Lec./Lab.(1 + 2)

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Safety & Principles of Sterilization in Microbiology Lab.

Laboratory techniques Laboratory techniques are the set of procedures that are used in natural sciences like chemistry, biology to conduct an experiment, all of them follow scientific methods; these procedures involve the use of laboratory equipment from (laboratory glassware to electrical devices), and others require more specific or expensive supplies.

Safety Rules in Microbiology laboratory:

⇒ **Treat all microorganisms as potential pathogens** especially unknown cultures as if they were pathogenic. A student who has a compromised immune system or has had a recent extended illness should talk with the instructor before working in the microbiology laboratory.

⇒ **Sterilize equipment and materials.** All materials, media, tubes, plates, loops, needles, pipetes, and other items used for culturing microorganisms should be sterilized by autoclaving. Otherwise, use commercially sterilized products. Understand the operation and safe use of all equipment and materials needed for the laboratory.

⇒ **Disinfect work areas before and after use.** Use a disinfectant, such as a 10% bleach or 70% ethanol solution, to wipe down benches and work areas both before and after working with cultures. Also be aware of the possible dangers of the disinfectant, as 70% ethanol can catch fire around open flame or high heat sources. Bleach, if spilled, can ruin your clothing. Either alcohol or bleach can be dangerous if splashed in the eyes. Students should know where the nearest eyewash station and sink are located.

⇒ **Wash your hands.** Use a disinfectant soap to wash your hands before and after working with microorganisms. Gloves may be worn as extra protection.

⇒ **Never pipette by mouth.** Use pipette bulbs or pipetting devices for the aspiration and dispensing of liquid cultures.

⇒ **Do not eat or drink in the lab, nor store food in areas where microorganisms are stored.** Never eat or drink in the laboratory while working with microorganisms. Keep your fingers out of your mouth,. Cover any cuts on your hands with a bandage.

⇒ **Label everything clearly.** All cultures, chemicals, disinfectants, and media should be clearly labeled with their names and dates. If they are hazardous, label them with proper warning and hazardous information.

⇒ **Autoclave or disinfect all waste material.** All items to be discarded, such as culture tubes, culture plates, swabs, disposable transfer needles, and gloves, should be placed in a biohazard autoclave bag and autoclaved. If no autoclave is available and you are not working with pathogens, the materials can be covered with a 10% bleach solution and allowed to soak for at least 1 to 2 hours.

⇒ **Clean up spills with care.** Cover any spills or broken culture tubes with a 70% ethanol or 10% bleach solution; then cover with paper towels. After allowing the spill to sit with the disinfectant for a short time, carefully clean up and place the materials in a biohazard autoclave bag to be autoclaved.

Wash the area again with disinfectant. **Never** pick up glass fragments with your fingers or stick your fingers into the culture itself; instead, use a brush and dustpan.

Principles of Sterilization in Microbiology Lab.

Sterilization: is defined as a process of complete elimination or **destruction of all microorganisms including the most resistant bacteria and spores**, which is carried out by various physical, mechanical, and chemical methods.

- **The aims of sterilization are:**

1. Eliminate the possibility of transmission of infectious agents to laboratory workers, the general public, and the environment.

2. Protect the cultures from environmental contaminants.

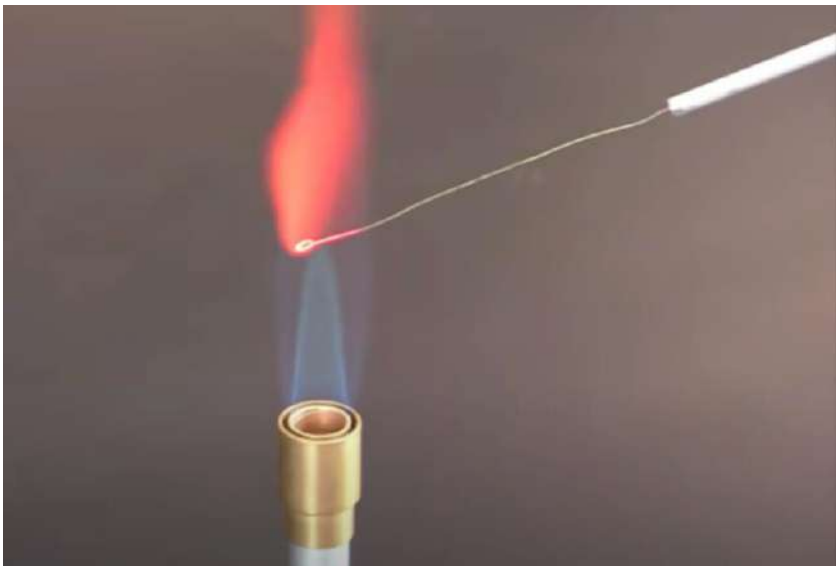
Methods of sterilization

1. Physical Method:

- **Heating:** Is the most rapid and best method of sterilization. which the material to be sterilized is stable enough to withstand the required temperature necessary to kill the microbes. The time needed for sterilization depends on the initial number of organisms present, type of materials to be sterilized (hence washed and cleaned items are easier to sterilize than dirty ones) and also on the temperature used. Spores need higher temperatures while vegetative bacteria can be destroyed at lower temperatures.

i. **Dry Heat:** Dry heat is frequently used for the sterilization of glassware and laboratory equipments. In dry heat sterilization, microbial cells are killed by oxidation of their constituents and protein denaturation (The essential cell constituents are destroyed and the organism dies). The temperature is maintained for almost an hour to kill the most difficult of the resistant spores. Dry heat is applied in the following ways:

a) Red heat: Inoculating wires, points of forceps and searing spatulas are sterilized by holding them in the flame of a bunsen burner until they are seen to be red hot.



b) Flaming: This method is used for sterilizing scalpels, needles, mouths of culture tubes, slides and coverslips. It involves passing them through the Bunsen flame without allowing it to become red hot.

FLAMING

c) Hot air oven: We can sterilize Glassware (like petri dishes, flasks, pipettes, and test tubes), Powder (like starch, zinc oxide, and sulfadiazine), Materials that contain oils, Metal equipment (like scalpels, scissors, and blades). To destroy microorganisms and bacterial spores, a hot air oven provides extremely high temperatures over several hours.

Temperature (°C)	Time (min)
170	30
160	60
150	150

ii. **Moist Heat:** Moist heat kills microorganisms mainly by hydrolysis & coagulation of proteins (denaturation), which is caused by breakage of the hydrogen bonds that hold the proteins in three-dimensional structure. The temperature at which denaturation occurs varies inversely with the amount of water (moist) present. Sterilization in saturated steam thus requires precise control of time, temperature, and pressure. The recommendation for sterilization in an **autoclave st is generally carried out at temperatures between 121°C - 134°C (273°F), under (1.0–2.0 bar) pressure, between 10 and 60 min, depending upon the material and the type of organism to be inactivated.**

emperature (°C)	Sterilization Time (min) for 1 Cycle
132–134	3–10
121	8–30
115	35–45
111	80–180

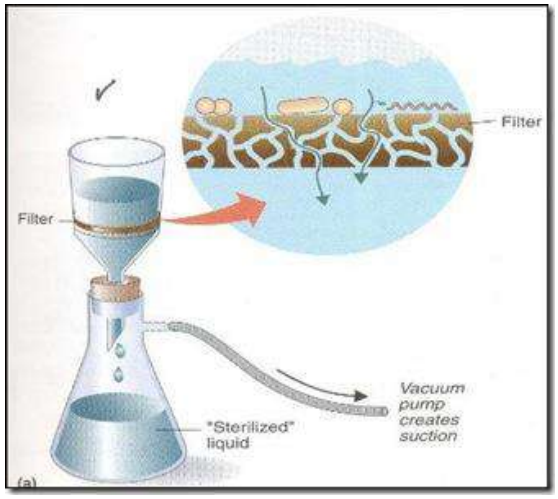
t is important to remove all the air from the autoclave before introducing steam because air is heavier than steam and will reduce the steam concentration (and hence the effectiveness) of the sterilization.

Rediation: By Damaging DNA. It can be Ionizing radiation (X rays, gamma rays) or Nonionizing radiation (UV). it is used for surface sterilization only. The main difference between radiations is their penetration. UV has limited penetration in air and sterilization occur in a small area around the UV lamp. However, it is relatively safe and useful for sterilizing small areas like hoods. x-ray has more penetrating power which make them more dangerous but very effective for large scale sterilization of plastic items (e.g syringes).

2. Mechanical Method:

Filtration: Is the quickest and effective way to sterilize solutions without heating. This method involves filtering with a pore size that is too small for microbes to pass through. It is particularly useful for solutions containing toxins, enzymes, drug, serum and sugars. Sugar solutions used for the cultivation of microorganisms tend to caramelise during autoclaving and so they are best sterilized by filtration. Filters with known pore sizes which are sufficiently small to hold back bacteria are

employed. Recently filters that can remove viruses are also available. Filtration is an excellent way to remove the microbial population from solution containing heat sensitive material.



3. Chemical Method:

Alcohol: Denaturing protein through a process that requires water therefore they should be diluted to 60-90% in water. Alcohol kills germs through a simple chemical process known as denaturation. Denaturation occurs when alcohol molecules break down the proteins present in the structure of germs. When the proteins break down and lose their structure, the cells can't function properly. For example, alcohol can eliminate common bacteria, such as *E. coli*, *salmonella*, and *Staphylococcus aureus*. Alcohol has also been shown to kill viruses such as herpes, hepatitis B, HIV, influenza, and coronaviruses, among others. Ethanol is widely used as a disinfectant and 70% isopropyl alcohol kills organisms by denaturing their proteins and dissolving their lipids. **(70% isopropyl alcohol can effectiveness cross over the cell membrane, thereby attacking the entire cell and killing the bacteria)** and is effective against most bacteria, fungi and many viruses**, but is ineffective against bacterial spores.**

Lec. 3

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Collection, Transportation, Examination & reporting of specimens

⇒ Collection and Transportation of Microbiology Specimens:

[1] Specimen collection & transportation are important factors, because any result generated by laboratory is limited by quality of specimen & its condition on arrival in the laboratory.

[2] The specimen should be obtained to minimize the possibility of introducing contaminating microorganisms that are not involved in the infectious process.

[3] Specimen collection is the crucial step in the investigation procedure of a pathological condition followed by the proper transportation of the specimen to the laboratory and then the identification procedure.

[4] The specimen should be collected according to the site of lesion or infection.

⇒ General Guidelines for Proper Specimen Collection &Transportation

1. All the samples should be collected in specific sterile containers by taking all the necessary precautions and under strict aseptic conditions to prevent the contamination.
2. Collect before antibiotic therapy whenever possible.
3. Clearly label the specimen container with the patient's name, identification number, date and time of collection, and source of specimen.
4. Collect an enough amount of specimen. Insufficient amounts of specimen may result in false-negative results.
5. Do not contaminate the external surface of the collection container and/or its accompanying paperwork.
6. Minimize direct handling of specimens in transit from the patient to the laboratory whenever possible. Use plastic sealable bags.
7. Transport all specimens to the laboratory immediately to ensure the survival and isolation of organisms and to prevent overgrowth by more hardy bacteria. This will provide a more accurate diagnosis of the infectious-disease process.
8. Specimens should always be transported in leak proof container inserted in a leak proof plastic bag.
9. It is important that culture specimens be processed as soon as possible after collection, preferably within 2 h. If longer delays are unavoidable, a suitable transport medium must be used. If urine samples will be

delayed, they should either be refrigerated, inoculated to primary isolation medium before transport, or transported in preservative solution.

⇒ Microscopic Examination of the Specimen in Microbiology Laboratory

1. direct examination of the specimen under the microscope by making a thin smear on the microscopic glass slide
2. staining it with a differential staining like Gram's Staining which gives some idea to the pathologist that what kind of a microbe is suspected to be present and the further procedure of the specimen examination is done accordingly.
3. The culture of the specimen is done to isolate the microbe in pure cultures. For this, the specimen is inoculated on the suitable sterile culture media plates and incubated at an optimum temperature in the incubator, commonly at 37°C. Various types of media are used in the microbiology laboratory for the isolation of microorganisms present in the specimen. Some of the commonly used media are as follows:

    - Nutrient Agar Medium
    - Blood Agar Medium
    - McConkey Agar Medium

4. the plates are incubated at the optimum temperature like for most of the bacteria is 37°C and for the fungi is 25°C for about 24 – 48 hours. The fungi are incubated for about 7-10 days as fungi take much longer time to grow on artificial culture media. After this the culture plates are examined for the colony morphology which may give a microbiologist a rough idea about the suspected microbe present.
5. the colonies obtained in the culture of the specimen are analyzed for the identification of true pathogen that is causing the pathology.

⇒ Reporting of specimens

1. Writing in a scientific style like that use in articles published.
2. To determine a significance of results, it requires knowledge of the patient's clinical status.
3. Advice & suggest.
4. The results in the report should not be confusing factor.
5. Should include comments to help clinicians distinguish infections from contamination

- **Components of a Typical Lab Report**

1. Patient name
2. Name and address of the laboratory location where the test was performed
3. Date report printed.
4. Name of doctor ordering the test(s)
5. Specimen source, when appropriate
6. Name of the test performed
7. Test result.
8. Abnormal test results. Lab reports will often draw attention to results that are abnormal or outside the reference range by setting them apart or highlighting them in some way. For example, “H” next to a result may mean that it is higher than the reference range. “L” may mean “low” and “WNL” usually means “within normal limits.”
9. Units of measurement
10. Reference ranges

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Culturing of organisms - Conventional microbiological techniques

Microbiological culture: is a method of multiplying microbial organisms under sterilized condition by allowing them to grow in specified culture medium. It is one of the major microbiological procedures used to diagnose the cause of infectious disease.

Purpose of Culturing

1. Isolation of Microorganisms from samples
2. Obtaining pure cultures.
3. Demonstrate their properties.
4. Determine sensitivity to antibiotics.
5. Count microbial cells
6. Maintain stock cultures.

A pure culture: contains only **one species** of microorganisms.

A mixed culture: contains **two or more species** of microorganisms.

If a bacterial culture is left in the same media for too long, the cells use up the available nutrients, excrete toxic metabolites, and eventually the entire population will die. Thus bacterial cultures must be regularly transferred, or **subcultured**, to new media to keep the bacterial population growing.

A culture media: is a special medium used in microbiological laboratories to grow different kinds of microorganisms. It is composed of different nutrients that are essential for microbial growth.

Compositions of culture media:

When culturing bacteria, it is very important to provide similar environmental and nutritional conditions that exist in its natural habitat. Hence, an artificial culture medium must provide all the nutritional components that a bacterium gets in its natural habitat.

A culture medium contains:

- Water
- Carbon Sources: Carbohydrate & Hydrocarbons
- Nitrogen Sources: (Ammonia, ammonium salts, & urea)
- Minerals: (Calcium, chlorine, magnesium, phosphorous, potassium & sulfur)
- Growth Factors: Vitamins, amino acids, and fatty acid

Classification of culture media: Bacterial culture media can be classified based on:

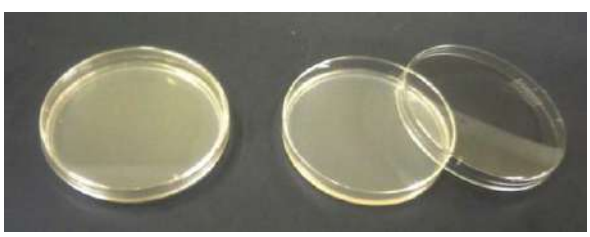
- **CONSISTENCY / PHYSICAL STATE**
- **CHEMICAL COMPOSITION**
- **PURPOSE or USE**

1) Classification based on consistency / Physical state:

A) Liquid media / Broth : When nutrients are dissolved in a solvent without any solidifying agent, then these types of media are known as **Liquid Media or Broth.** In liquid medium, bacteria grow uniformly producing general turbidity. These are available for use in test-tubes, bottles or flasks. In liquid medium, bacteria grow uniformly producing general turbidity.



B) Solid media / Agar: Any liquid medium can be made by the addition of certain solidifying agents (agar) at a concentration of (1.5 – 2.0)%. Agar is a polysaccharide obtained from the cell membranes of some species of red algae.

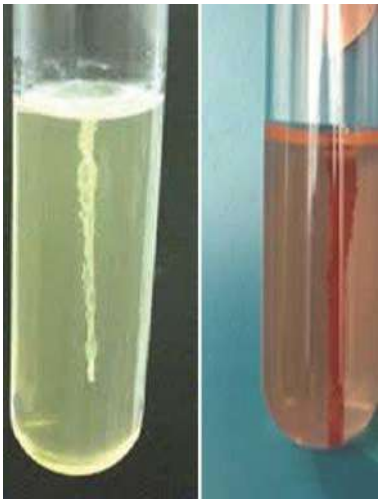



Advantages of solid media:

- **Colony identification**
- **Isolation and enumeration of microbial cells**
- **Determining the colony characteristics of the isolate.**
- **Mixed bacteria can be separated**
- **Antibiotics sensitivity tests.**

C) Semi-solid agar: contain a low percentage (less than 1%) of agar, which can be used for:

- **Motility testing**
- **Separating motile from non-motile strains**
- **Cultivation of microaerophilic bacteria.**



2) Classification based on chemical composition

a. **Synthetic or chemically defined medium**
b. **Non-synthetic or chemically undefined medium**

3) Classification based on purpose or use:

A. **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.**
B. **Enriched Media:** Addition of extra nutrients in the form of blood, serum, egg yolk, etc, to the basal medium makes an enriched medium. Enriched media are used to grow nutritionally exacting (fastidious)

bacteria. Blood agar, chocolate agar, etc are a few examples of enriched media. **Blood agar** is prepared by adding 5-10% (by volume) blood to a blood agar base**. Chocolate agar** is also known as heated blood agar.

C. **Selective media:** Selective medium is designed to inhibit the growth of some microorganisms while allowing the growth of others. To make a medium selective include antibiotics, dyes, chemicals, alteration of pH or a combination of these can be used. **Examples of selective media include: Mannitol Salt Agar MacConkey's Agar.**

D. **Differential/ Indicator Media:** bacteria can be recognized on the basis of their colony colour. An indicator is included in the medium. A particular organism causes change in the indicator, e.g. blood, neutral red, tellurite. Examples: Blood agar and MacConkey agar are indicator media.

- **Transport media:** Clinical specimens must be transported to the laboratory immediately after collection to
- **prevent overgrowth of contaminating organisms or commensals**,
- **prevent drying of specimen**

This medium maintains all the organisms in the specimen without altering their concentration.These types of medium **only contain buffers and salts**. Examples of these media: **Cary-Blair medium**, **Amies medium,** & **Stuart medium.**

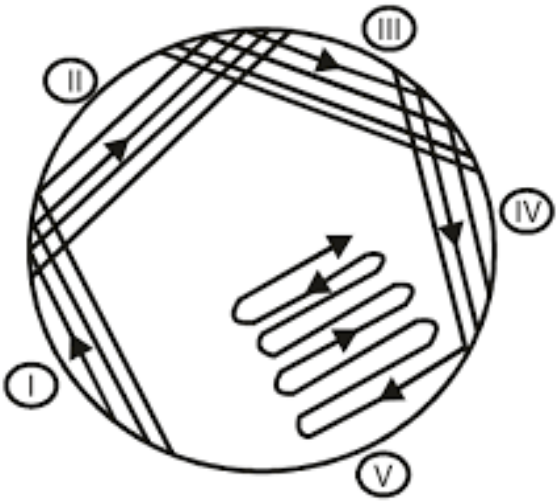
Methods of Culturing

Various methods are used for culturing of bacteria. These include :

a. **Streak Culture:** The streak plate method is a microbiological laboratory technique used to **:**

1. obtain a pure culture of bacteria from a mixed culture
2. obtain well-isolated colonies
3. propagate bacteria

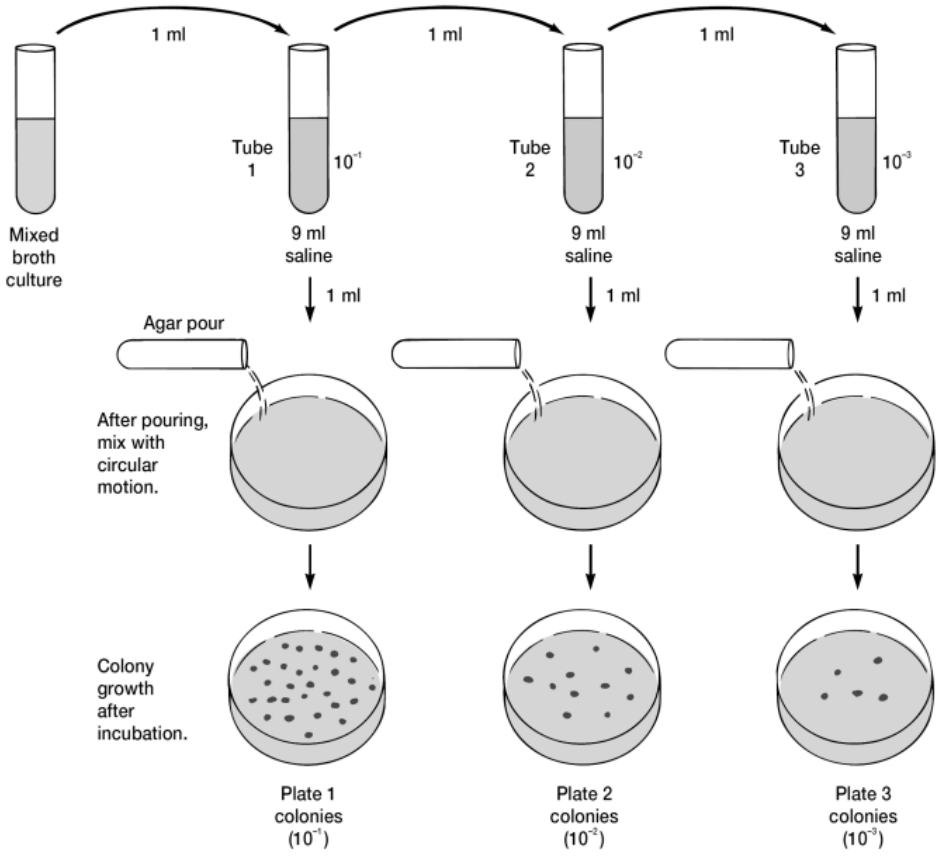
Single isolated colonies obtained by this method are very useful to study various properties of bacteria.



