reduceing bowel movements in **diarrhea-predominant IBS patients**, and reducing the incidence of **antibiotic-, traveler's-, and HIV/AIDS-associated diarrheas**.

C-Nutritional supplements

Nutritional yeast is a **deactivated** yeast, usually ***S. cerevisiae***. It is naturally low in **fat** and **sodium** as well as an excellent source of **protein** and **vitamins**, especially most **B-complex** vitamins, as well as other **minerals** and **cofactors** required for growth.

Pathogenic yeasts

Some species of yeast are **opportunistic pathogens** that can cause infection in people with **compromised immune systems**. ***Cryptococcus neoformans*** and ***Cryptococcus gattii*** are significant pathogens of immunocompromised people. They are the species primarily responsible for **cryptococcosis**.

Cryptococcosis: is a **fungal disease** that occurs in about one million **HIV/AIDS** patients, causing over 600,000 deaths annually.

Lab 20 Protozoa (Phylum: Sarcodina)

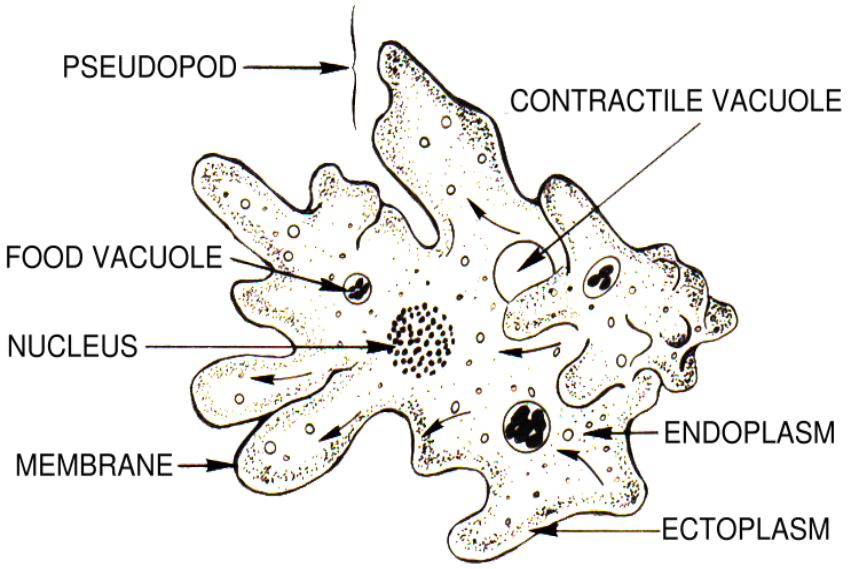
Amoeba (plural amoebas/amoebae) is a genus that belongs to Kingdom protozoa. Generally, the term is used to describe single celled organisms that move in a primitive crawling manner (by using temporary "false feet" known as pseudopods).

Amoebas are eukaryotes, which means that their genetic material are well organized and enclosed within a membrane (nuclear membrane)

Amoeba Microscopy

Amoebas are simply single celled organisms. As such, they can only be viewed using a microscope. There are methods that can be used to observe these organisms.

The first and simplest methods involves viewing amoebas under the microscope without staining. This is a simple method that allows students to view them live as they move around. The second method involves fixing and staining to get a better view of the structure and organelles of the organism.



1. Simple (Direct) Method

Amoebas can be found freely living and thriving in shallow pond waters with organic material.

To view amoebas under the microscope, students will need the following:

- A sample of water collected from a pond with organic material
- Pondweed from a pond
- Petri dish
- A compound light microscope
- Water
- A dropper

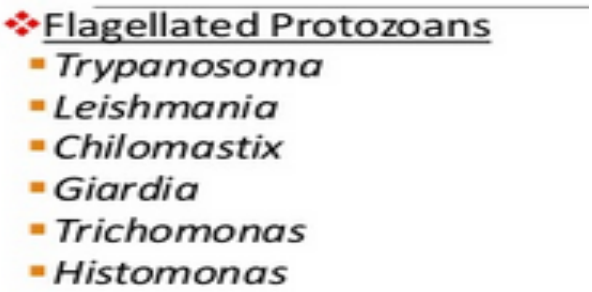
For this technique, the student may either observe a sample of pond water directly to identify the organism or conduct a simple culture to grow and increase the number of amoebas

Observation

When viewed, amoebas will appear like a colorless (transparent) jelly moving across the field very slowly as they change shape. As it changes its shape, it will be seen protruding long, finger like projections (drawn and withdrawn).

Labs 21&22 Protozoa (Flagellated Protozoa)

Movement of the flagellates is accomplished by the presence of flagella in their trophozoite form, this characteristic that distinguishes flagellates from the other groups of protozoa. In flagellates' life cycles that consist of both the trophozoite and the cyst stages.