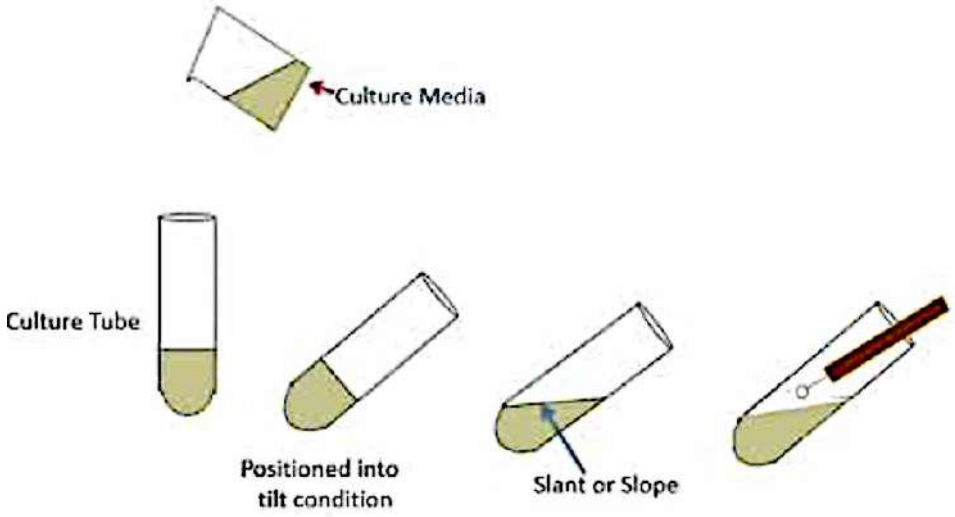
Inoculation procedure (flame loop between streaks)

Formation of discrete colonies

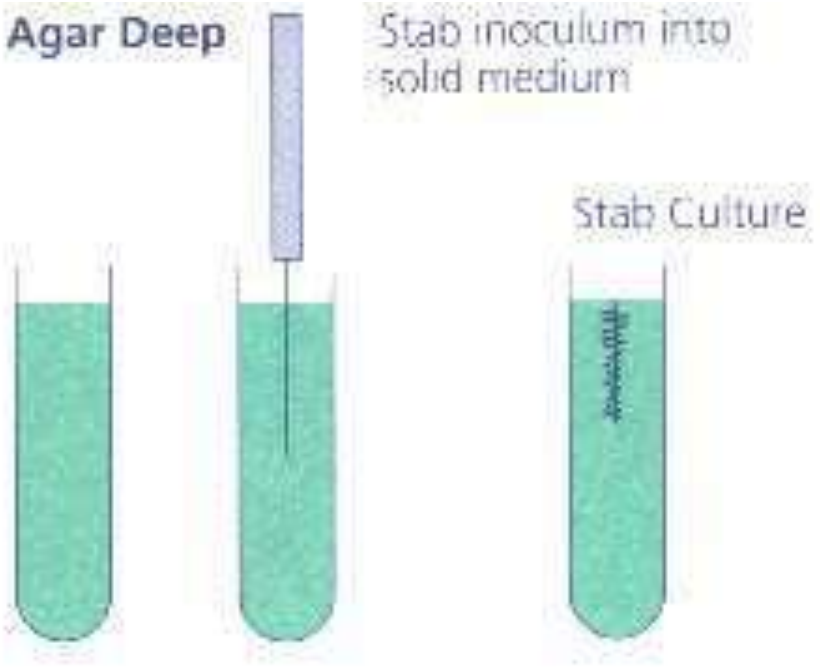
b. **Pour-Plate Culture:** This method used for counting the number of colony-forming bacteria present in a liquid specimen. This method is carried out in tubes, each containing 9 mL of molten agar. The molten agar in tubes is left to cool in a water bath at 45°C. The inoculum to be tested is diluted in serial dilution. Then 1 mL each of diluted inoculum is added to each tube of molten agar and mixed well. The contents of tubes are poured into sterile Petri dishes and allowed to set. After overnight incubation of these Petri dishes at 37°C, colonies are found to be distributed throughout the depth of the medium, which can be counted using a colony counter.



c. **Stroke Culture:** It is carried out in tubes usually containing nutrient agar slopes. It is carried out in tubes usually containing nutrient agar slopes. In this type of culture method, liquified culture media is poured in culture tubes, these tubes are placed in inclined position to increase the upper surface area of culture.



d. **Stab Culture:** Stab culture is prepared by stabbing the medium in tubes with a long, straight wire and incubating at 37°C.



e. **liquid culture:** Liquid culture is prepared in a liquid media enclosed in tubes, flasks, or bottles. The medium is inoculated by touching with a charged loop or by adding the inoculum with pipettes or syringes and incubating at 37°C, followed by subculture on to solid media for final identification. A major disadvantage of liquid culture is that it does not provide pure culture of the bacteria and also the bacterial growth does not exhibit special characteristic appearances (Identification of bacteria is not possible).

Lec. 5

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Biochemical Testing of Microorganisms

Biochemical tests are the most important methods for microbial identification. Microbial biochemistry tests shorten the time required to identify microbes, reduce costs, and ensure or enhance the accuracy of identification of an unknown sample.

Catalase Test

The catalase test tests for the presence of catalase, an enzyme that breaks down the harmful substance hydrogen peroxide into water and oxygen. It is used to differentiate those bacteria that produce an enzyme catalase, such as staphylococci, from non-catalase-producing bacteria such as streptococci. Normally 3% H2O2 is used for the routine culture while 15% H2O2 is used for detection of catalase in anaerobes.

Principle of Catalase Test

The enzyme catalase mediates the breakdown of hydrogen peroxide into oxygen and water. The presence of the enzyme in a bacterial isolate is evident when a small inoculum is introduced into hydrogen peroxide, and the rapid elaboration of oxygen bubbles occurs. The lack of catalase is evident by a lack of or weak bubble production.

Bacteria thereby protect themselves from the lethal effect of Hydrogen peroxide which is accumulated as an end product of aerobic carbohydrate metabolism.

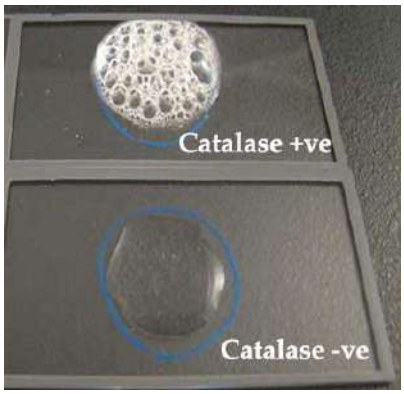
Uses

1. Used to differentiate between Streptococcus (catalase -ve) & Staphylococcus (catalase +ve)
2. Used to differentiate strains of Clostridium, which are catalase-negative, from Bacillus species, which are positive.

Procedure of Catalase Test

Slide Method

1. Use a loop or sterile wooden stick to transfer a small amount of colony growth in the surface of a clean, dry glass slide.
2. Place a drop of 3% H_2O_2 in the glass slide.
3. Observe for immediate bubble formation



Positive: The formation of bubbles is considered a positive result

Examples: *Staphylococci, Corynebacterium diphtheriae, E. coli, Enterobacter, Klebsiella, Shigella, Proteus, Salmonella*, *Pseudomonas, Mycobacterium tuberculosis, Aspergillus.*

Negative: No or very few bubbles produced.

Examples: *Streptococcus and Enterococcus* spp

Precautions of Catalase Test

- The test organisms should not be taken from blood agar culture. Red Blood cells contain catalase and their presence will give a false positive test.
- Hydrogen peroxide must be fresh as it is very unstable.
- Iron wire loop should not be used. Iron containing loops will cause false positive test results if exposed to hydrogen peroxide.

Oxidase Test

Purpose

Tests for the presence of the enzyme indophenol oxidase.

Principle

Indophenol oxidase, in the presence of atmospheric oxygen, oxidizes the phenylenediamine oxidase reagent to form a dark purple compound, indophenol.

Uses

The oxidase test is based on the production of an enzyme called indophenols oxidase. This enzyme oxidizes a redox dye (present in the reagent) which results in a color change of yellow to dark purple.

Procedure of Oxidase test

Oxidase test can be performed in various ways. These include:

- **Filter paper test**
- **Filter paper spot test**
- **Direct plate method**
- **Test tube method**

Filter Paper Test Method

1. Soak a small piece of filter paper in 1% Kovács oxidase reagent and let dry.
2. Use a loop and pick a well-isolated colony from a fresh (18- to 24- hour culture) bacterial plate and rub onto treated filter paper
3. Observe for color changes.

Results:

- **Oxidase positive:** color changes to dark purple within 5 to 10 seconds.
- **Delayed oxidase-positive**: color changes to purple within 60 to 90 seconds.

- **Oxidase negative**: color does not change or it takes longer than 2 minutes.

Uses of oxidase test:

- Helpful in screening colonies suspected of being a member of the ***Enterobacteriaceae*** family; all the members of the Enterobacteriaceae **family including *E. coli* are oxidase negative.**
- Oxidase test is especially important in **separating *Aeromonas* from *Enterobacteriaceae.***
- Used as a major characteristic for the identification of Gram-negative rods that are not in the *Enterobacteriaceae* family.
- Colonies suspected of belonging to other genera ***Aeromonas, Pseudomonas, Neisseria, Campylobacter*, and *Pasteurella* are oxidase positive.**

Limitations:

- Any delayed reactions should be considered negative.
- Do not add excess reagent, at it may cause the reaction to fade on oxides-positive organisms.
- Steel loop, nichrome loop, and wire loop containing iron may give a false-positive reaction. A platinum loop or wooden applicator stick is recommended.

Motility Test

Uses

- Used for detecting motility of microorganisms.

Principle:

Motility is apparent by the presence of diffuse growth away from the line of inoculation.

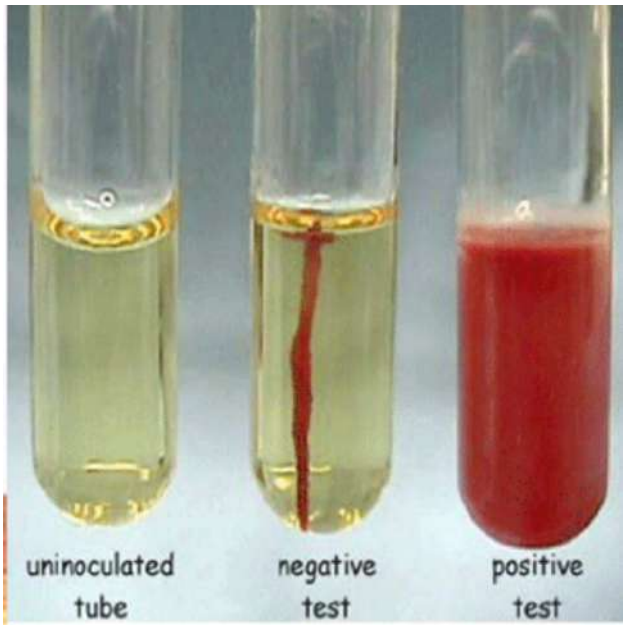
The medium mainly used for this purpose is SIM medium (Sulphide Indole Motility medium) which is a combination differential medium that tests three different parameters, Sulfur Reduction, Indole Production and Motility. This media has a very soft consistency that allows motile bacteria to migrate readily through them causing cloudiness. Bacterial motility is evident by a diffuse zone of growth extending out from the line of inoculation. Some organisms grow throughout the entire medium, whereas others show small areas or nodules that grow out from the line of inoculation. The non-motile bacteria will only grow in the soft agar tube and only the area where they are inoculated.

Test Procedure

1. Inoculate with growth from an 18-24 hour culture by stab inoculation with a needle.
2. Incubate at a temperature and duration appropriate for the organism being tested.
3. Examine tubes for growth and signs of motility.

Results

- **Positive:** Diffuse, hazy growths that spread throughout the medium rendering it slightly opaque.
- **Negative:** Growth that is confined to the stab-line, with sharply defined margins and leaving the surrounding medium clearly transparent.



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Molecular Diagnostic Techniques

Molecular Diagnostics Techniques

The process of identifying a disease by studying molecules, such as proteins, DNA, and RNA, in a tissue or fluid from an affected individual.

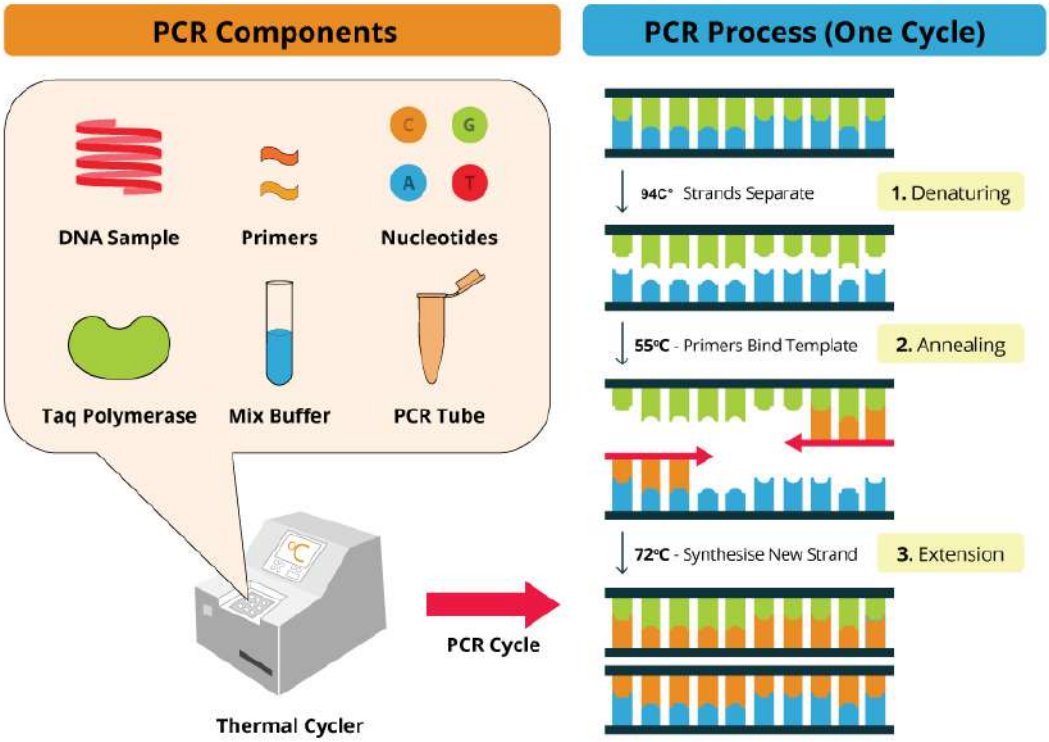
Applications of Molecular Diagnostics Techniques

1. Classification of organisms based on genetic relatedness (genotyping).
2. Identification & confirmation of isolate obtain from culture.
3. Early detection of pathogen in clinical samples.
4. Rapid detection of antibiotic resistance.
5. Detection of mutations.
6. Differentiate toxigenic from nontoxigenic strains.
7. Detection of microorganisms that impossible, dangerous, & costly to culture.
8. Diagnosis of non-culturable agents: e.g., Human papillomavirus, Hepatitis B virus etc.
9. Detection of slow-growing microorganisms: e.g., Mycobacterium tuberculosis
10. Detection of microorganisms when present in **small numbers in clinical samples** e.g., detection of HIV in antibody-negative patients, intra-ocular fluid in case of **(uveitis) eye inflammation.**

Polymerase Chain Reaction (PCR) – (Molecular Photocopy)

It is a fast and inexpensive technique used to amplify a single copy or a few copies of a specific segment of DNA to generate thousands to millions of copies of a particular DNA sequence.

PCR Reaction Components:



DNA template: DNA template is DNA target sequence. At the beginning of the reaction, high temperature is applied to separate both the DNA strands from each other so that primers can bind during annealing (**the process of heating then cooling slowly**)

DNA polymerase: is an enzyme acts to add nucleotides complementary to template strand ~~at~~ 3'-OH of the bound primers and synthesizes new strands of DNA complementary to the target sequence.

The most commonly used **DNA polymerase is Taq DNA polymerase (from Thermus aquaticus, a thermophillic bacterium- (species of bacteria that can tolerate high temperatures), because of high-temperature stability.**

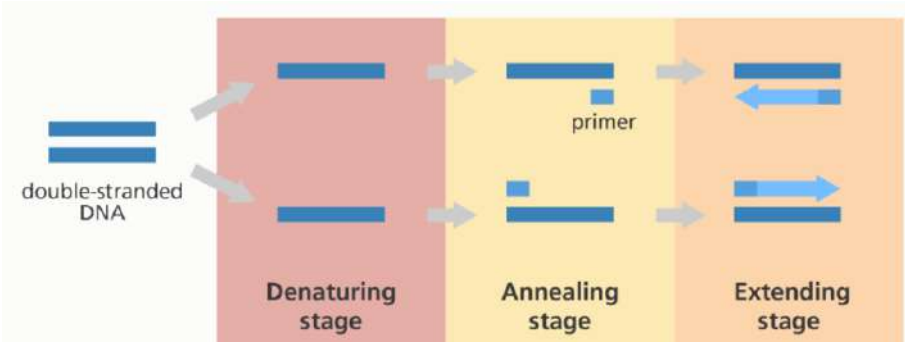
Mg2+ ions: in the buffer act as **co-factor for DNA polymerase enzyme** and hence are required for the reaction.

Primers: Primers are synthetic DNA strands of about 18 to 25 nucleotides complementary to 3' end of the template strand. DNA polymerase starts synthesizing new DNA from the 3' end of the primer. Two primers must be designed for PCR; the **forward primer** and the **reverse primer. (A primer is a short strand of RNA or DNA that serves as a starting point for DNA synthesis).**

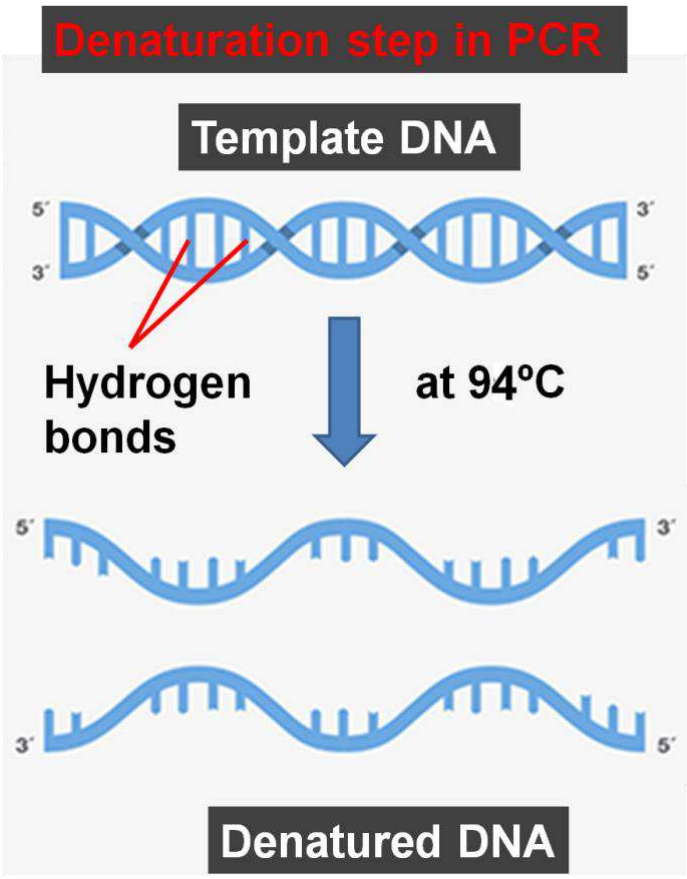
Nucleotides (dNTPs or deoxynucleotide triphosphates): All types of nucleotides are "building blocks" for new DNA strands and essential for reaction. It includes Adenine(A), Guanine(G), Cytosine(C), Thymine(T) or Uracil(U).

PCR Steps

1. **Denaturing** – when the double-stranded template DNA is heated to separate it into two single strands.
2. **Annealing** – when the temperature is lowered to enable the DNA primers to attach to the template DNA.
3. **Extending** – when the temperature is raised and the new strand of DNA is made by the Taq polymerase enzyme.



1. **Denaturation at 94°C**: During the heating step (denaturation), the reaction mixture is heated to 94°C for 1 min, which causes separation of DNA double stranded. Now, each strand acts as template for synthesis of complementary strand.

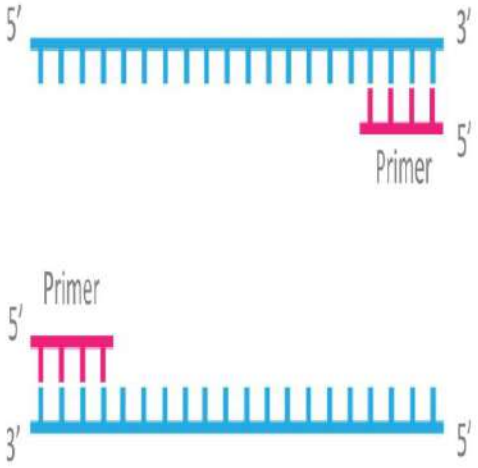


- **The high temperature** causes the hydrogen bonds between the bases in two strands of template DNA to break and the two strands to separate.
- This results in two single strands of DNA, which will act as templates for the production of the new strands of DNA.
- It is important that the temperature is maintained at this stage for long enough to ensure that the DNA strands have separated completely.

2. **Annealing at 55°C** : This step consist of cooling of reaction mixture after denaturation step to **55°C**, which causes **hybridization (annealing)** of primers to separated strand of DNA (template). The length and **GC** content (**G**uanine-**C**ytosine content) of the primer should be sufficient for stable binding with template.

⇒ **Primers serve as the starting point for DNA synthesis. Only once the primer has bound can the polymerase enzyme attach and start making the new complementary strand of DNA from the loose DNA bases.**

⇒ **The two separated strands of DNA are complementary and run in opposite directions.**

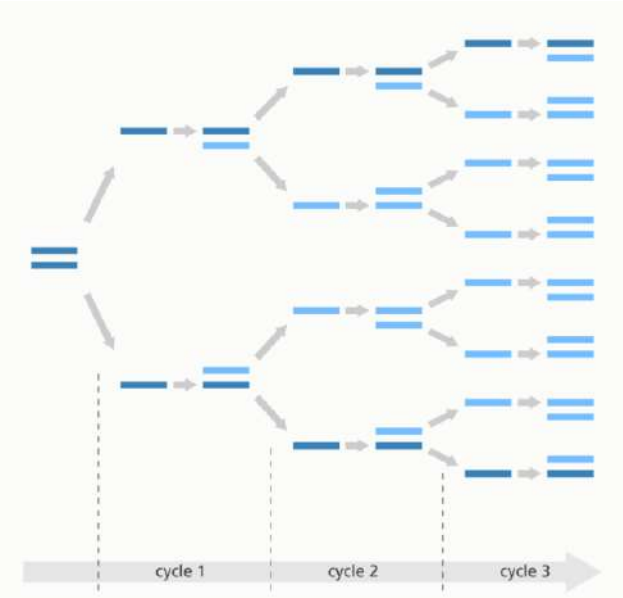


Annealing

3. **Extension at 72°C**: The reaction mixture is heated to 72°C which is the (**OPTIMAL temperature for the Taq polymerase working)**. The polymerase adds nucleotide (dNTP's) complimentary to template thereby extending the new strand.

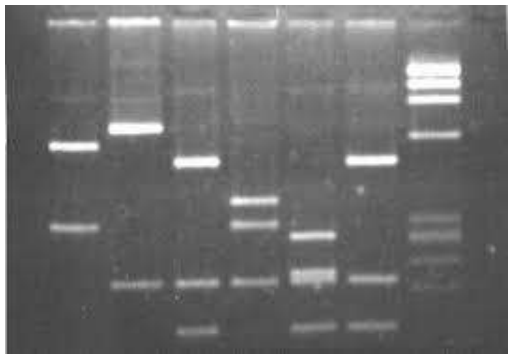
Final hold: First three steps are repeated 35-40 times to produce millions of exact copies of the target DNA. Once several cycles are completed, during the hold step, 4–15 °C temperature is maintained for short-term storage of the amplified DNA sample.