Genus: *Giardia lamblia* Was for many years considered as nonpathogenic. This organism is now considered to be the only known pathogenic intestinal flagellate It is causing disease called (Giardiasis), the natural habitate to this parasite is duodenum of human intestine.

1– Trophozoite stage: This form is described as pear or teardrop in shape (in front view), whereas in (lateral view) it is spoon shaped. The broad anterior end of the organism tapers off at the posterior end. Its' motility resemble a falling leaf. The appearance is bilaterally symmetrical. Containing two ovoid to spherical nuclei , each with a large karyosome usually centrally located . Peripheral chromatin is absent. This stage is supported by an axostyle made up of two axonemes, two slightly curved rod-like structures known as median bodies (parabasal bodies). Typical trophozoite containing four pairs of flagella (one pair at the anterior end, one pair at the posterior end and two pairs are located laterally which are extended from the axoneme in the center of the body). Also this stage has sucking disc covering one half to three quarters of the ventral surface, the sucking disc serves as nourishment point of entry by attaching to the intestinal villi of an infected human.

2– Cyst stage: It's ovoid in shape and have two median bodies. Immature cysts have 2 nuclei, mature cysts have 4 nuclei and 4 median bodies, central karyosomes

are present but no peripheral chromatin. Mature cysts contain twice as many anterior flagellar structures.

Laboratory Diagnosis

1.Stool examination: The stool sample is the specimen of choice for the recovery of Giardia lamblia trophozoites and cysts.

2. Stool antigen detection: available tests use either an immunofluorescent antibody (IFA) assay or enzyme-linked immunosorbent assay (ELISA) against cyst or trophozoite antigens, these examinations are limited to the detection of Giardia lamblia

.3. Stool culture: not useful for diagnosing Giardiasis because the organism cannot be grown from patient samples.

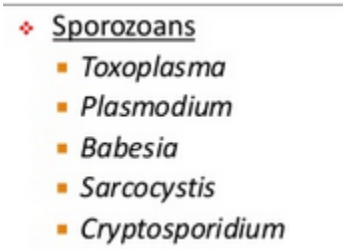
4. Serum antibody detection:

5. String test (entero-test)

Labs23 &24 **Protozoa (Sporozoans)**

General characteristics:

All sporozoa are obligate parasites, they form temporary non-motile spores which contain infective cells.



Genus: *Plasmodium*

This group includes the most pathogenic parasites of man. The word malaria literally means “bad air” reflecting the ancient belief that the disease was contracted by breathing bad air (swamp gas). Human malaria parasites belong to one of four species;

- *Plasmodium vivax,*
- *P. falicparum,*
- *P. malariae,*

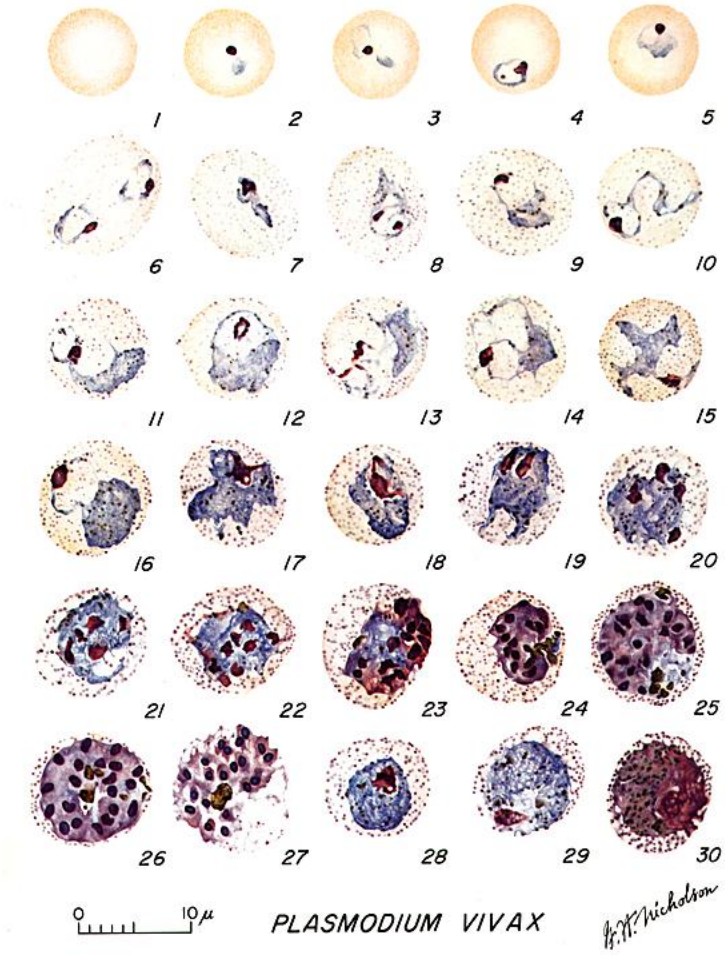
- *P. ovale*.