A result **is a huge number of copies of the specific DNA segment produced in a short period of time.**

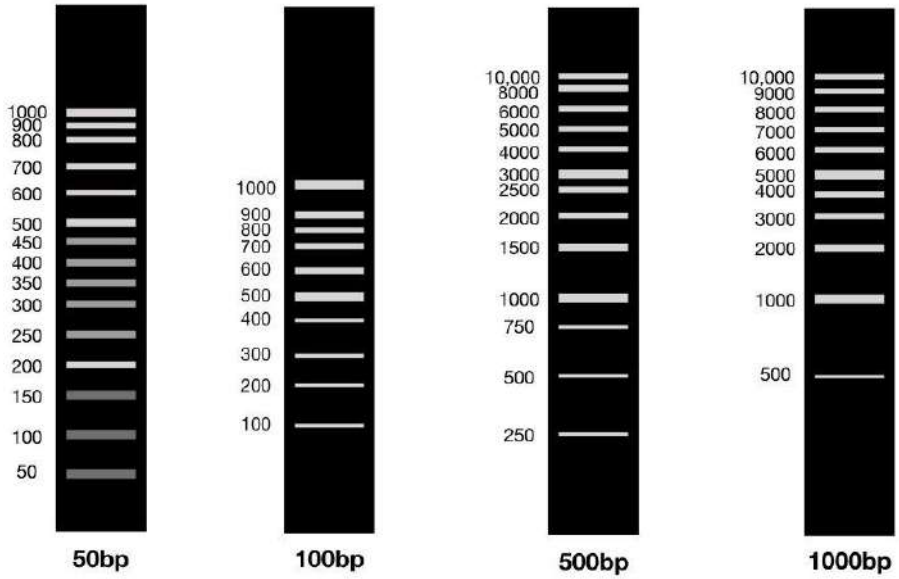


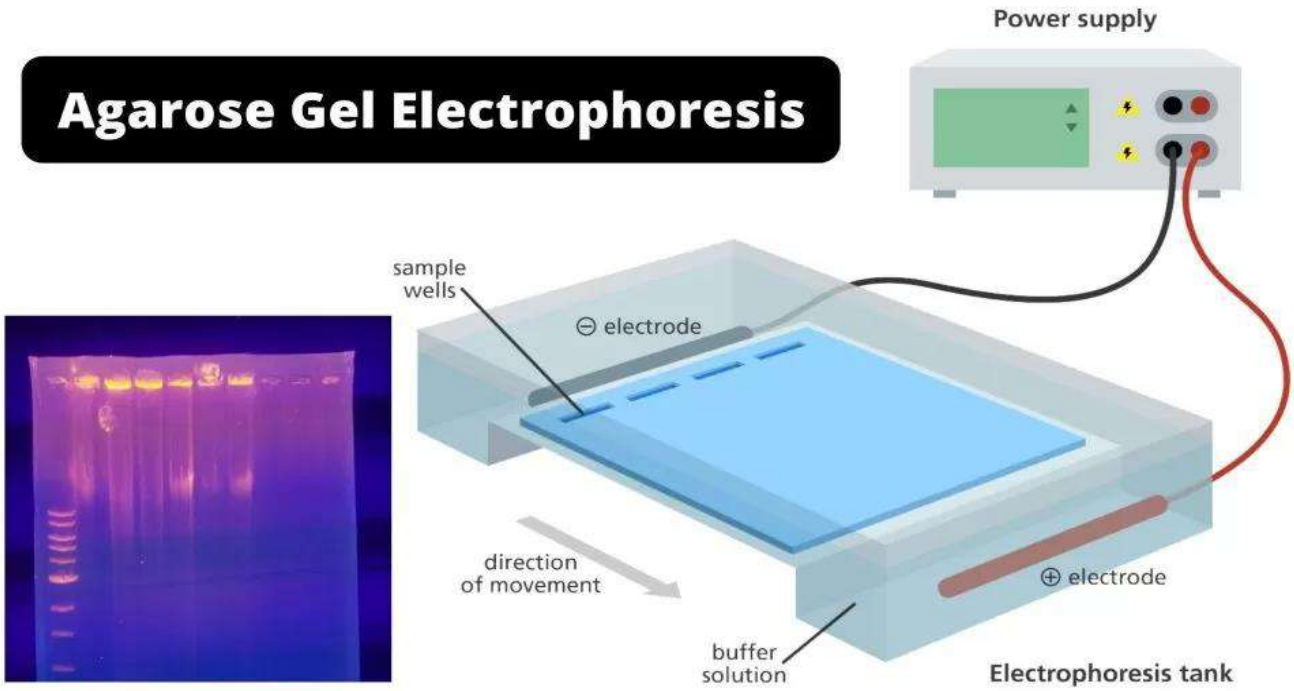
Agarose Gel Electrophoresis

Gel electrophoresis is the standard lab procedure for separating DNA by size (e.g., length in base pairs) for visualization and purification. Electrophoresis uses an electrical field to move the negatively charged DNA through an agarose gel matrix toward a positive electrode. Shorter DNA fragments migrate through the gel more quickly than longer ones. Thus, you can determine the approximate length of a DNA fragment by running it on an agarose gel alongside a DNA ladder (a collection of DNA fragments of known lengths).



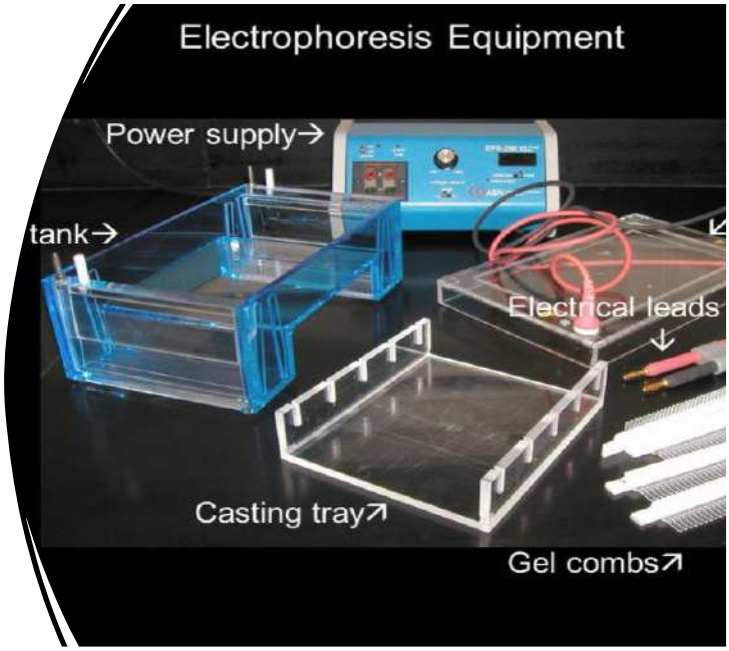
"The DNA ladder is a standard-sized molecular marker or fragments of DNA applied to determine the size of PCR amplicons. 50bp, 100bp, 1000bp and 3000bp are several commercially available and popular markers."





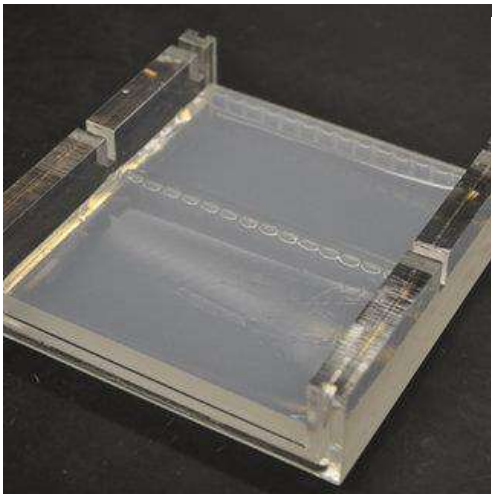
Equipments

- Casting tray
- Well combs
- Voltage source
- Gel box
- UV light source
- Microwave



Reagents

- **TAE**
- **Agarose**
- **Ethidum bromide**



Procedure

https://www.youtube.com/watch?v=TIZRGt3YAug

Lec. 8

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Examination of Swabs

Collection of swabs for culture

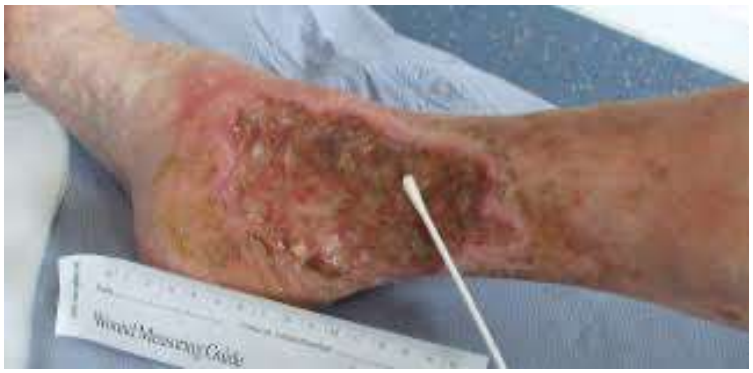
Swabs from the genital tract, throat, eye, ear, nose, and superficial wounds (e.g., sores, boils, and rashes) should be transported to the laboratory in the transport medium **Amies medium with charcoal.**

Swabs C&S- Amies Transport Medium
Charcoal or Clear Gel

Collection of WOUND swabs

- Gentle cleansing of a skin wound prior to sample collection is recommended to reduce commensal flora contamination.
- Swabs must be used to collect purulent exudates.
- Place the swab into the transport media.
- Label swab with patient's full name, source of specimen and date and time of collection.

- Maintain swabs at **room temperature** and **submit them to the laboratory WITHIN 24 hours of collection.**

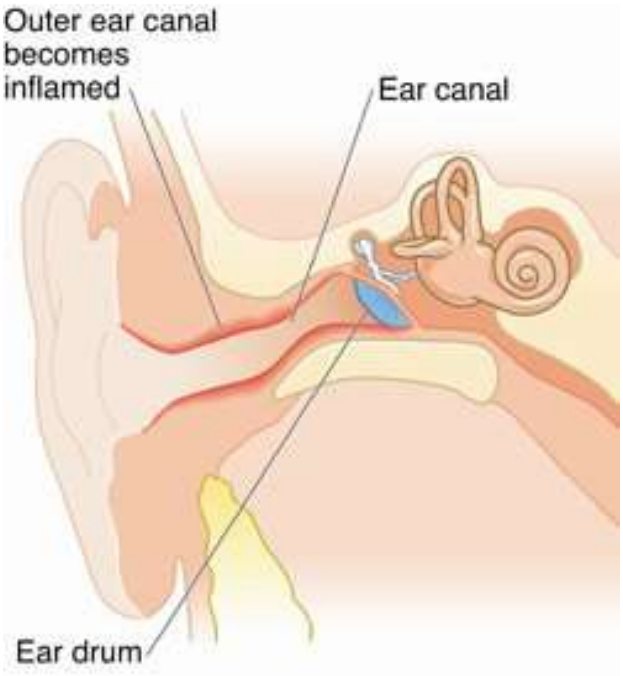


Collection of EYE swabs

- Collect before topical or anesthetics are applied.
- Swab pus or purulent discharge taken from the lower inverted lid.
- Place the swab into the transport media.
- Label swab with patient's full name, date of birth or health card number, source of specimen and date and time of collection.
- Maintain swabs at **room temperature** and **submit to the laboratory WITHIN 24 hours of collection.**

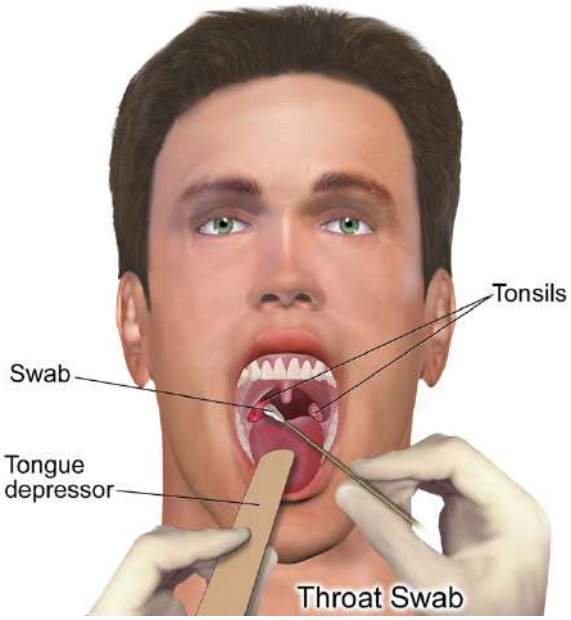
Collection of Ear swabs

- Swab the external ear canal.
- Place the swab into the transport media.
- Label swab with patient's full name, source of specimen and date and time of collection.
- Maintain swabs **refrigerated at 2-8 oC** and **submit to the laboratory WITHIN 24 hours of collection.**



Collection of Throat swabs

- Swab the tonsillar area and/or posterior pharynx.
- Place the swab into the transport media.
- Label swab with patient's full name, date of birth or health card number,source of specimen and date and time of collection.
- Maintain swabs at **room temperature** and **submit to the laboratory WITHIN 24 hours of collection. Genital Swab Cultures include.**



Collection of Urethral specimens for culture of Neisseria gonorrhea

- Exudates from the urethra should be collected on a swab.Place the swab into the transport medium.
- Label swab with patient's full name, source of specimen and date and time of collection.
- Maintain swabs at room temperature and submit to the laboratory WITHIN 24 hours of collection.

Collection of Vaginal swabs for yeast, bacterial vaginosis & Trichomonas

Bacterial Vaginosis: Bacterial vaginosis is a type of vaginal inflammation caused by the overgrowth of bacteria naturally found in the vagina, which upsets the natural balance.

Symptoms:

- Thin, gray, white or green vaginal discharge
- Foul-smelling "fishy" vaginal odor
- Vaginal itching
- Burning during urination

- Wipe away any excessive amount of secretion.
- Using a swab, collect secretions from the mucosal membrane.
- Place the swab into the transport medium.
- Label swab with patient's full name, date of birth or health card number, source of specimen and date and time of collection.
- Maintain swabs at room temperature and submit to the laboratory WITHIN 24 hours of collection.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Lec. 9
Cell and Tissue Culture

The culture of animal cells and tissues is a generally and widely used technique that involves isolation of cells, tissues and organs from animals and growing them in an in vitro or artificial environment. The term culture means to keep alive and grow in an appropriate medium.

The list of different cell types which can now be grown in culture include connective tissues such as

- fibroblasts
- skeletal, cardiac and smooth muscle
- epithelial tissues
- neural cells
- endocrine cells and
- many different types of tumor cells.

In vitro culture has been proven to be the most valuable method **to study the functions and mechanism of operations of many cells.**

APPLICATIONS OF ANIMAL CELL CULTURE

- Cancer research
- Vaccine manufacturing
- Recombinant protein production
- Drug selection and development
- Gene therapy

APPLICATIONS OF ANIMAL CELL CULTURE

- Stem cell biology
- In vitro fertilization technology
- Genetic engineering
- Toxicity testing
- Virology

LIST OF MAIN COMPONENTS FOR CELL CULTURE

1. Bio Safety Cabinets: It offers protection from contaminants during culture.

CO_2 Incubators: Cells are grown in an atmosphere of 5%-10% CO2. It keeps constant level of humidity

Microscopes: Inverted microscopes are used for this purpose.

Culture vessels: These consist of petri dishes, multi-well plates, microtiter plates, roller bottles, screw cap flasksT-25, T-75, T150.

Centrifuges: Cells are centrifuged at low temperature and low speed.

Freezer: For freezing and short-term storage

Hemocytometer: To determine the cell counts before or after culture.

Water bath with shaker: For cell dissociation and trypsinization

Liquid N2 Cylinder provides a very stable ultra-low temperature environment. Thus, it is used for long-term storage

pH meter: To determine the pH of the medium.

Primary culture:

Freshly isolated cultures are known as primary cultures until they are passaged or subculture. The first step in obtaining the primary culture is isolation of tissues from the whole part or organ, followed by disaggregation of cells from the tissues. This is done by addition of trypsin to the tissue for disintegration and isolation of cells. The cells obtained after trypsin digestion are incubated in a medium.

Subculture culture:

A subculture is a new culture taken from a primary culture and grown separately in the culture medium. Subculture allows the expansion of the culture (it is now known as a cell line).

Procedure:

1- https://www.youtube.com/watch?v=Qv-Lo2bMjQc
2- https://www.youtube.com/watch?v=pP0xERLUhyc

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Lec. 10

Advanced Lab. Techniques

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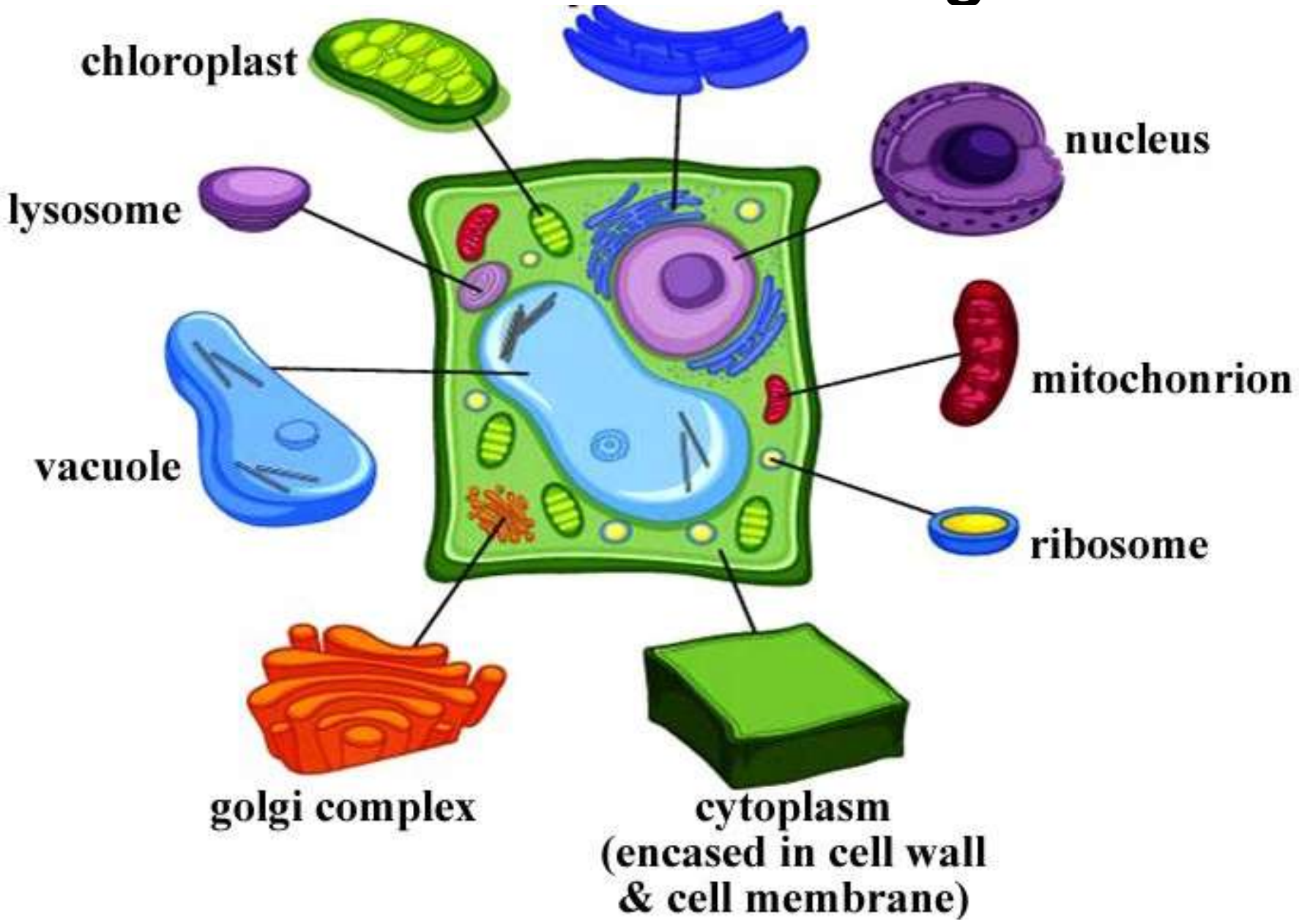
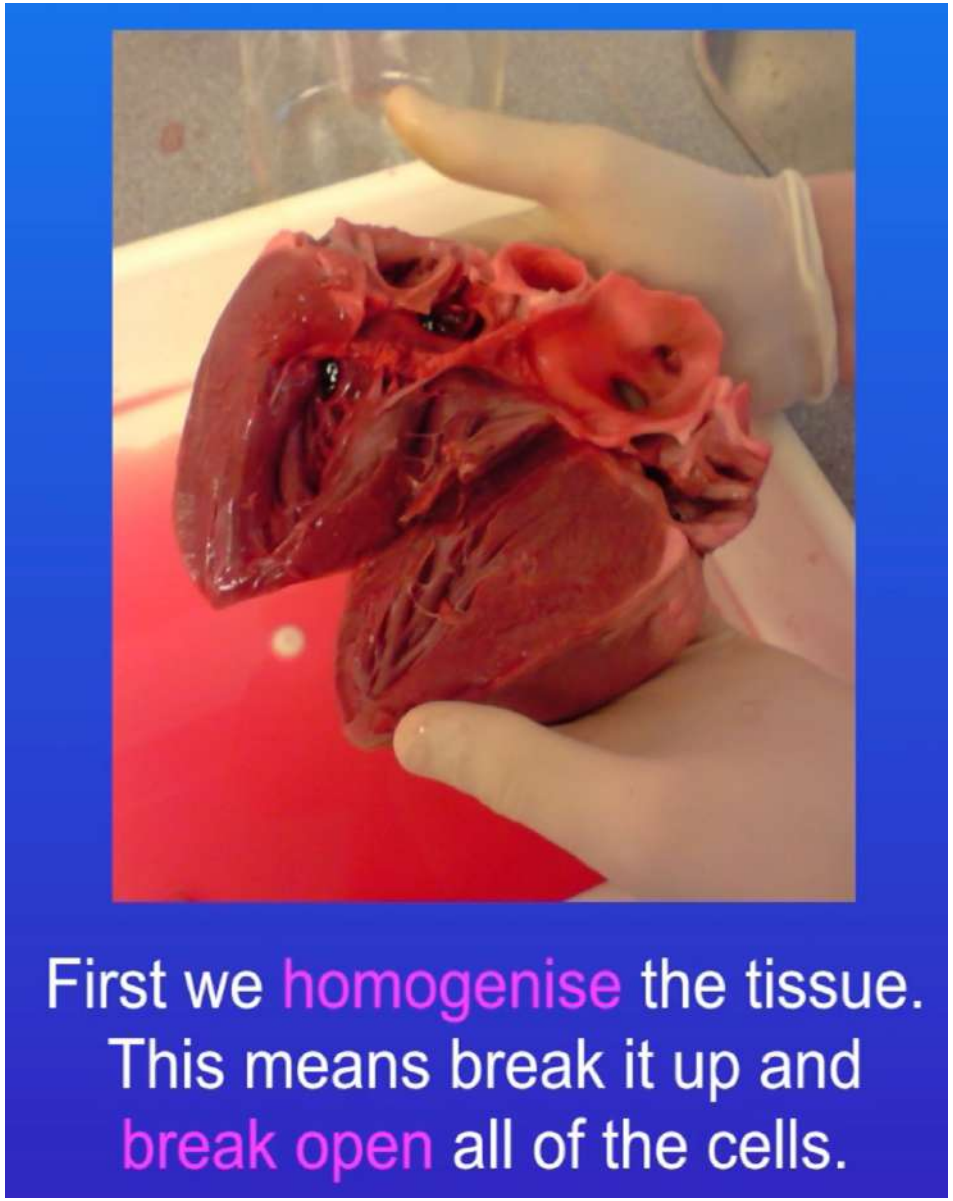
Cell Homogenization & Fractionation

Cell Fractionation: is the process used to separate cellular components while preserving functions of each component

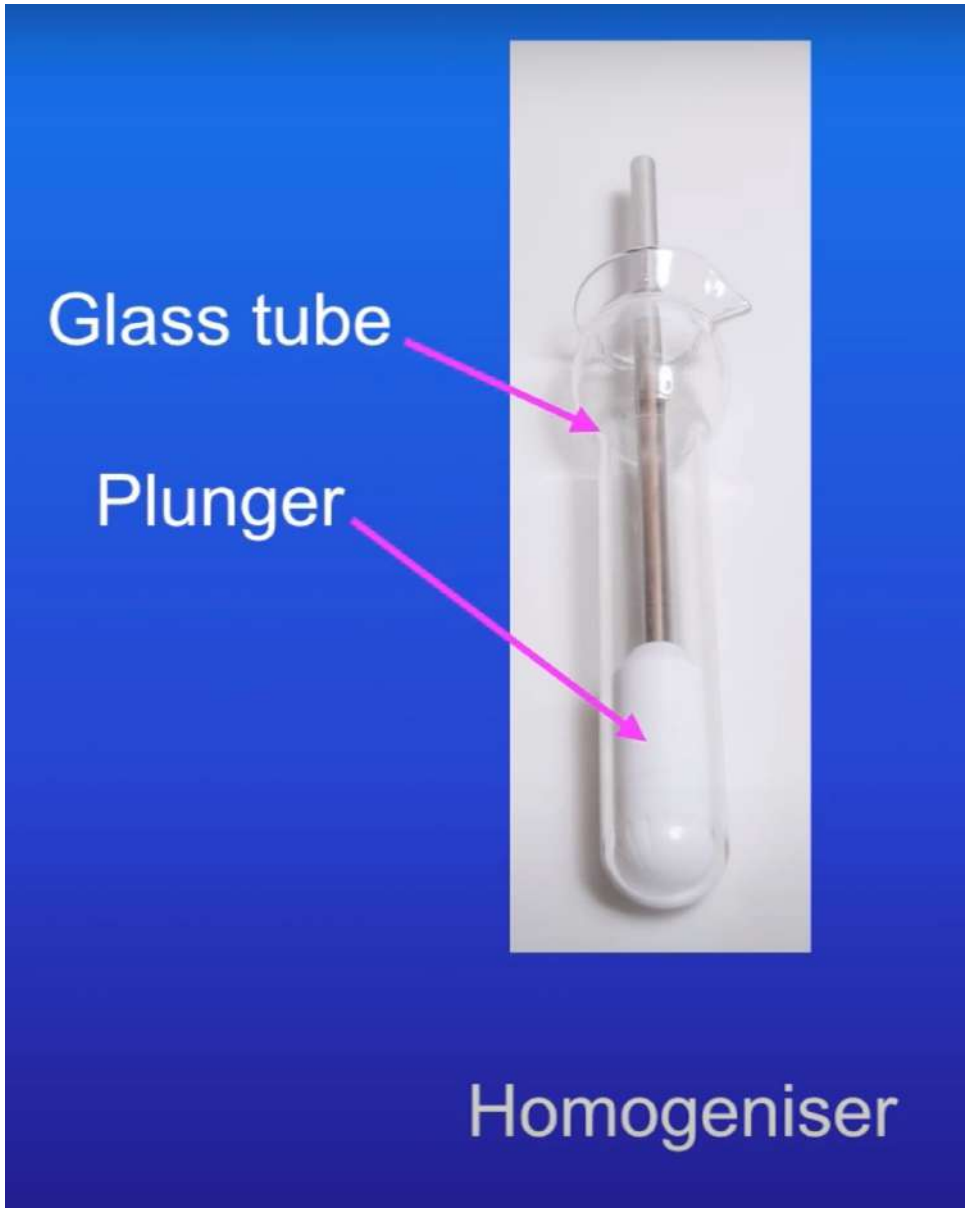
Cells contain different organells



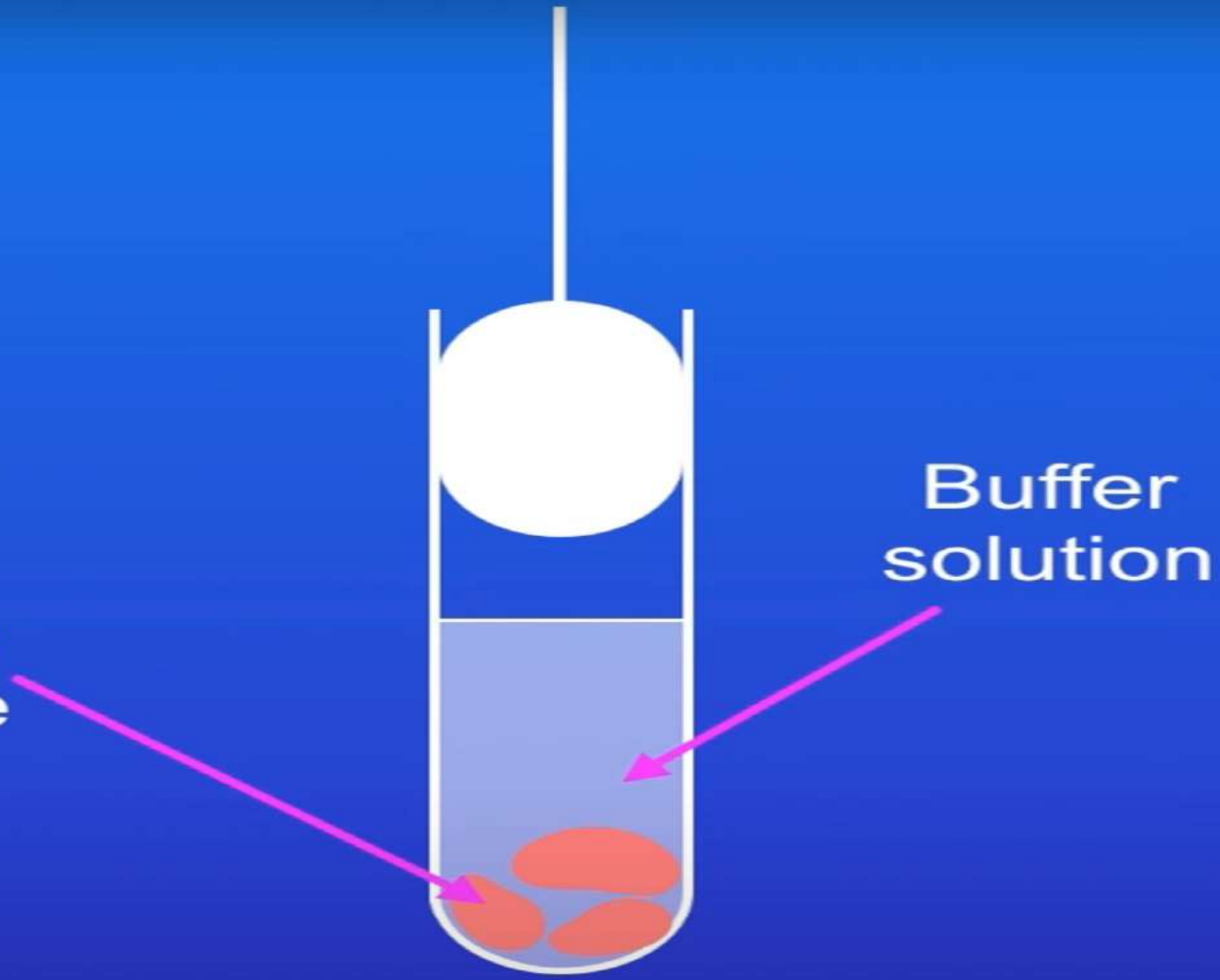
- **In the first stage we take a sample of tissure : for example, Heart Muscle Tissue**



Homomogenizer : Is a glass tube containg plunger



blender

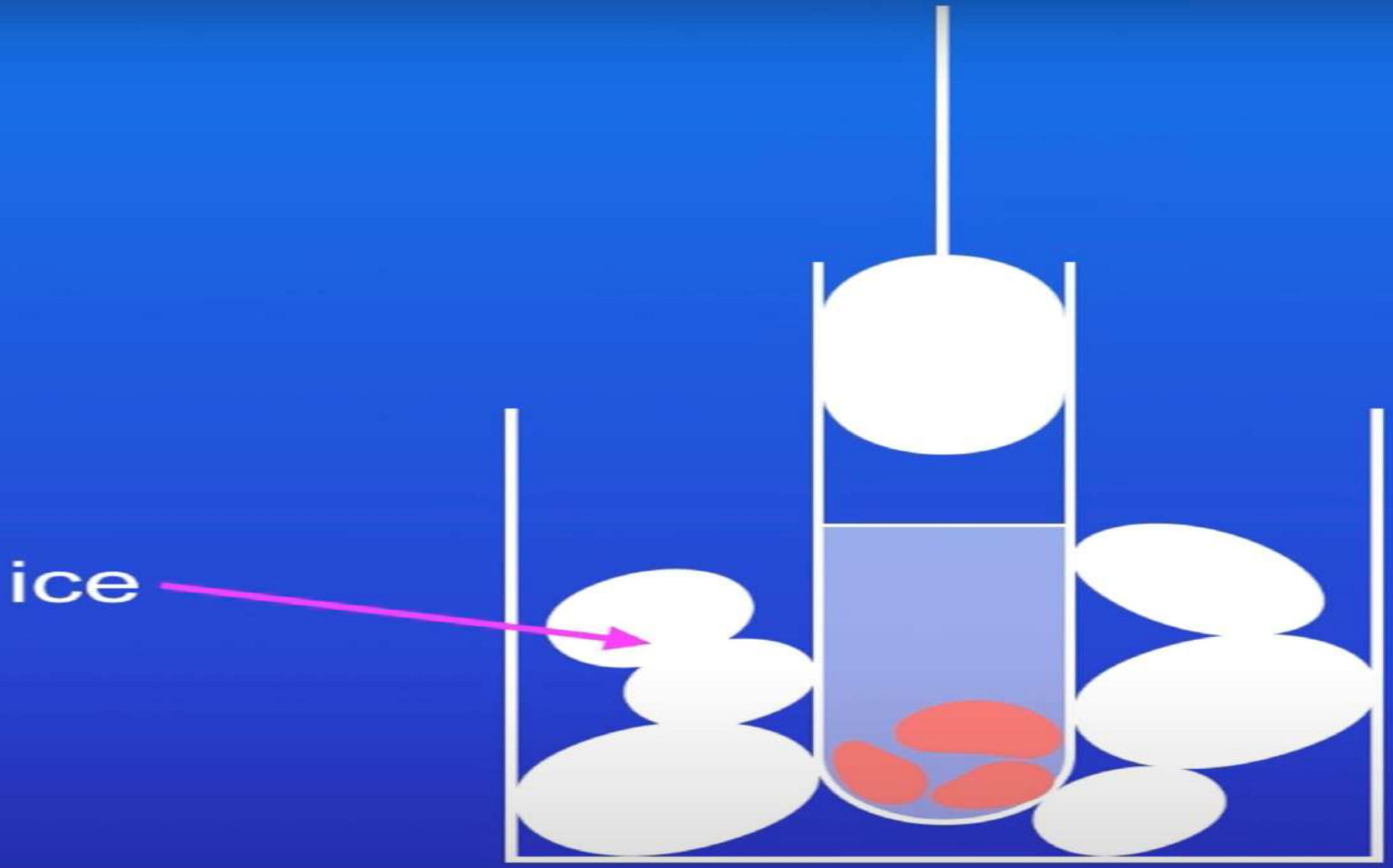


Cover with a buffer solution to keep the pH constant. If the pH changed, enzymes in the cell's organelles could denature.

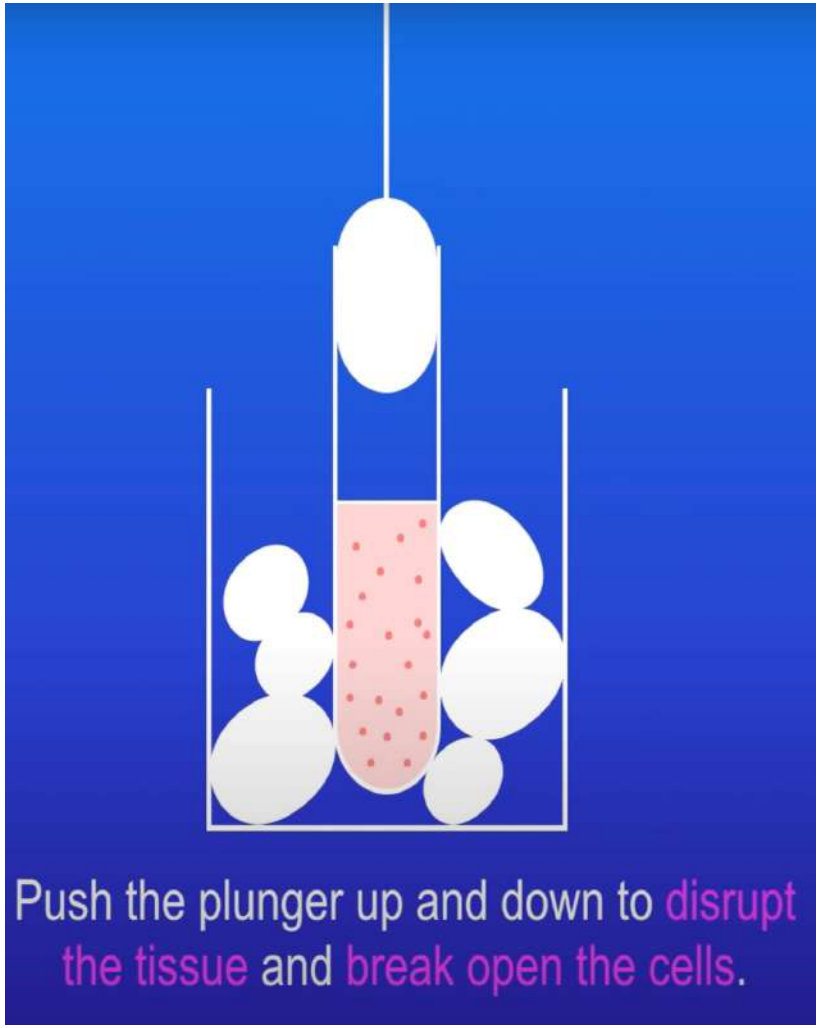
Buffer solution

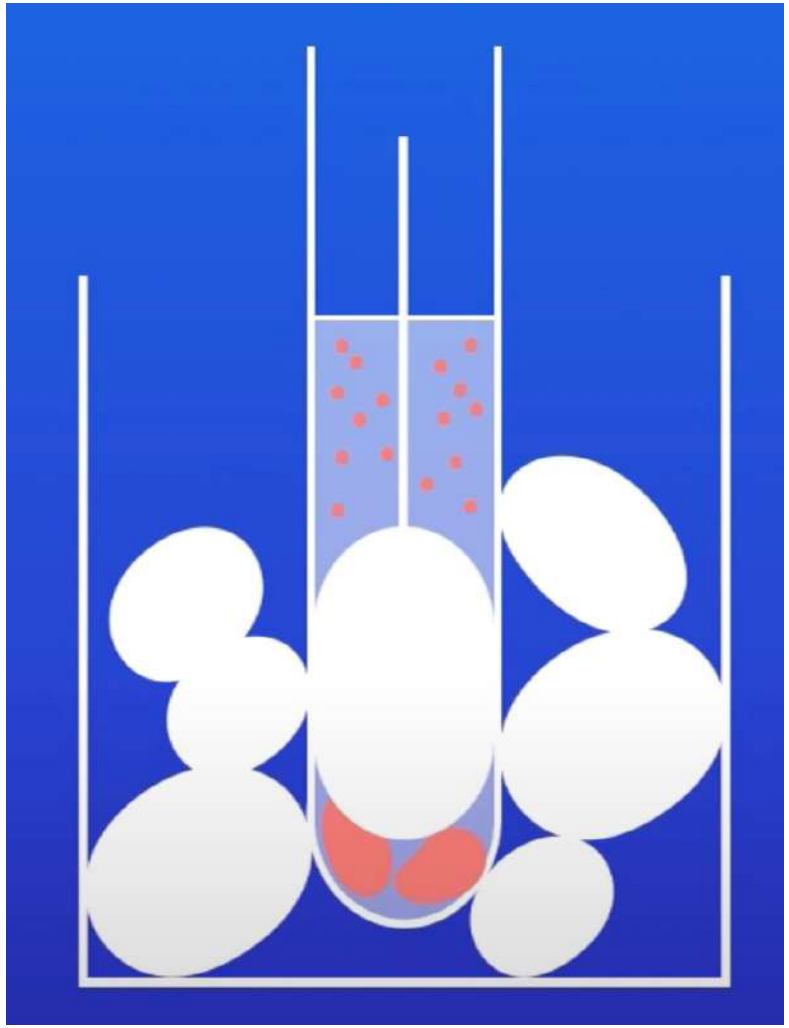
Tissue sample

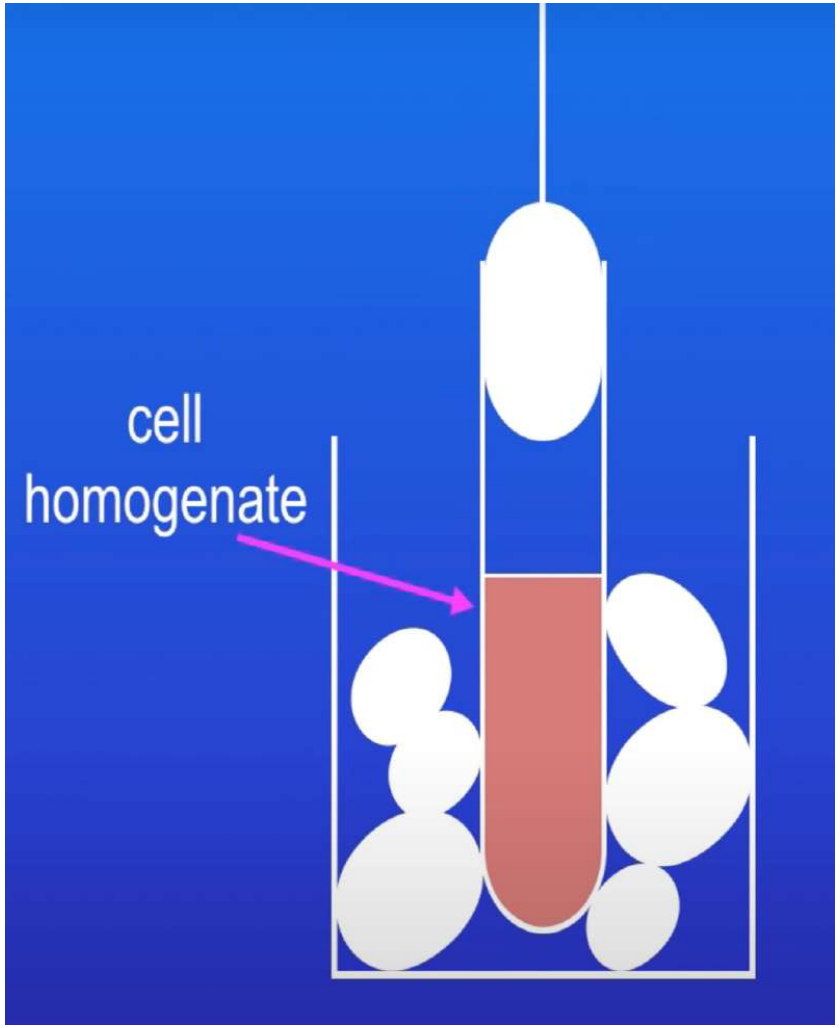
The water potential of the buffer is the same as the cell. This prevents water from moving into the organelles by osmosis and bursting them.



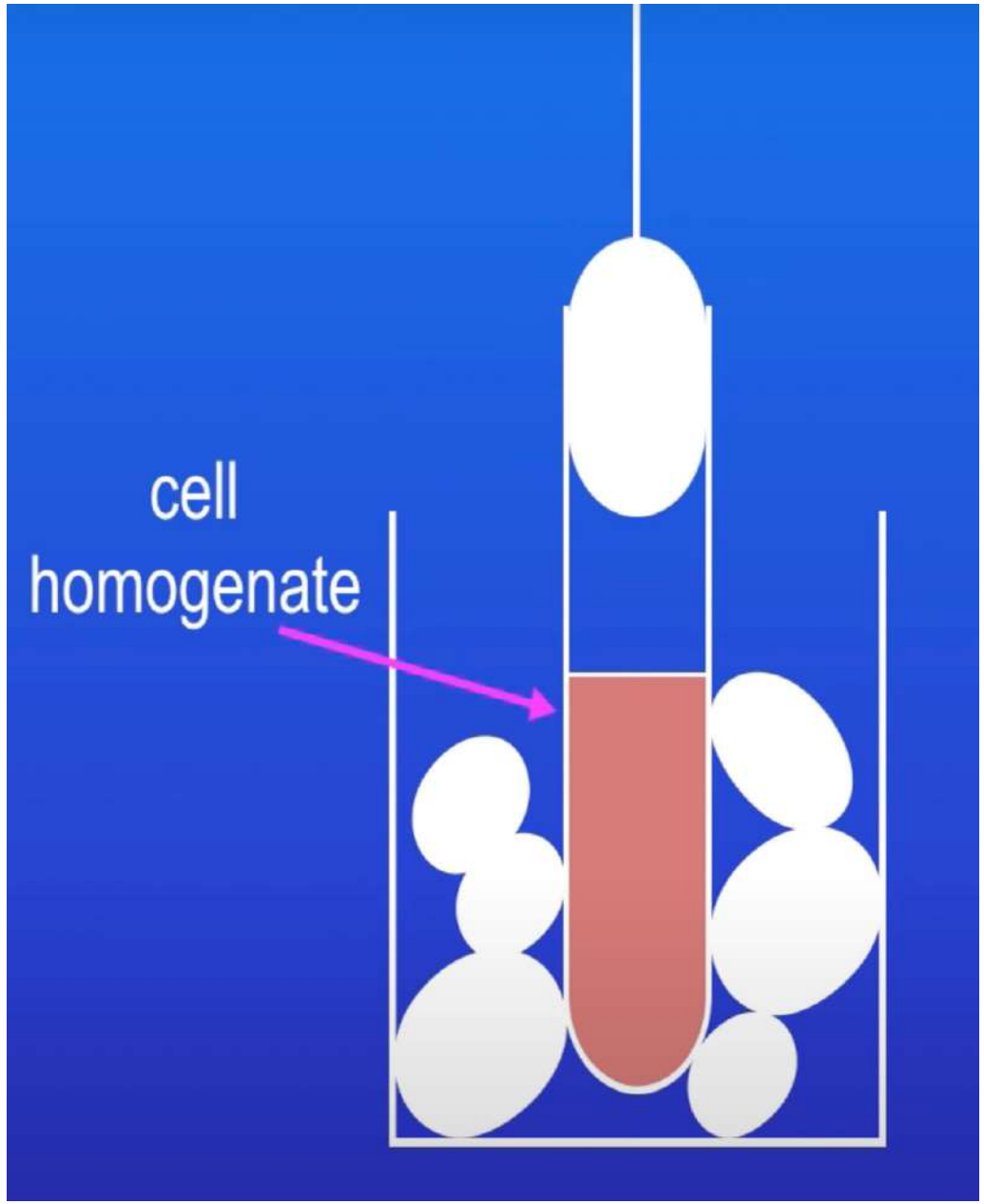
Place the homogeniser on ice. so that enzymes work more slowly, preventing them from destroying organelles.