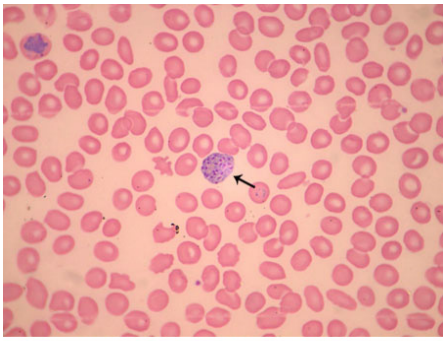
Life cycles: Transmission to humans occurs when infected mosquitoes take a blood meal. Sporozoites in the salivary glands of the vector are introduced into the vertebrate host during feeding.

- Sporozoites remain in the blood for 30 minutes. These travel via the lymphatics and blood system to the liver where they enter a liver cell, transform into meronts, and undergo a series of schizogonic cycles (this cycle is called the **exoerythrocytic phase**, or EE phase).
- Eight days after sporozoite infection, merozoites derived from the EE cycle begin circulating in red blood cells (RBCs). The merozoites in the RBCs undergo another series of schizogonic cycles (this phase is called the **erythrocytic phase**). Inside RBCs, merozoites "round-up" to form the well-known "**ring-form" meront** (or **ring-form trophozoite**).
- Each meront will divide to produce 12-18 "daughter" merozoites. Schizogonic cycles eventually become synchronized so that the release of merozoites resulting from the bursting of red blood cells occurs at regular intervals (every 48 hours) causing a recurrent succession of fever and chills. Eventually, merozoites enter new RBCs, forming trophozoites that develop into micro- and macrogametocytes.
- These must be picked up by the vector to continue development. In the vector, the microgametocyte exflagellates, and the macrogametocyte is fertilized, developing into a motile zygote, the **ookinete**. The ookinete penetrates into the stomach of the vector and develops into an oocyst in which thousands of sporozoites develop. These travel to the salivary glands where they await transmission to the next host.

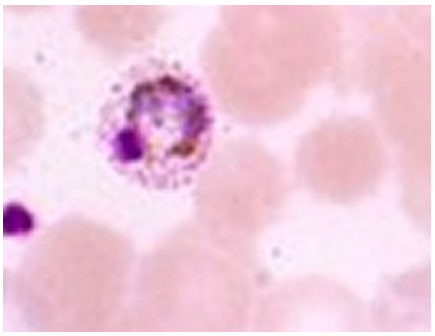
Be able to identify the different stages of *Plasmodium*. Keep in mind the types of slides (particular preparation) you are examining and where in the life cycle the parasite is likely to be when inside these tissues.

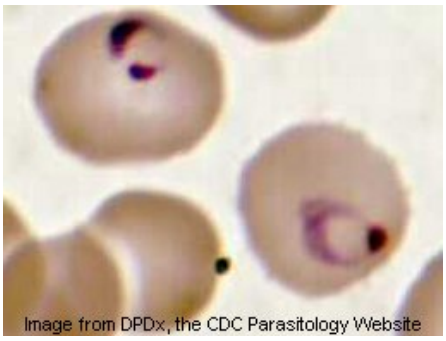
Slide: **Blood smear of *Plasmodium vivax*:** *Plasmodium vivax* can be recognized by its variable ring stage. Schizonts contain about 16 merozoites and the infected cell is enlarged and contains **Schuffner's dots**. The disease caused by this *Plasmodium* is mild and known as **benign tertian malaria** (fever paroxysms typically every 48 hours).