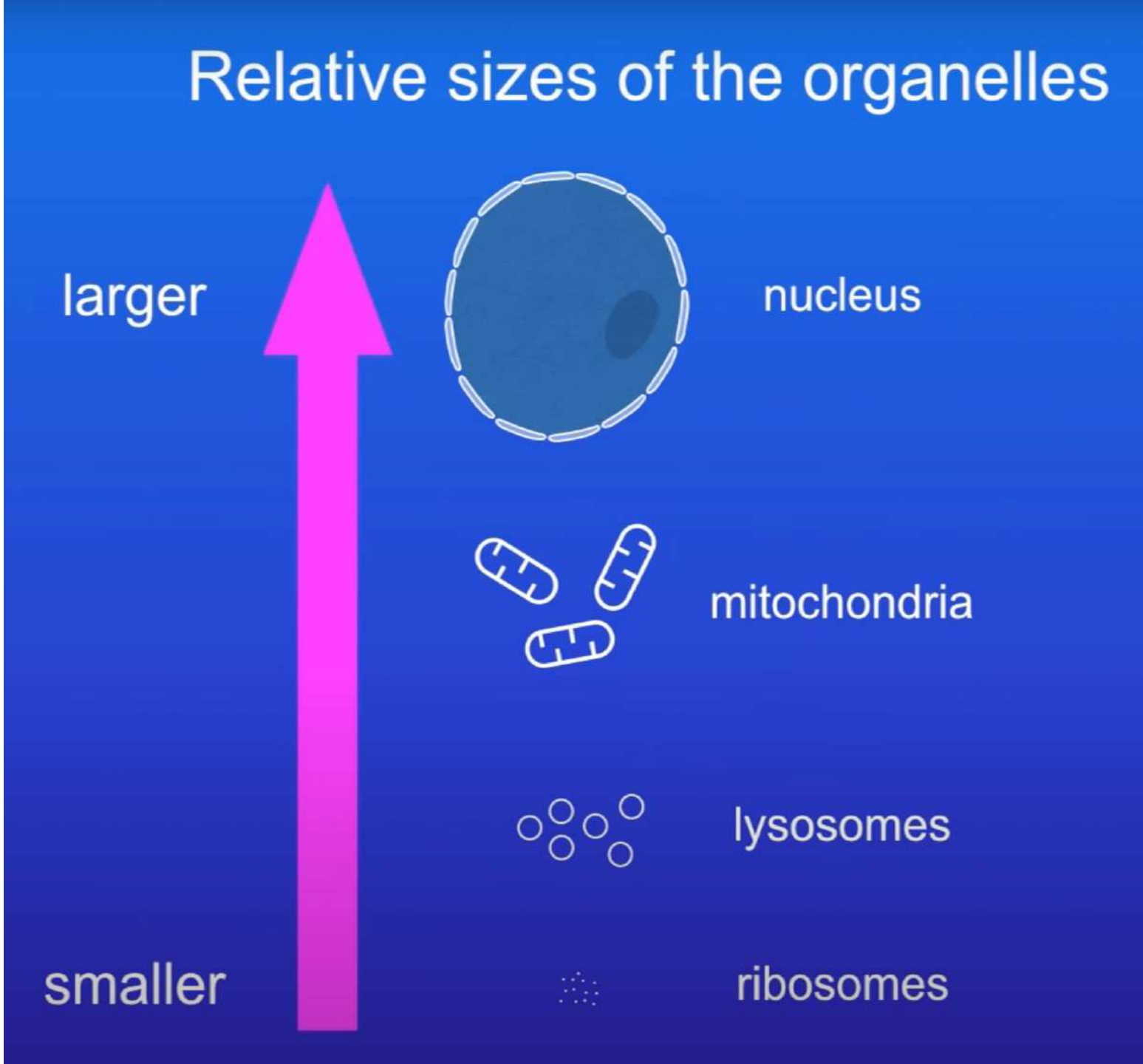


Push the plunger up and down to disrupt the tissue and break open the cells.

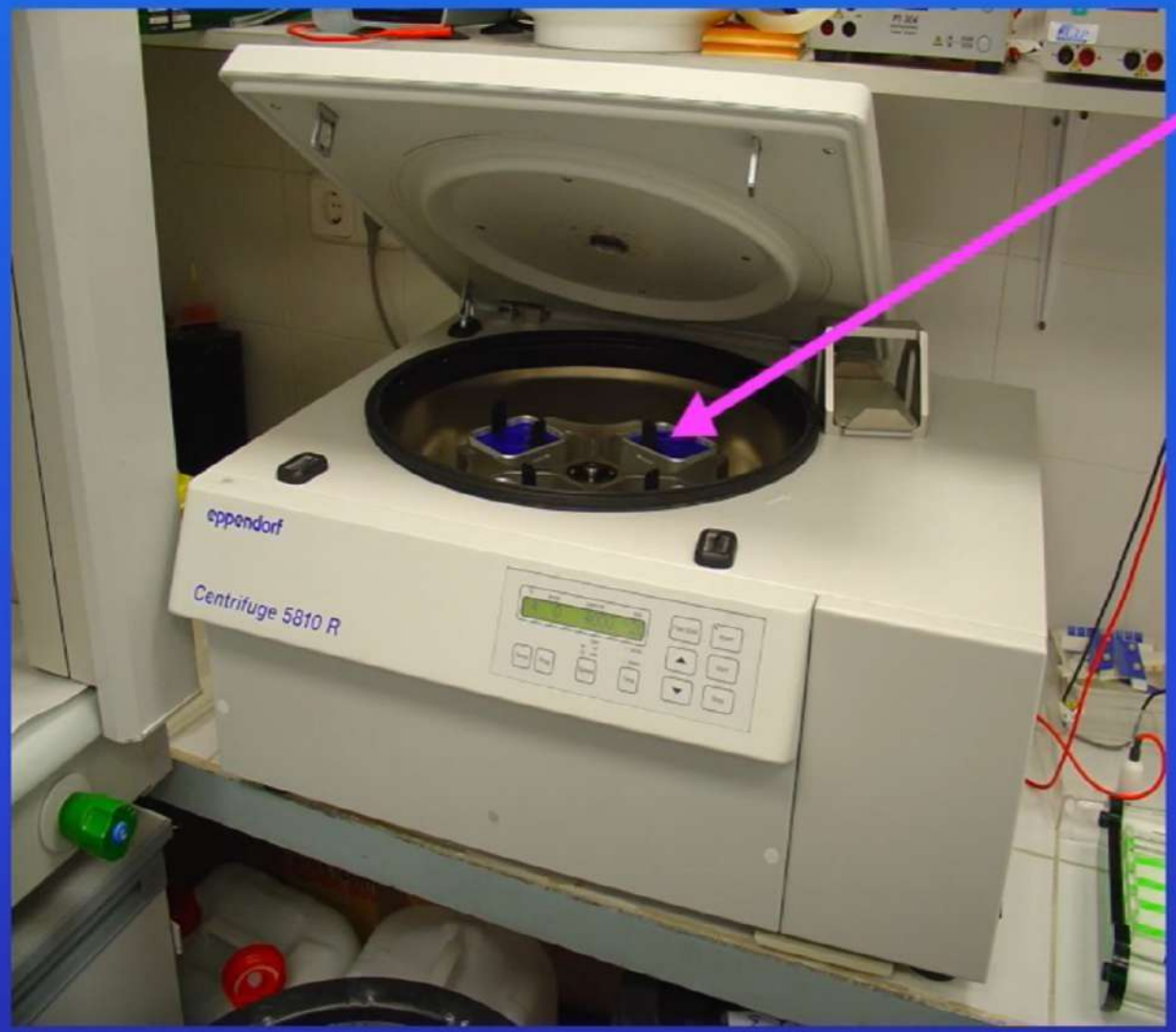


Cell homogenate contain all the organelles of the cell

we need to separate the organells in order know its function..

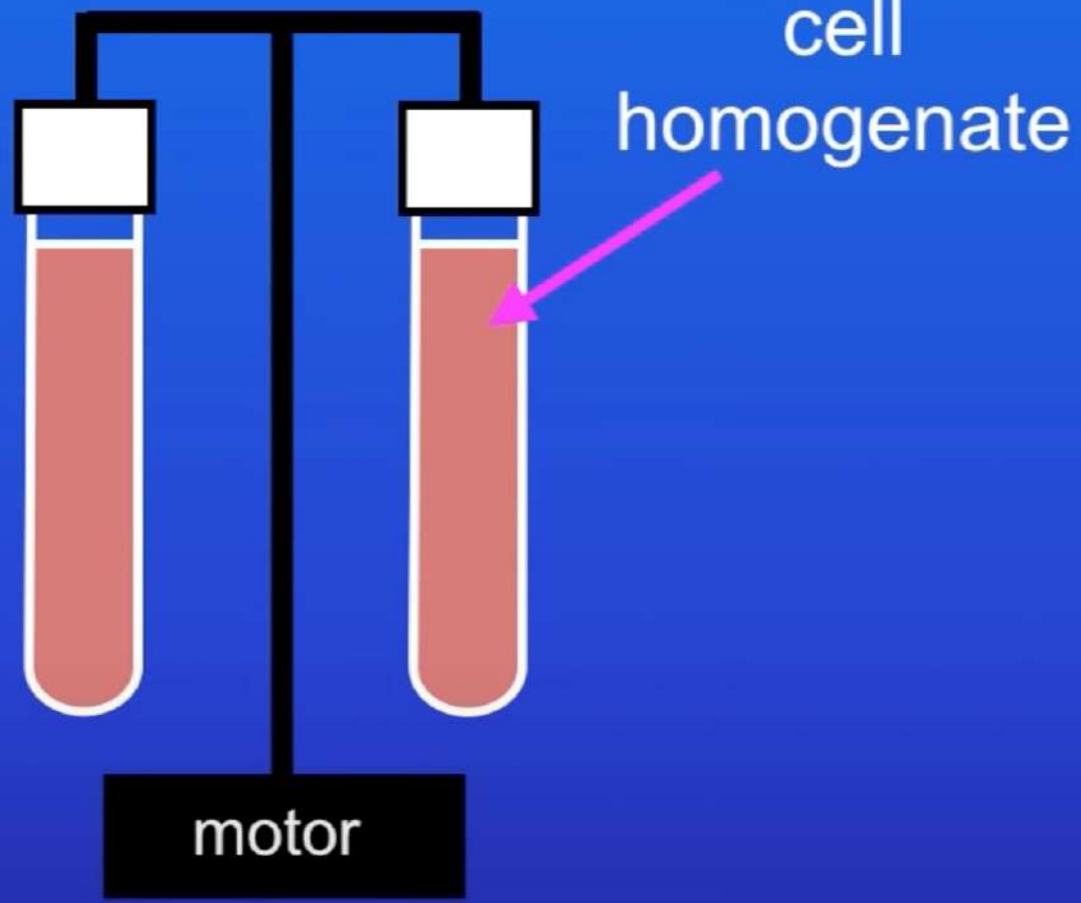


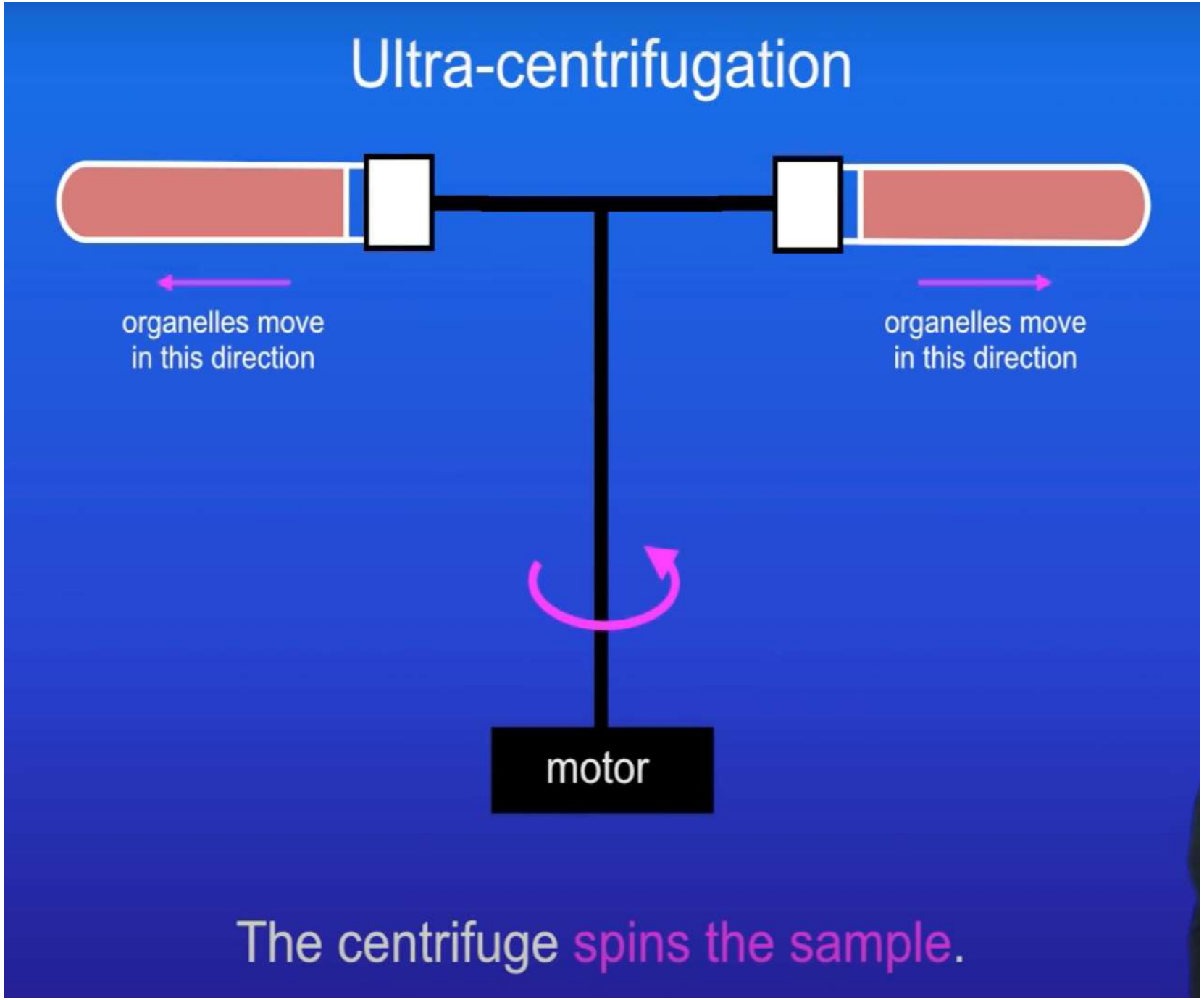
Ultra-centrifugation



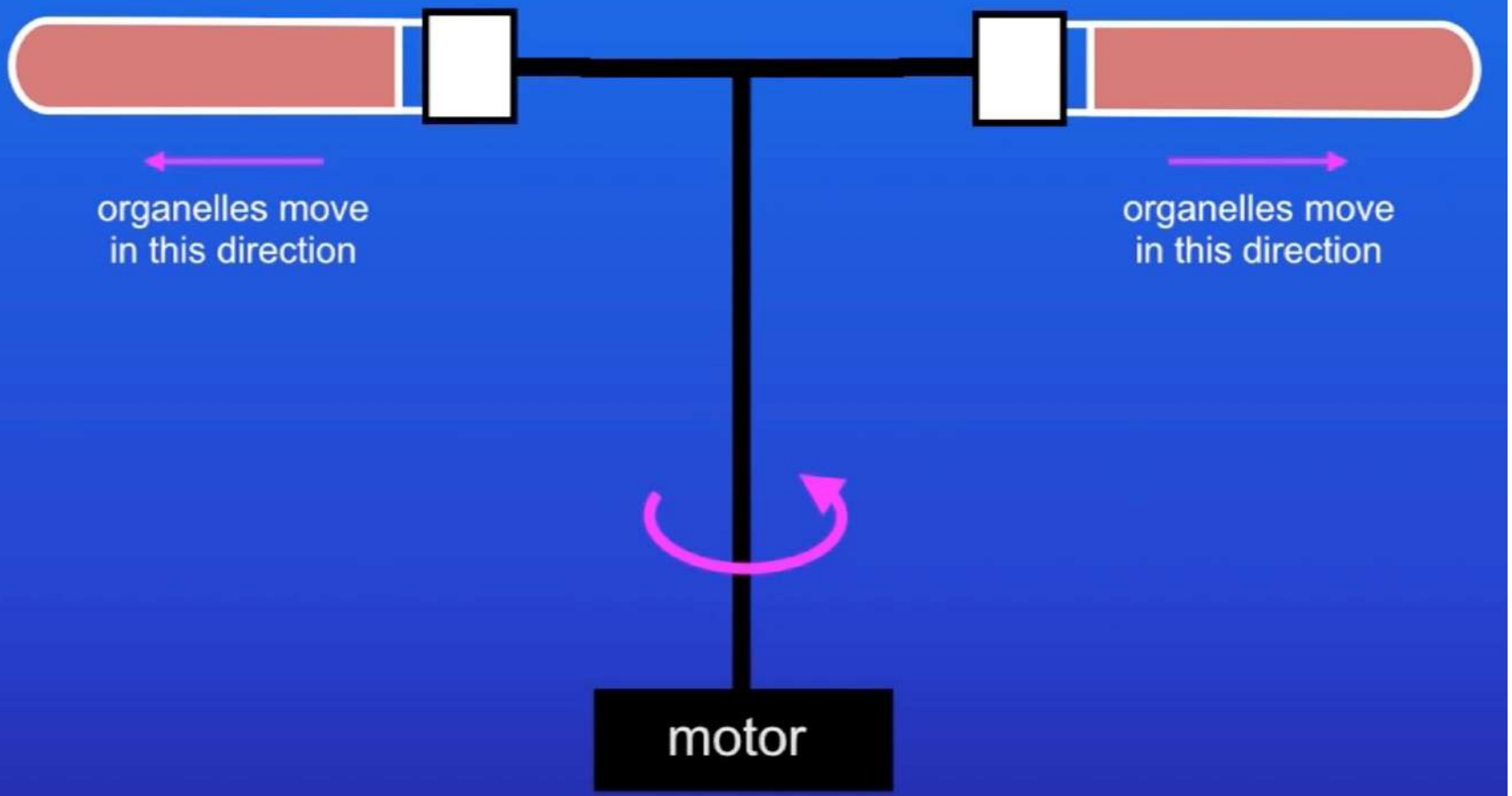
Separation of diff. part of the cell called fractionation.. using a mechine called centrifuge

Ultra-centrifugation



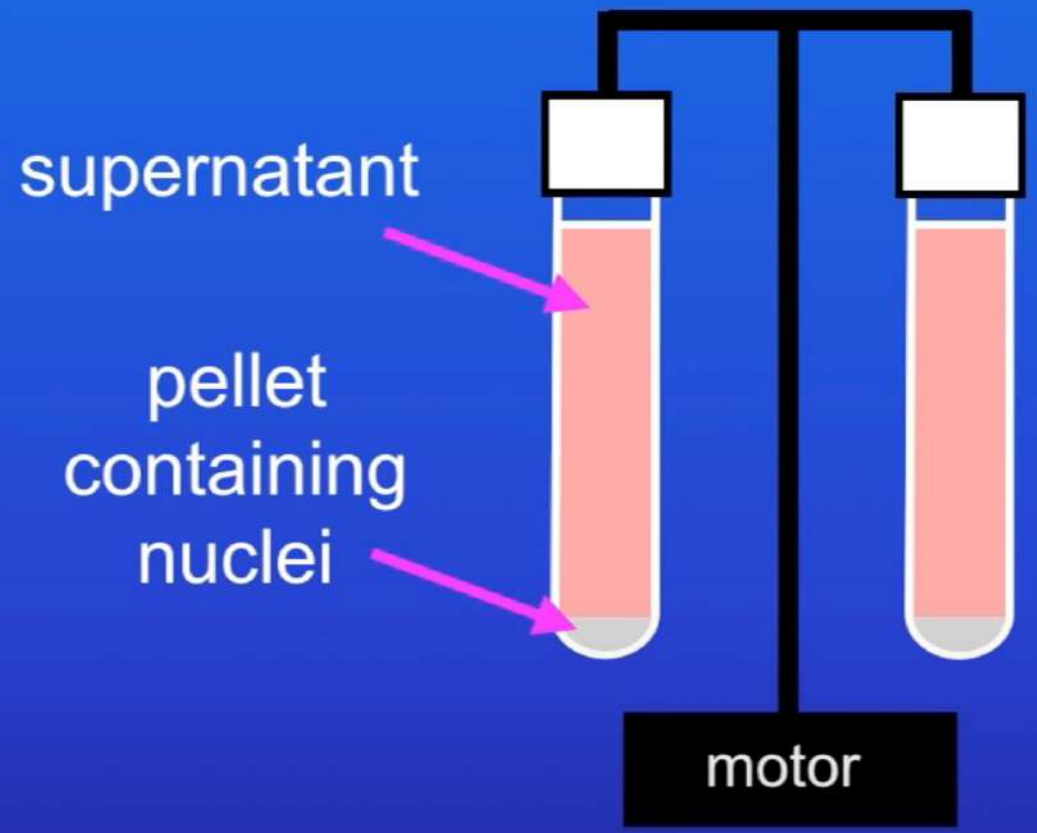

Ultra-centrifugation
organelles move in this direction
organelles move in this direction
motor
The centrifuge spins the sample.

Ultra-centrifugation



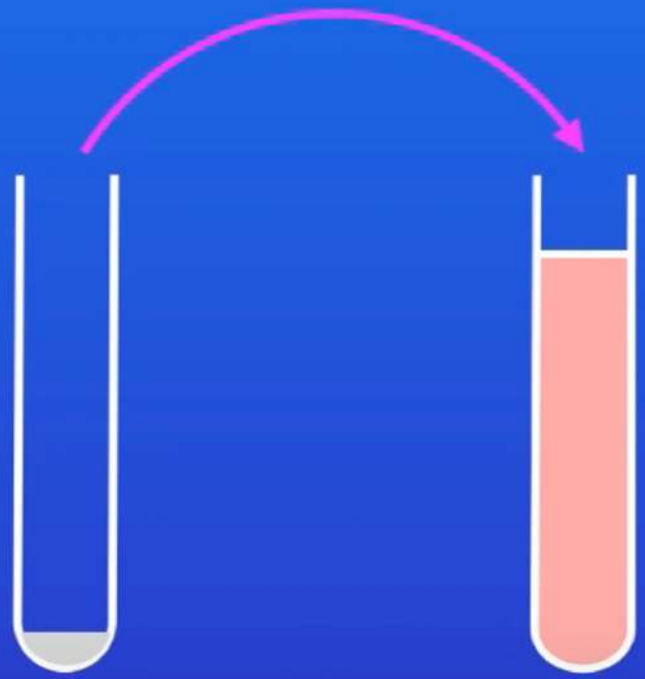
Larger organelles such as the nuclei experience a greater force and move towards the bottom of the tube faster than smaller organelles.

Ultra-centrifugation



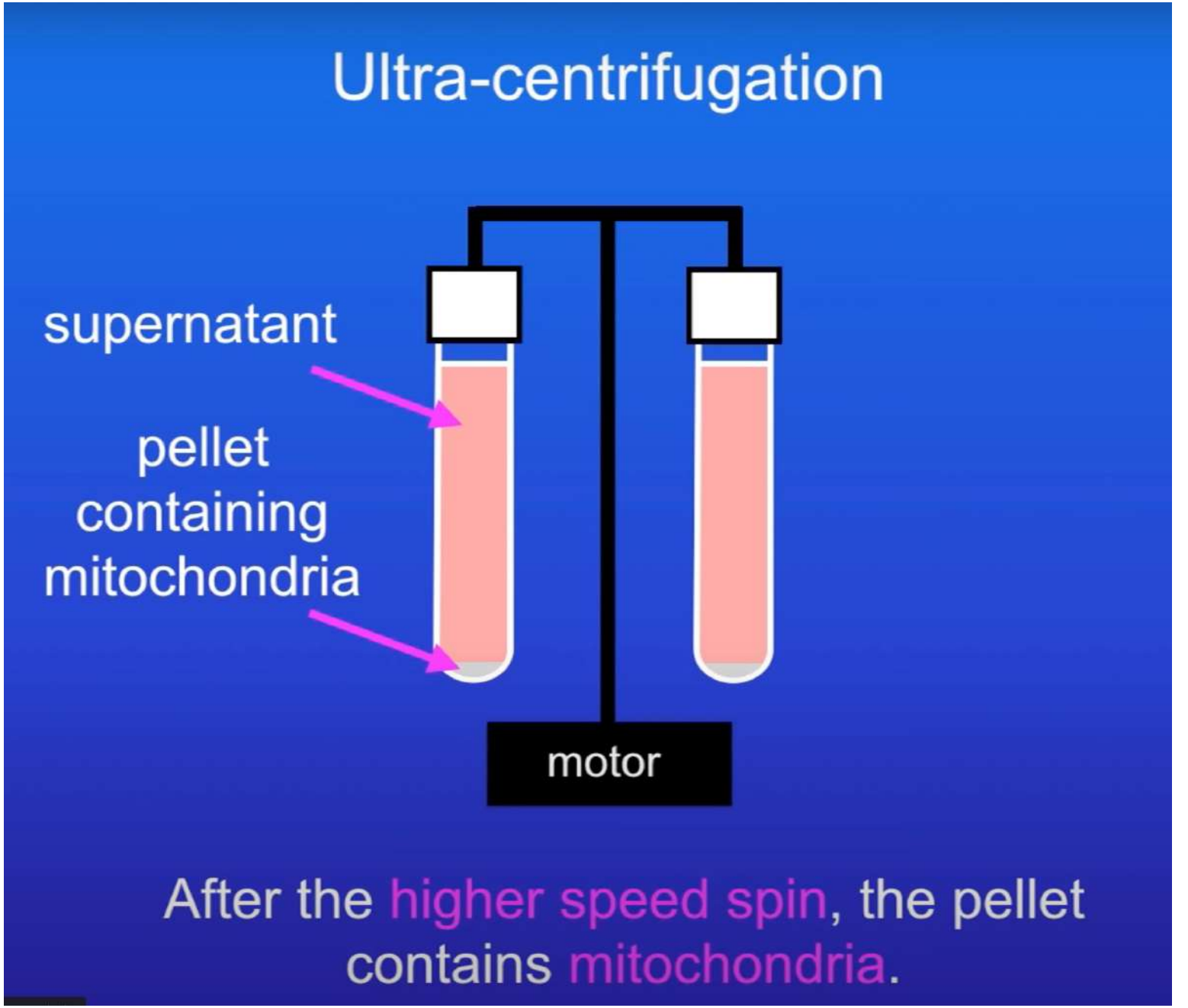
Start with a relatively low-speed spin. The nuclei form a pellet at the bottom of the tube.

Ultra-centrifugation



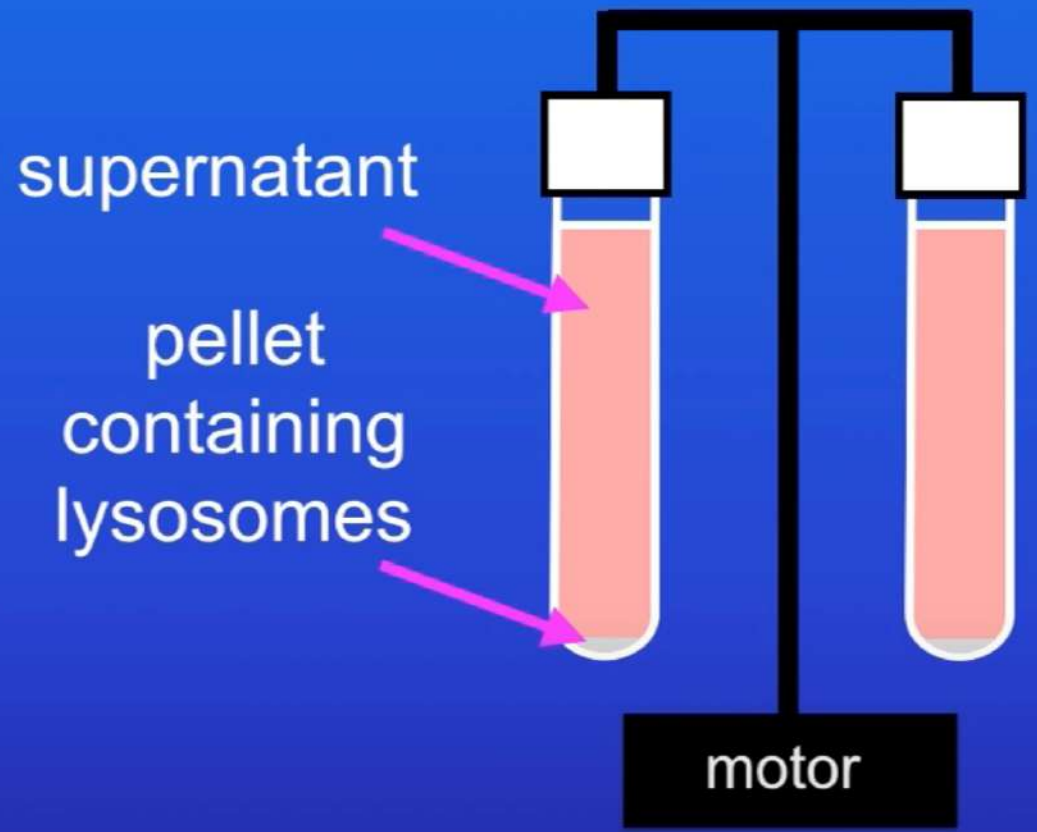
We now transfer the supernatant into a new tube and centrifuge at a higher speed.

Ultra-centrifugation



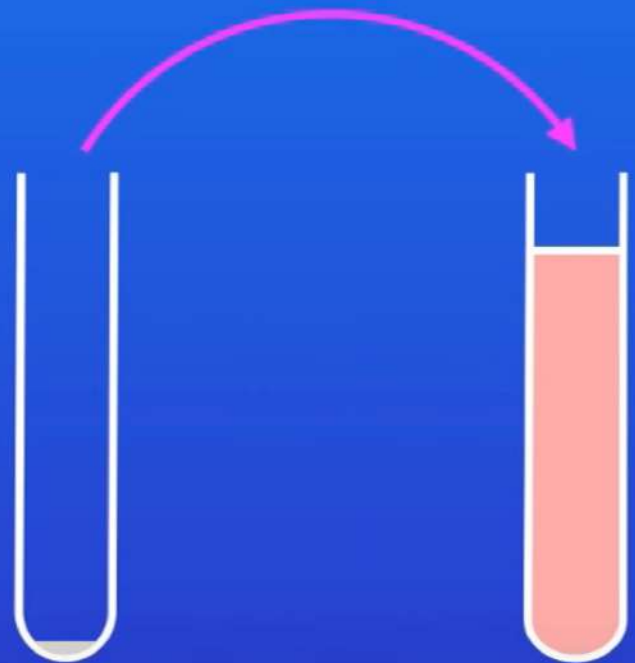
After the higher speed spin, the pellet contains mitochondria.

Ultra-centrifugation



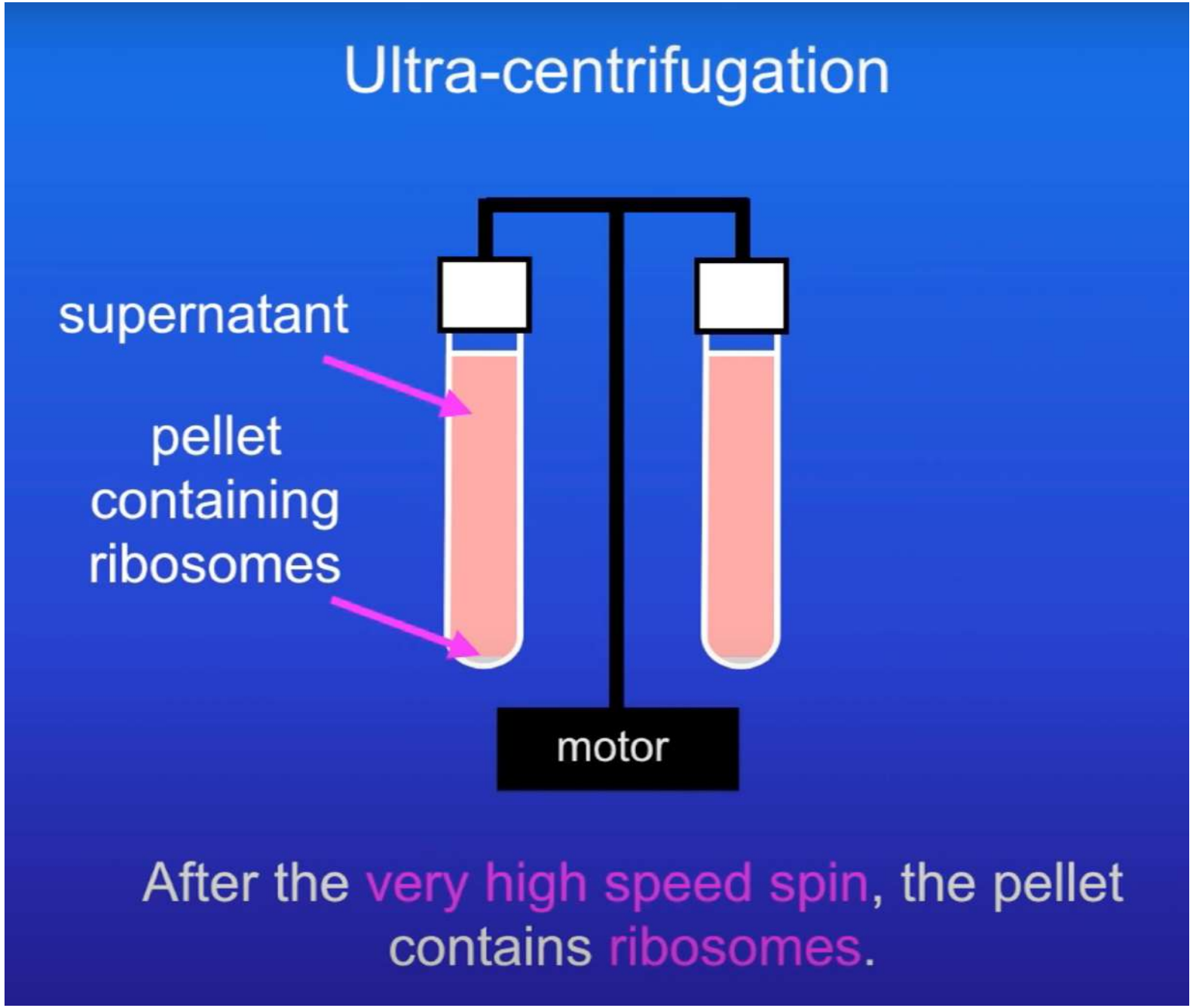
After the higher speed spin, the pellet contains lysosomes.

Ultra-centrifugation

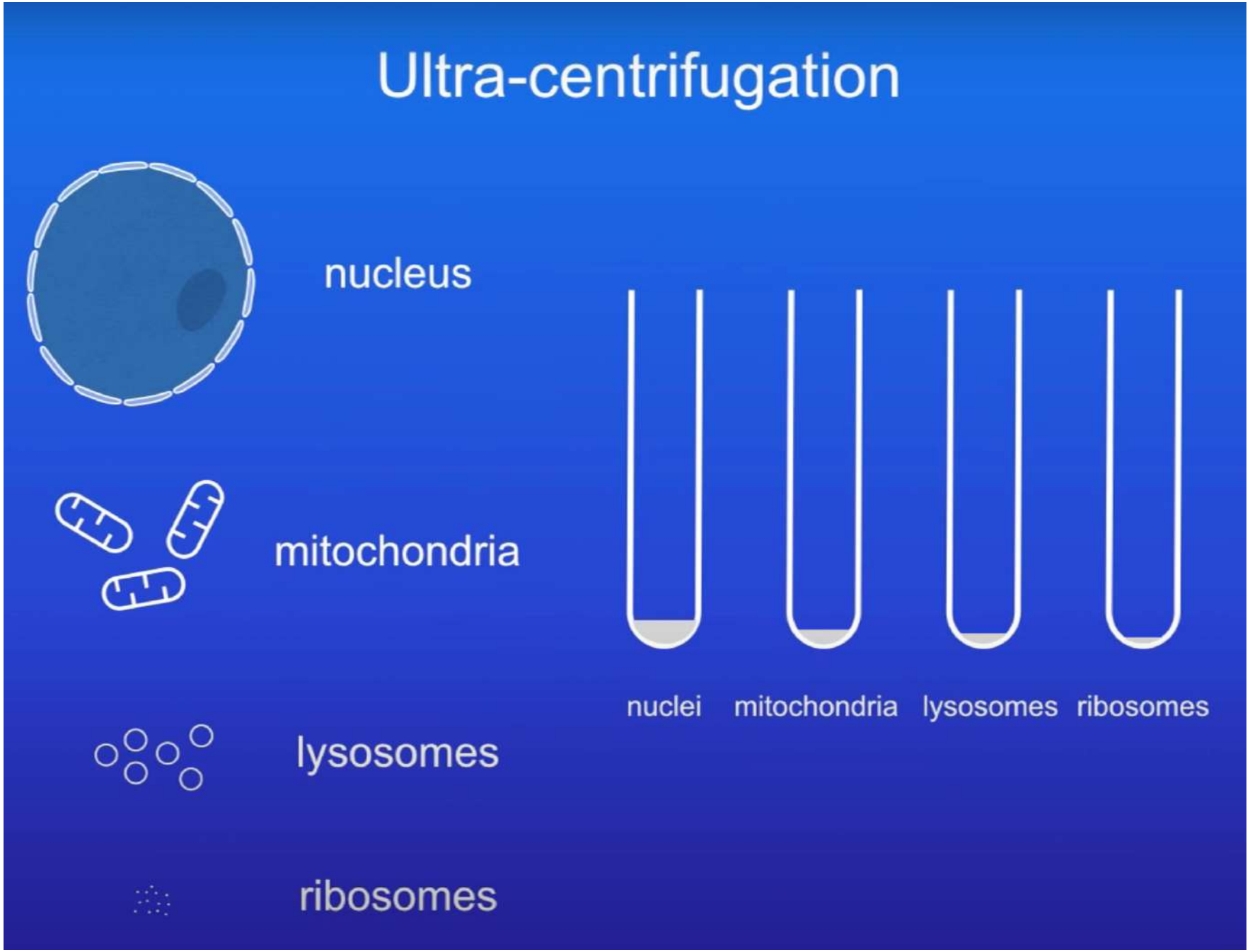


We now transfer the supernatant into a new tube and centrifuge at a higher speed.

Ultra-centrifugation



After the very high speed spin, the pellet contains ribosomes.



Now can test each fraction to detemin how the organells work

Keep the pellets on ice until we use them. This is to slow down enzymes which might damage the organelles

It's extremely difficult to separate the organelles fully.

Other organelles such as the endoplasmic reticulum and Golgi body might be present in your fractions.

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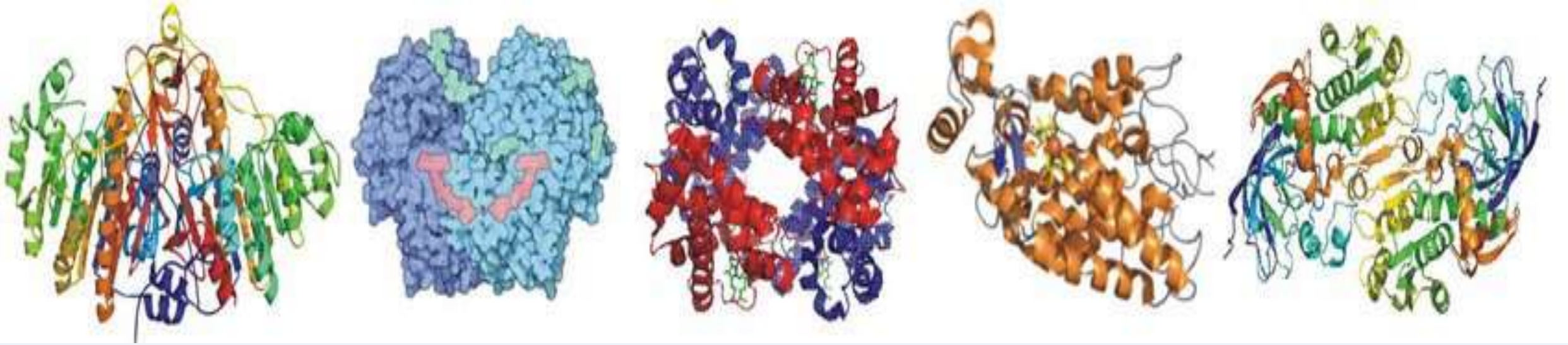
Lec. 11

Enzyme Assay Techniques

Enzymes are a special kind of protein found in cells of living organisms. They're made up of long chains of amino acids held together by peptide bonds. No two types of enzymes have the same amino acid structure, and each enzyme has its own unique shape.

Enzymes are essential for life and are one of the most important types of protein in the human body.



Enzyme Assay

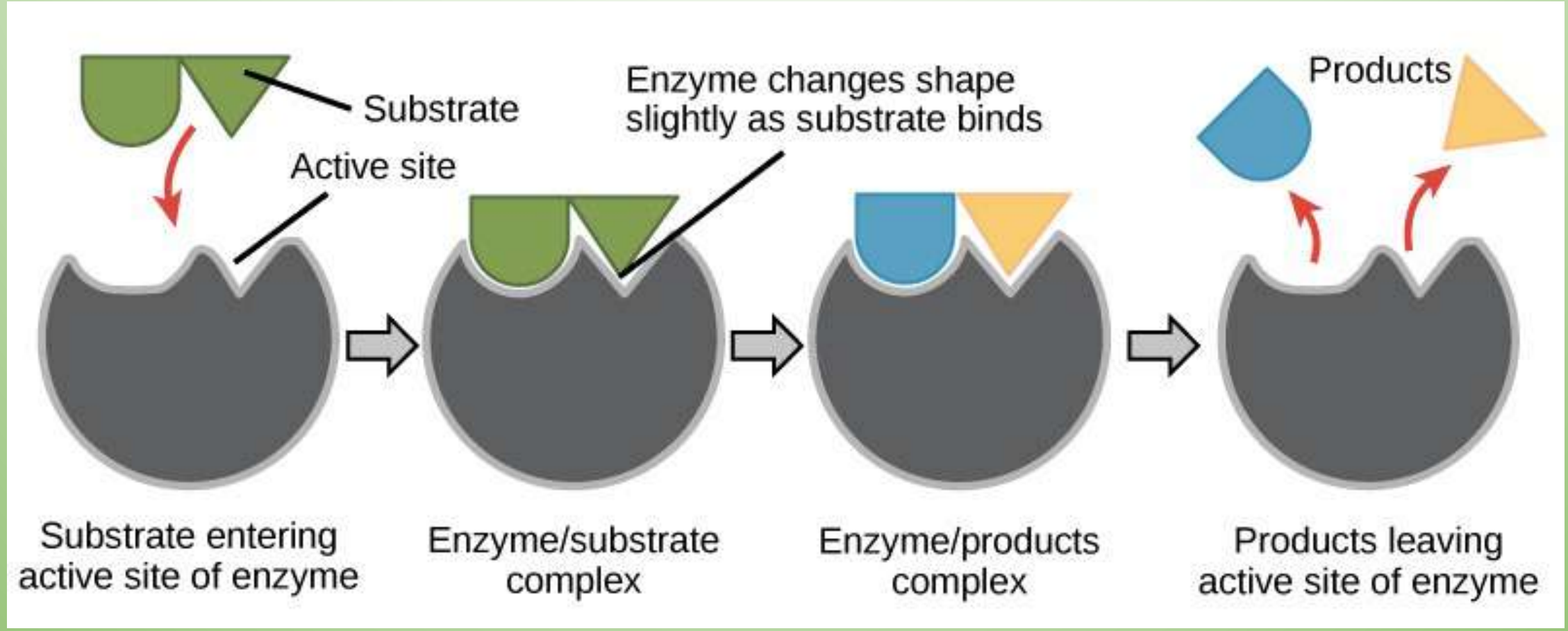
laboratory technique that measures enzyme activity

Why Do We Use Enzyme assays ?

Enzyme assays can be used for a variety of purposes, which include:

1- Identifying the presence of an enzyme
2- Investigation of specific enzyme kinetics
3- The activity of inhibition within a sample

Enzyme activity : is the rate of enzyme reaction generally expressed as units of substrate converted (or product formed) per time unit.



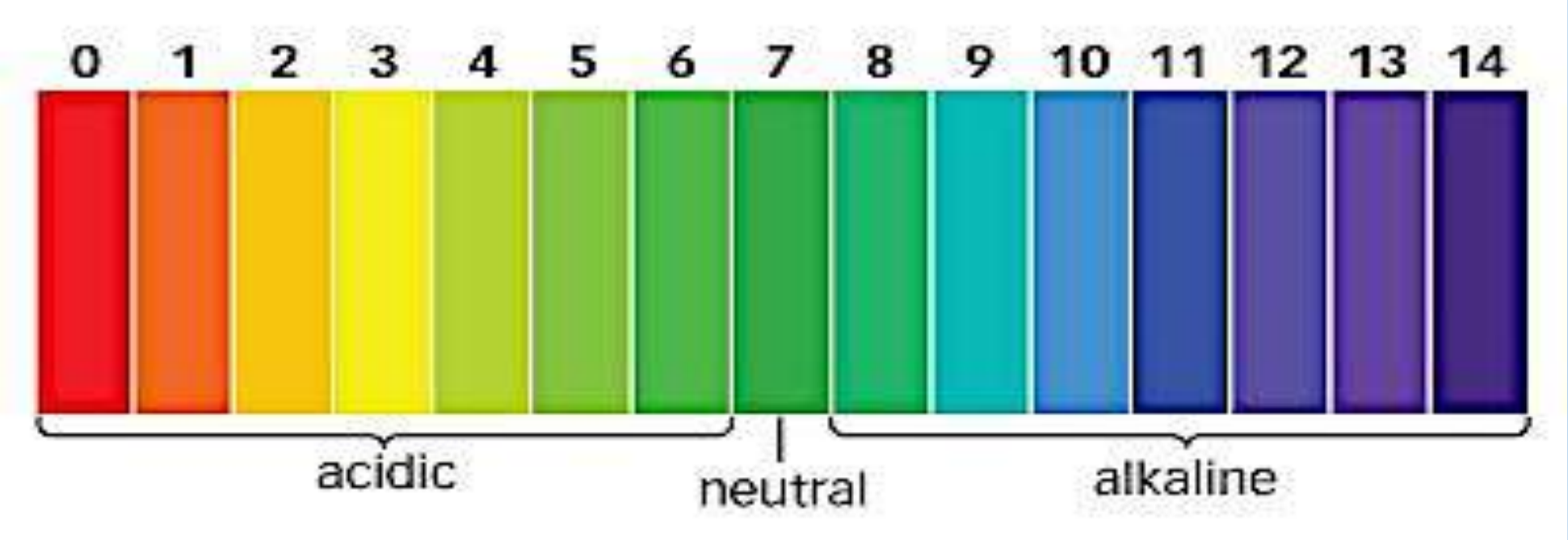
- **Enzyme kinetics :** is the study of the chemical reactions that are catalyzed by enzymes.