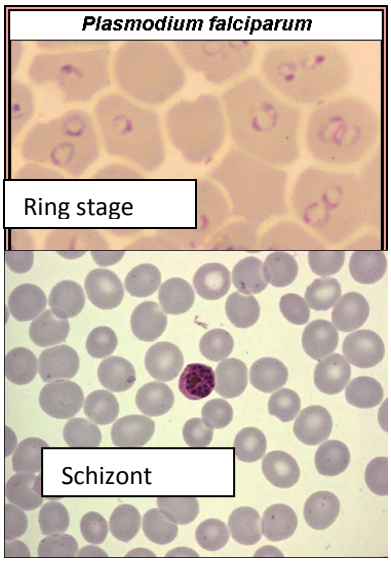


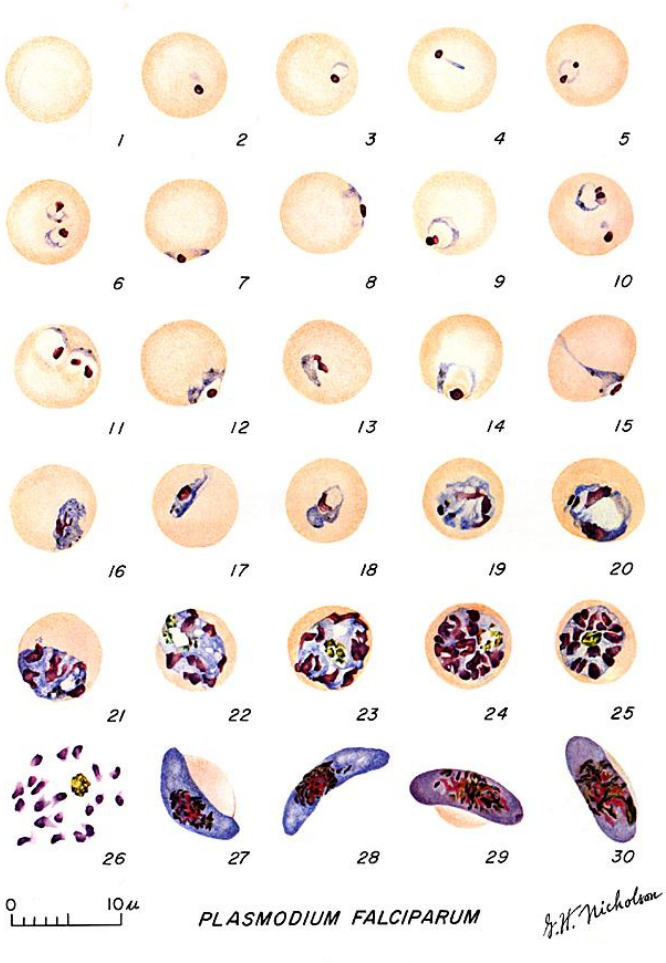
Source: Lichtman MA, Shafer MS, Felgar RE, Wang N: *Lichtman's Atlas of Hematology*: http://www.accessmedicine.com



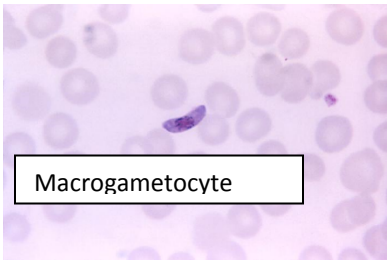


<u>Slide</u>: Blood smear of *Plasmodium falciparum*: has a very neat ring stage trophozoite. Multiply infected cells are common. Schizonts are rare in the peripheral blood. Gametocytes are crescent shaped. The disease caused by this organism is severe and known as **malignant tertian malaria** (fever paroxysms every 48 hours). It is this species that kills the vast majority of humans that die of malaria.





Lab 25



Virology

What is a virus?

- Particle composed of inner core of either DNA or RNA surrounded by a protein coat
- Must replicate within the cell
- Replicate in a different manner from cells (not binary fission)

Methods of Diagnosing Viral Infections

- 1. Virus isolation
- 2. Direct detection of the virus
  - a. Detection of the virus Ag (antigenaemia assay, ELISA, latex agglutination)
  - b. Immune EM
  - c. Detection of the virus nucleic acid (PCR, In situ hybridization)
  - d. Immunocytochemistry, and immunohistochemistry
- 3. Indirect (Serology) … IgM, IgG (titer)

Virus Isolation Using three living systems

1. **Tissue culture**
2. **Embryonated egg**
3. **Animals lab**

Types of tissue culture:

1. Primary tissue culture: from adult human or animal eg. (monkey kidney), can be sub-cultured once or twice.

- Advantages
- -Normal cells
- -Contain the original number of chromosomes
- Disadvantages
- -Can be sub-cultured once or twice
- -Should be prepared freshly each time

-Requires continuous organ supply

2. Semi-continuous: from human fetal tissue, eg. human embryo lung fibroblasts, MRC-5, W138, can be sub-cultured 20-50X.

- Advantages
- 20-50 passages
- Susceptibility for many viruses

- Can grow fastidious viruses

- Disadvantages
- Difficult to be obtained

3. Continuous (Cell line): from human or animal tumors, eg. Vero, HEp-2, HeLa, Hepatoma cell line, can be sub-cultured indefinitely.

- Advantages
- Can be sub-cultured indefinitely
- Easy to obtain
- Disadvantages
- Malignant cells (could express altered phenotype, different number of chromosomes)