- Studying enzyme kinetics provides information about the diverse range of reactions in the human body, which we can use to understand and predict the metabolism of all living things.

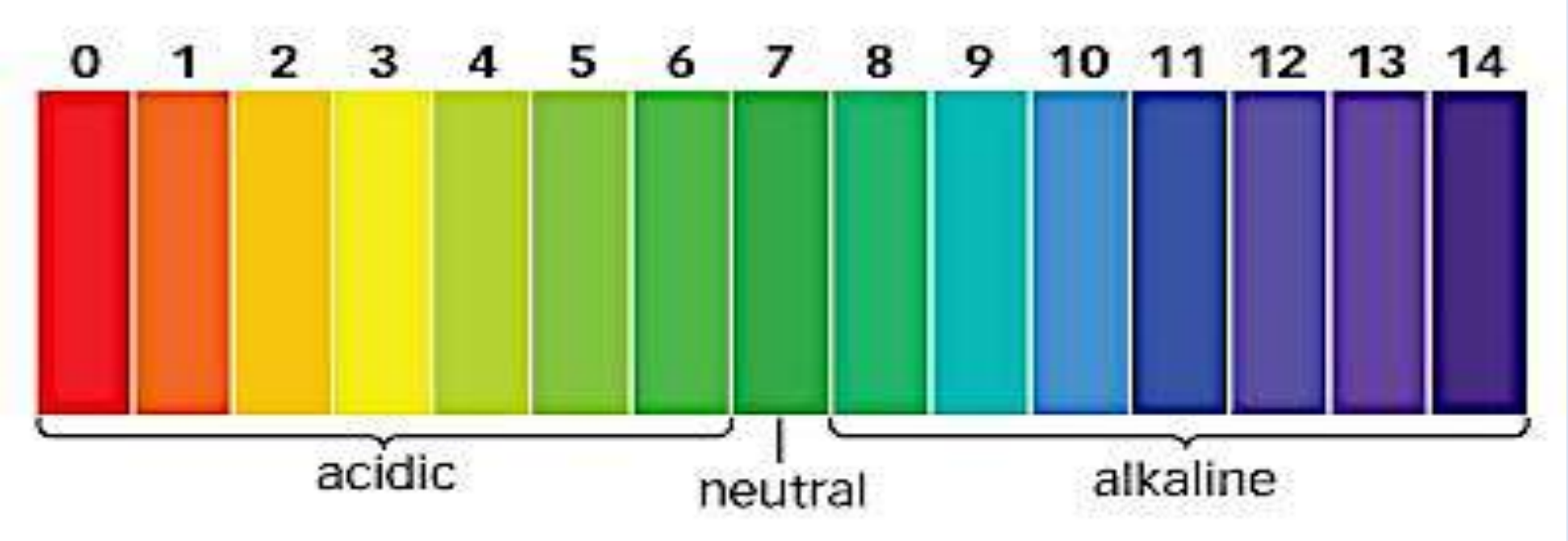
Factors That Affect Enzyme Assay

pH

Most enzymes are sensitive to pH and have specific ranges of activity. All enzymes have an optimum pH. The pH can stop enzyme activity by denaturation (altering) the three dimensional shape of the enzyme by breaking ionic, and hydrogen bonds.

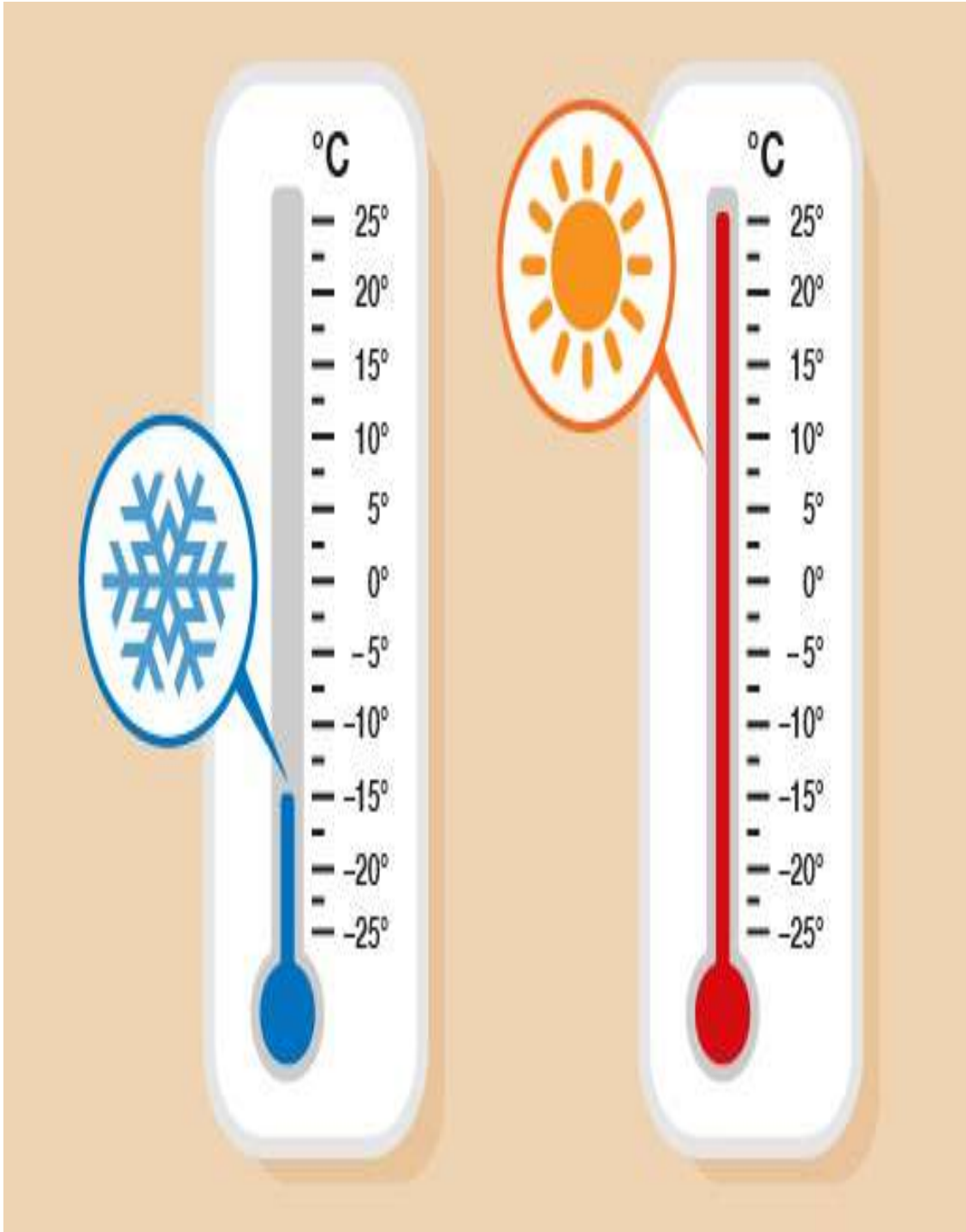


Most enzymes function between a pH of 6 and 8; however pepsin in the stomach works best at a pH of 2 and trypsin at a pH of 8.

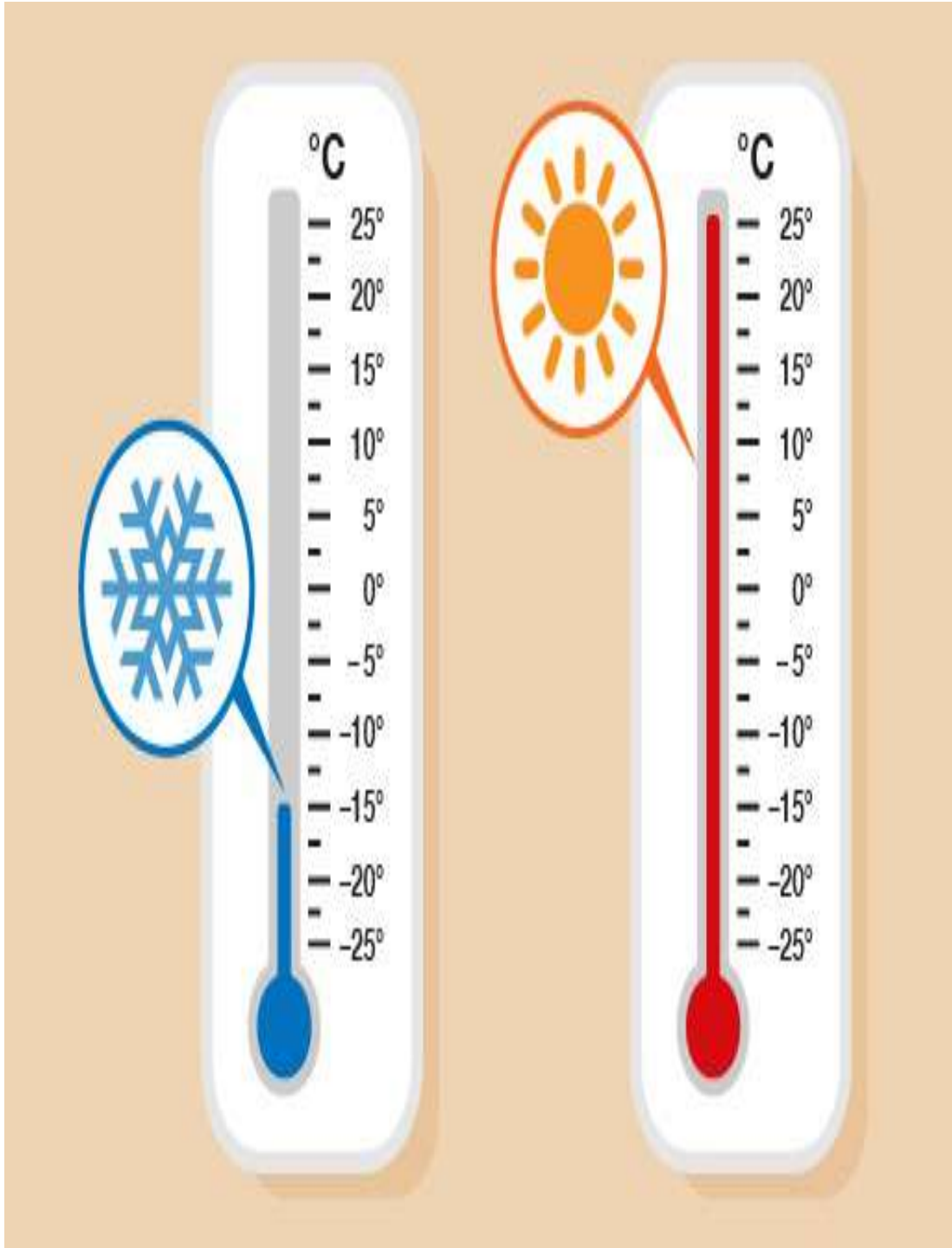


Temperature

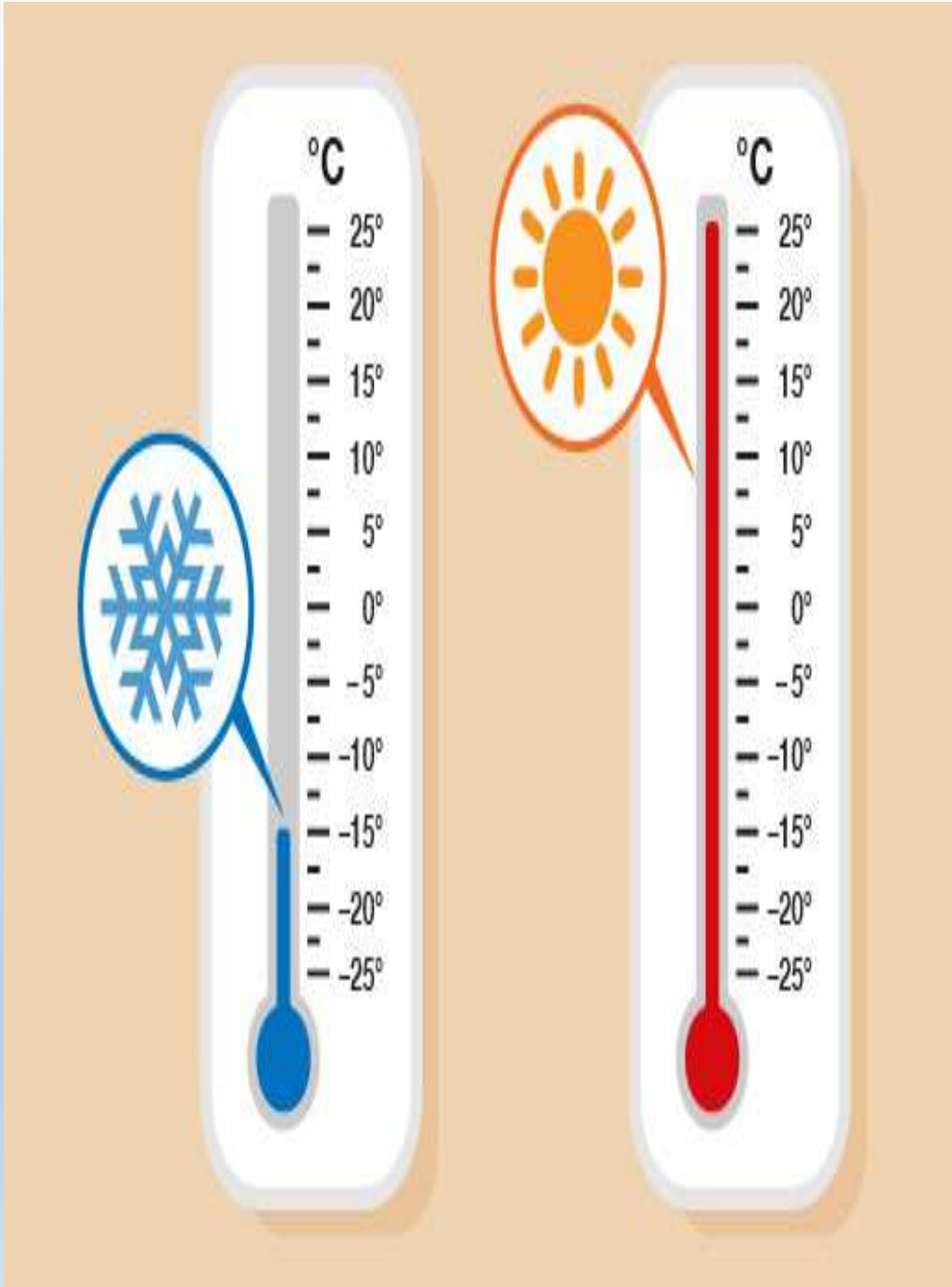
All enzymes work within a range of temperature specific to the organism. Increases in temperature generally lead to increases in reaction rates



There is a limit to the increase because higher temperatures lead to a sharp decrease in reaction rates. This is due to the denaturation (alteration) of protein structure resulting from the breakdown of the weak ionic and hydrogen bonding that stabilize the three dimensional structure of the enzyme.



The "optimum" temperature for human enzymes is usually between 35 and 40 °C. The average temperature for humans is 37 °C. Human enzymes start to denature quickly at temperatures above 40 °C. Enzymes



Substrate Saturation

Increase in substrate concentration will increase the rate of reaction, but only up to a certain point. At the point of saturation, the reaction rate is at its upper limit and will not increase, regardless of how much substrate is added.

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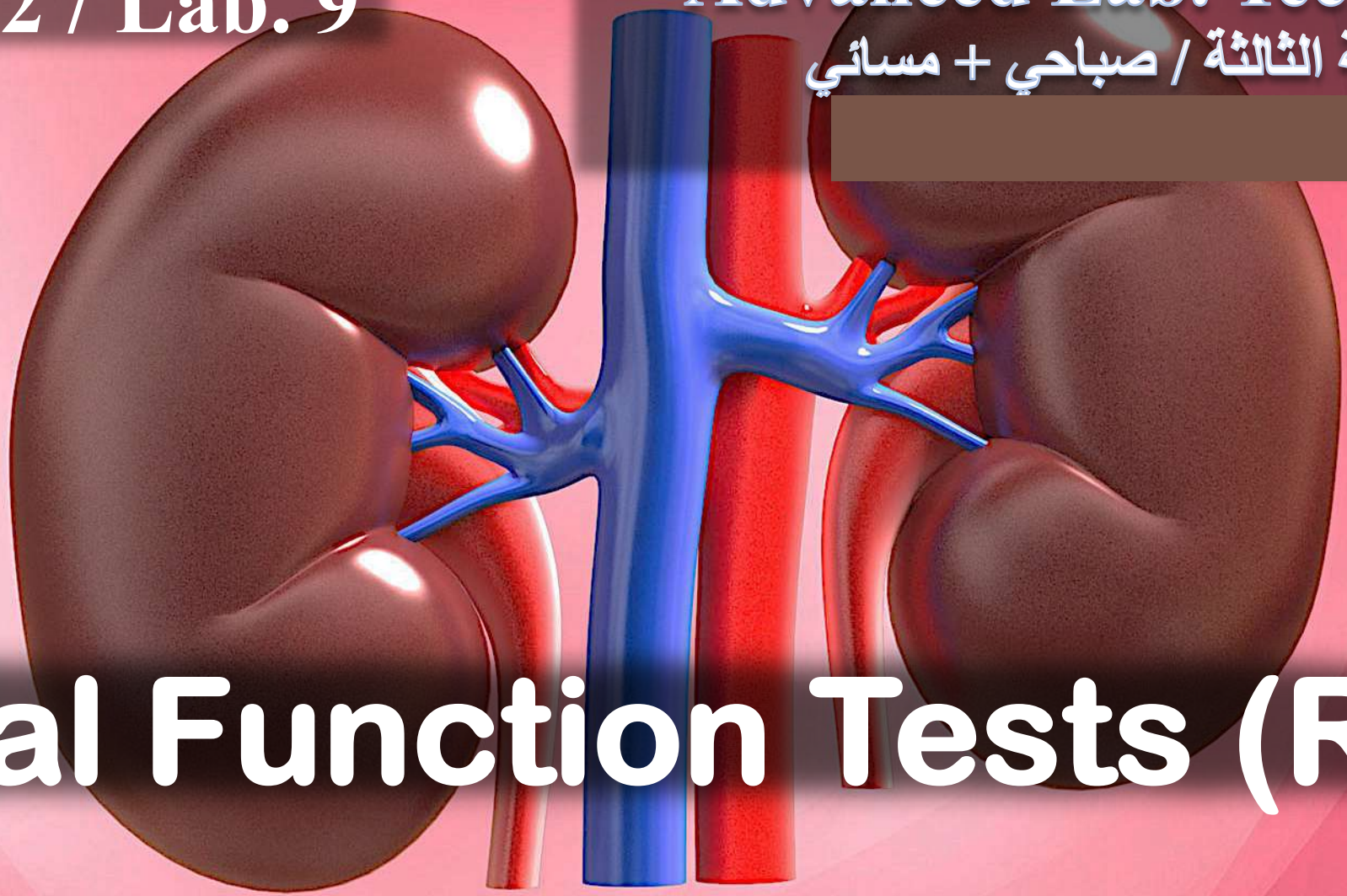
Advanced Lab. Techniques

المرحلة الثالثة / صباحي + مسائي

Lec. 12 / Lab. 9



Renal Function Tests (RFTs)

The main function of the kidneys is to excrete toxic waste found in blood through the removal of urine. Diseases such as diabetes, high blood pressure can damage the kidney untreated. Medication including traditional medicines without the advice of a doctor is also a cause of kidney failure

If the kidneys fail to function properly will cause severe health problems (renal or kidny failure). Thus, serious renal patients need to undergo dialysis for cleaning up blood from toxic substances that are dangerous. Dialysis machine serves as a substitute kidney.

- Acute kidney failure occurs when kidneys suddenly become unable to filter waste products from blood. When kidneys lose their filtering ability, dangerous levels of wastes may accumulate, and your blood's chemical makeup may get out of balance. Acute kidney failure — also called acute renal failure or acute kidney injury — develops rapidly, usually in less than a few days.

- Intake of supplements such as multi-vitamin, mineral salts or traditional medicine excessively could possibly cause kidney failure.

Signs and symptoms of acute kidney failure (renal failure) may include:

- Decreased urine output, although occasionally urine output remains normal
- Fluid retention, causing swelling in your legs, ankles or feet
- Shortness of breath
- Fatigue
- Confusion
- Nausea
- Weakness
- Irregular heartbeat
- Chest pain or pressure
- Seizures or coma in severe cases

Renal function tests (RFT) are a group of tests that may be performed together to evaluate kidney (renal) function. The tests measure levels of various substances, including **several minerals, electrolytes, proteins, and glucose (sugar)** in the blood, to determine the current health of the kidneys. **If the kidneys are not functioning properly, waste products can accumulate in the blood and fluid levels can increase to dangerous volumes, causing damage to the body or a potentially life-threatening situation.** Numerous conditions and diseases can result in damage to the kidneys. The most common causes of and main **risk factors for kidney disease** are **diabetes** and **hypertension.**

Typical Renal Function Tests

The individual tests included in a kidney function panel can vary by laboratory, but the tests typically performed include:

Electrolytes: electrically charged chemicals that are vital to normal body processes, such as nerve and muscle function; among other things, they help regulate the amount of fluid in the body and maintain the acid-base balance. Electrolytes include:

Na+

Sodium: Sodium helps your **nerves and muscles work properly**. Once your body takes in enough sodium, the kidneys get rid of the rest in your urine. If your sodium **blood levels are too high or too low**, it may mean there is a problem in your **kidneys**, **dehydration**, or another **medical condition**.

k+

Potassium: helps the **heart and muscles work properly**. A potassium level that is **too high or too low may weaken muscles** and **change your heartbeat**. The test may also be used to monitor or diagnose conditions related to abnormal potassium levels. These conditions include **kidney disease**, **high blood pressure**, and **heart disease**.

Cl^-

Chloride : A blood test measures the amount of chloride in your blood. It is often measured along with other electrolytes to diagnose or monitor conditions such as **kidney disease, heart failure, and high blood pressure**.

Minerals

Phosphorus (PO4): a mineral that is vital for, muscle and nerve function, **energy production**, and **bone growth**; it also plays an **important role as a buffer**, helping to **maintain the body's acid-base balance**.

Calcium (Ca++): one of the **most important minerals** in the body; it essential for the proper **functioning of muscles**, **nerves**, and **heart** and is required in **blood clotting** and **in the formation of bones.**

Protein

Albumin – a protein that makes up about 60% of protein in the blood and has many roles such as keeping fluid from leaking out of blood vessels and transporting hormones, vitamins, drugs, and ions like calcium throughout the body.

Waste products

Urea: Urea nitrogen is a normal waste product in the blood that comes from the breakdown of protein from the foods you eat and from your body metabolism; It is normally (filtered) removed from the blood by kidneys, but when kidney function slows down, the urea level rises – BUN - BU

Urea test is used for the following purposes:

- Suspects of kidney disease or damage
- If your kidney function needs to be evaluated, especially if you have a chronic condition such as diabetes or high blood pressure
- To help determine the effectiveness of dialysis treatment if you're receiving hemodialysis or peritoneal dialysis
- To help diagnose a number of other conditions, such as liver damage, urinary tract obstruction, congestive heart failure or gastrointestinal bleeding — although an abnormal Blood urea test result alone doesn't confirm any of these conditions

Generally, a high urea level means kidneys aren't working well. But high level of urea can also be due to:

1. Dehydration, resulting from not drinking enough fluids or for other reasons
2. Urinary tract obstruction
3. Congestive heart failure or recent heart attack
4. Gastrointestinal bleeding
5. Severe burns
6. Certain medications, such as some antibiotics
7. A high-protein diet

Creatinine – a waste product in the blood that comes from muscle activity; It is normally removed from the blood by kidneys, but when kidney function slows down, the creatinine level rises.

Creatinine test is used for the following reasons:

1. To make a diagnosis if there are signs or symptoms of kidney disease
2. To screen for kidney disease if you have diabetes, high blood pressure or other conditions that increase the risk of kidney disease
3. To monitor kidney disease treatment or progression
4. To monitor for side effects of drugs that may include kidney damage or altered kidney function
5. To monitor the function of a transplanted kidney

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Lec. 13/Lab. 10

Separation Techniques

A **separation process** is a method that converts a mixture into two or more product mixtures. In other words, it's a scientific process of distinguishing to two or more substances in order to obtain purity.

Complete & incomplete separation

Some types of separation require complete purification of a certain component.While an incomplete separation process may specify an output to consist of a mixture instead of a single pure component.

FILTRATION

Filtration is the most often used method for removing solid particles. The substance which is left behind in the filtration medium is called **residue.** The liquid which passes through the filtration medium is called **filtrate.**

Applications/examples:

1- To purify compounds

2- HEPA in air-conditioning to remove particles from air

CENTRIFUGATION

** It is used to separate mixtures where the solid particles don't settle faster, and which are of very small size and can't be separated by filtration.

** Centrifugation is a technique which in involves the application of centrifugal force to separate particles from a solution according to their (size, shape, density, viscosity of the medium & rotor speed)

** A centrifuge is a device that separates particles from a solution using a rotor.

Applications/examples:

** Microcentrifuges are used to process small volumes of biological molecules, cells, or nuclei.

** Used in diagnostic laboratories for blood and urine tests.

** Aids in separation of proteins using purification techniques such as salting out.

** Differential Centrifugation used to separate organelles and membranes found in cells.

CRYSTALLIZATION

**The formation of crystals from a solution typically, a heated solution is cooled to reduce its solubility, resulting in a pure solid. By this process of Crystallization, the main component is separated from the other mixture.

**Crystallization is the (natural or artificial) process by which a solid forms, where the atoms or molecules are highly organized into a structure known as a crystal.

**Used to separate a dissolved heat-liable solid (will decompose upon heating and hence can sublime) solid (solute) from a solution.

Applications/examples:

**Purification of drugs

**Fractional Crystallization: It is possible to separate mixtures of different ionic compounds having identical chemical composition by dissolving them in water and adjusting the temperature of the solution so that one compound crystallizes out and the other does not.

EVAPORATION

Can be used as a separation method to separate components of a mixture with a dissolved solids in a liquid. The liquid is evaporated, meaning it is converted from its liquid state to a gaseous state. This often requires heat. Once the liquid is completely evaporated, the solid is all that is left behind.

Applications/examples:

**Recovering salts from solution.

**Demineralization of water.

Lec.14 / Lab 12

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Glucose Test

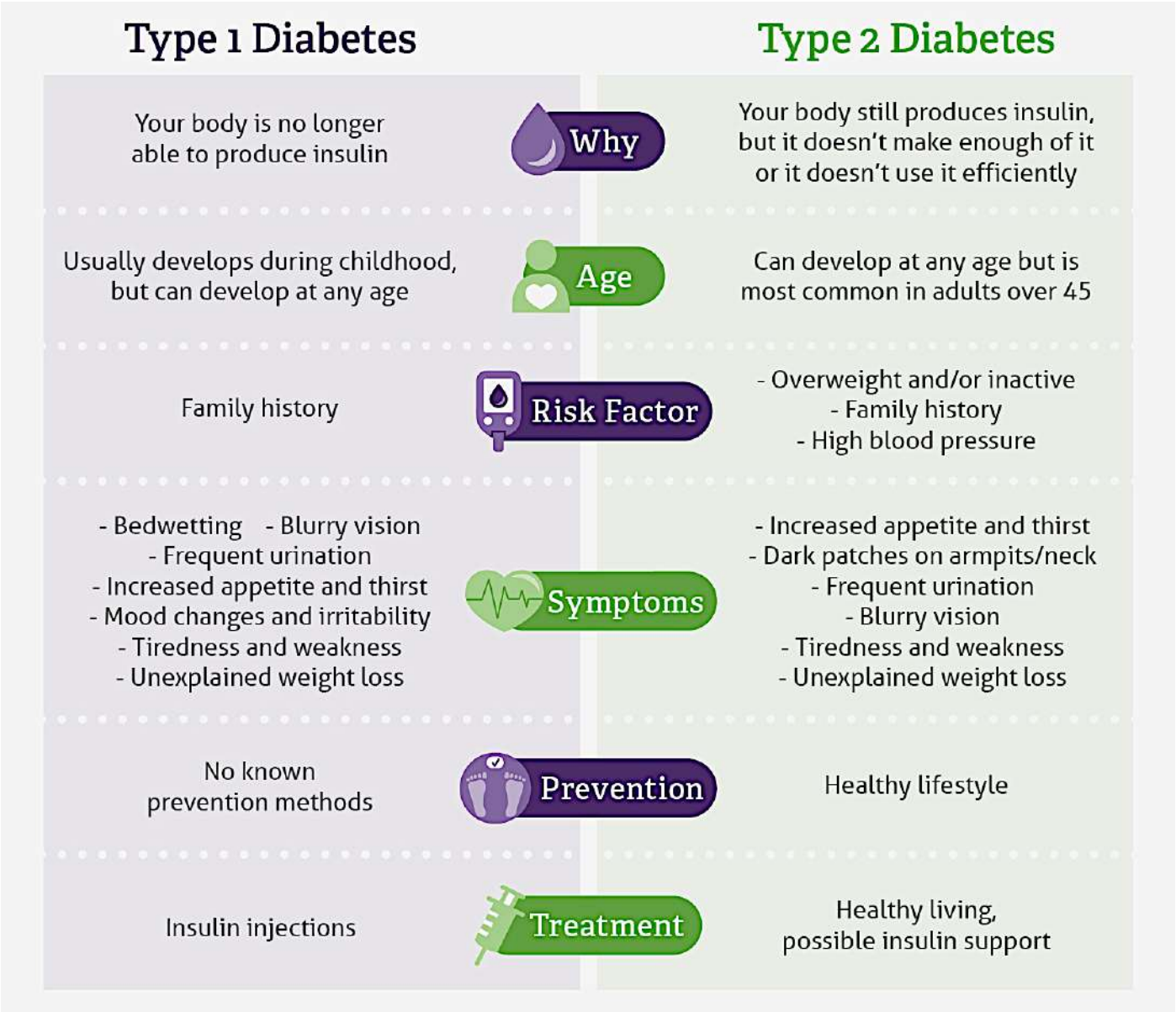
Blood glucose level is the concentration of glucose in the blood at a precise point in time. As this test only provides an indicator of blood glucose, increased levels of glucose are found in diabetes mellitus, hyperthyroidism, pancreatitis, and renal failure. Decreased levels are found in insulinoma, hypothyroidism, hypopituitarism, and liver disease. In contrast, the HbA1c test provides an overall marker of an individua'ls average blood glucose over the last 3 months. This provides a better reflection of how the levels of sugar in the blood are being controlled over time. However, as the reliability of the HbA1c test depends on red blood cells, it is important to remember that certain medical conditions, such as anemia, can affect the results.

Type 1 Diabetes

The main difference between the two types of diabetes is that type 1 diabetes is a genetic disorder that often shows up early in life, and type 2 is largely diet-related and develops over time. If you have type 1 diabetes, your immune system is attacking and destroying the insulin-producing cells in your pancreas.

Type 2 Diabetes

With type 2 diabetes, your body still produces a small amount of insulin, but it isn't effective enough. The pancreas can't keep up with the high blood sugar levels resulting from poor diet and lack of exercise. Some people with type 2 diabetes actually have "insulin resistance," which means the pancreas produces insulin but the body does not recognize it (this is different than type 1, in which the insulin-producing cells are being attacked by the immune system).



HbA1c test

Analysis of glycated hemoglobin (HbA1c) in blood provides evidence about an individual's average blood glucose levels during the previous two to three months. The HbA1c is now recommended as a standard of care for testing and monitoring diabetes, specifically the type 2 diabetes.

HbA1c testing relies on hemoglobin. Hemoglobin is the part of the red blood cell that carries oxygen throughout the body. When you have glucose in blood, it glycates (sticks) to hemoglobin. The more glucose in blood, the more it sticks. And it can stay there for around three months, or about how long the average red blood cell lives. [The Hb A1c test measures the average amount of glucose that's been attached to hemoglobin over time. So the HbA1c test provides more information about blood sugar over a longer period of time].

An HbA1c test result gets reported as a percentage. The number represents the portion of hemoglobin proteins that are glycosylated, or holding glucose. The higher the percentage, the higher your blood sugar levels have been over the last few months.

- Less than 5.7% means you don't have diabetes.
- 5.7% to 6.4% signals pre-diabetes.
- 6.5% or higher means a diabetes diagnosis.
- 7% or lower is the goal for someone trying to manage their diabetes.

Glucose Test

Procedure:

	Standard	Sample
Reagent	1000 µL (1ml)	1000 µL (1ml)
standard	10µL	-
Sample	-	10 µL
Mix well & incubate at 37C for 5 min. or at 20-25 for 10 min.		
Record the absorbance at 505 nm		

Calculation:

$$\frac{\text{Sample}}{\text{St.}} * 100$$

(N: Fasting blood sugar :70-120)
(N: 2hr. post prandial BS: Less than 180)

Conversion factor:
mg/dl * 0.0555 = mmol/L

Lec. 15

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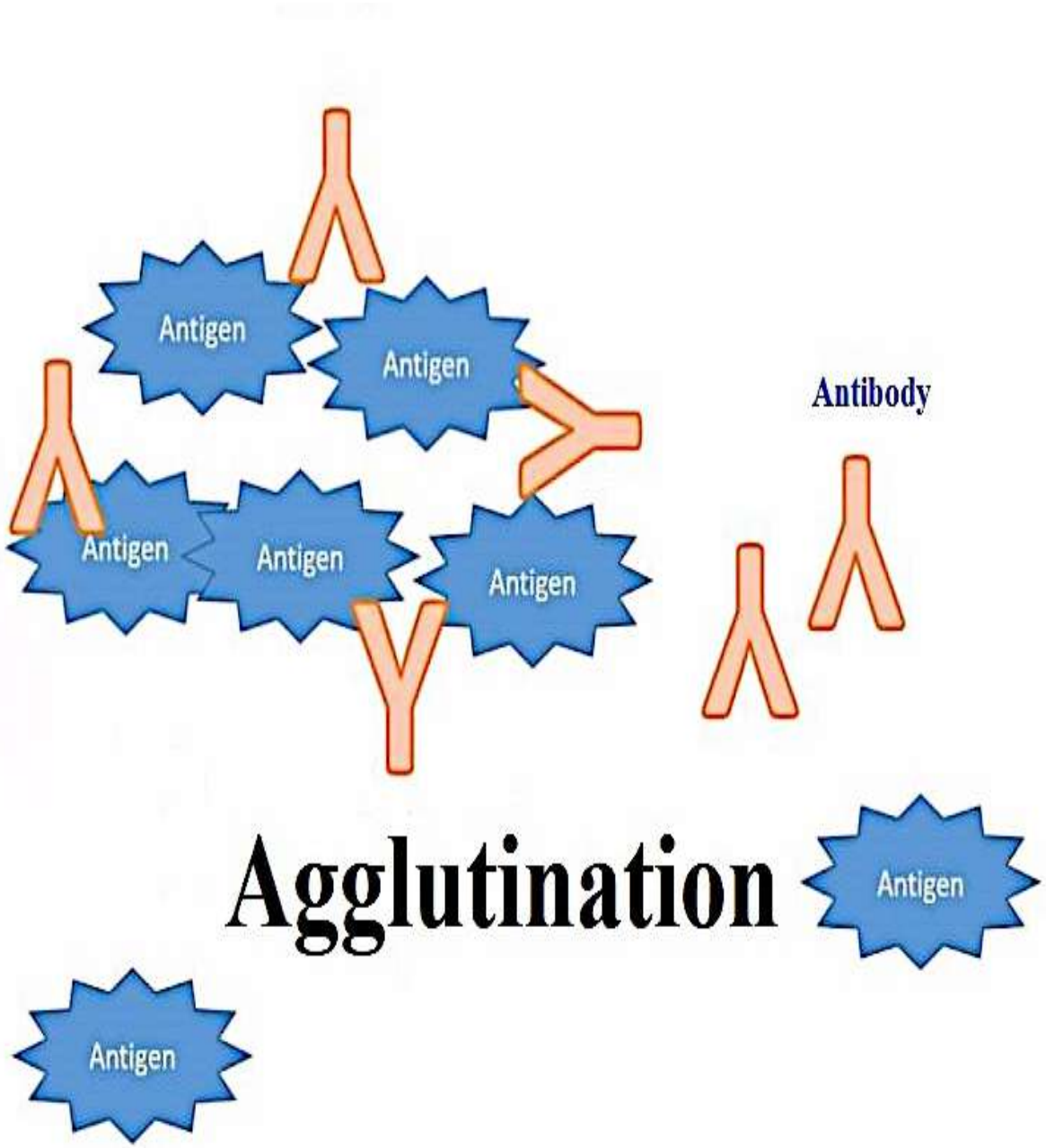
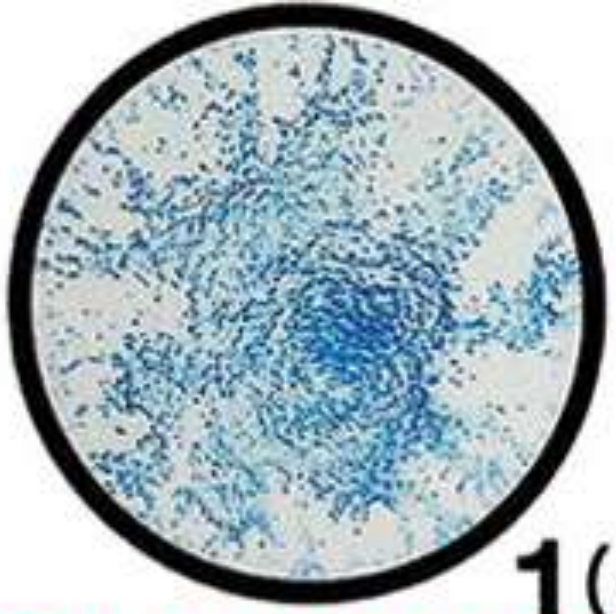
Agglutination

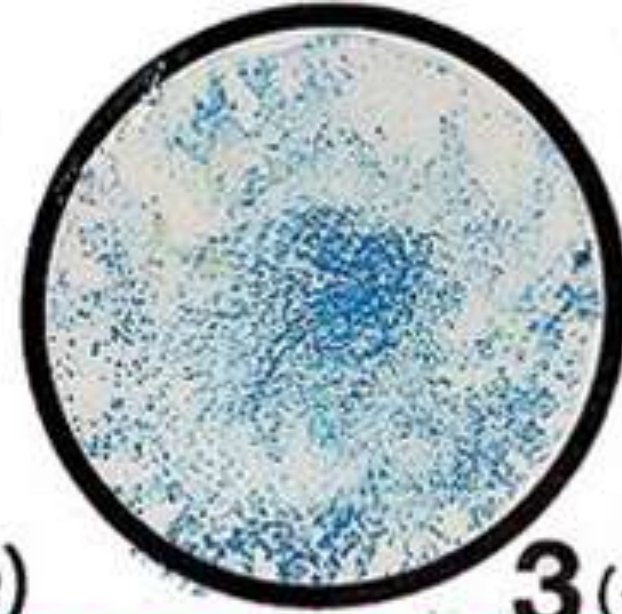
The reaction between a particulate antigen & an antibody results in visible clumping called agglutination. A positive reaction can be detected in a short time. However, the antigen-antibody complex may be seen with the naked eye if the complex size is large.



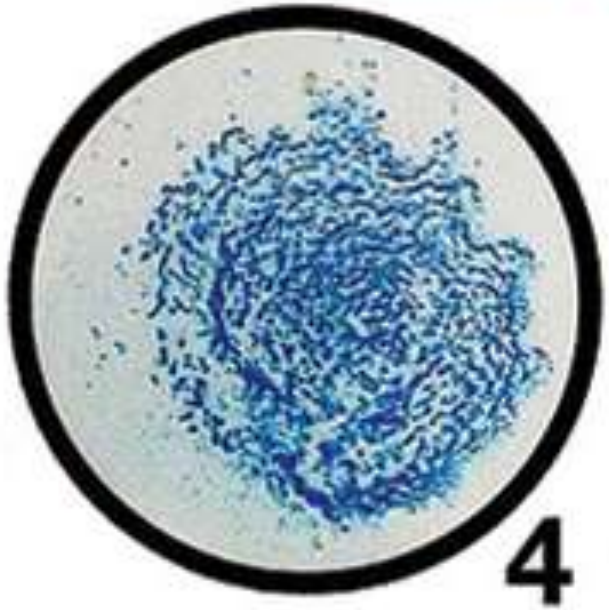


1 (+ve)

2 (+ve)



3 (+ve)



4

(+ve)

5

(+ve)

6

(-ve)

Types Of Agglutination Reactions

Active agglutination

- Direct agglutination (active agglutination): Cells (such as bacteria, fungus, and erythrocytes) and their insoluble particulate antigens can be directly agglutinated by their specific antibodies.

- example: Bacterial agglutination (Widal test) and Hemagglutination (blood group).

Passive agglutination

- Passive agglutination employs carrier particles that are coated with soluble antigens.
- Antigen is attached to certain inert carrier thereby, particles or cells gets agglutinated when corresponding antibody reacts. Latex particles, Carbon particles, and Bantonite are used as inert carriers.
- Example: Antigens coated in latex particles used in ASO test

Application of agglutination test

1. For blood grouping and cross matching.
2. For identification of unknown microbial culture
3. For detection of antigen and antibody in clinical sample
4. For serological diagnosis of certain microbial diseases such as typhoid (Widal test).

The ROSE BENGAL plate agglutination test :

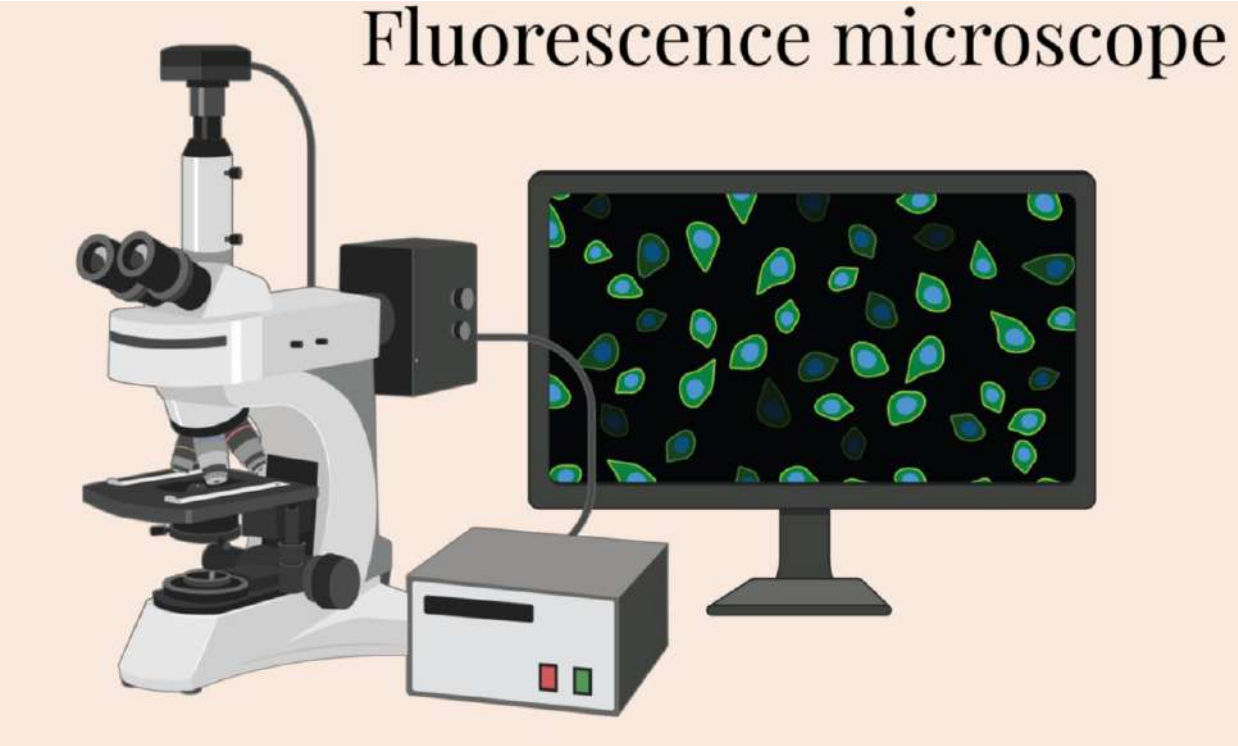
Is a rapid test which was designed originally for screening use in veterinary medicine but is now often used for the diagnosis of human brucellosis, Its **high sensitivity**, **ease**, and **speed of use**, as well as its **low cost**. The rose Bengal-stained Brucella antigen is used for early detection of brucella agglutinins (***Brucella Abortus***, ***Brucella Meliteness***, & ***Brucella Suis)***.

Immunofluorescence (IF) Technique

- Is a powerful technique that utilizes fluorescent-labeled antibodies to detect specific target antigens. This allows to see proteins that would otherwise be invisible, even under a high-powered microscope. The biological samples include tissue and cells.

Principle of Immunofluorescence

- Specific antibodies bind to the protein or antigen.
- Antibodies could be labeled with fluorescence molecules (fluorochromes): is a fluorescent dye which emit greenish fluorescence under UV light.
- When light of one wavelength falls on fluorochrome, it absorbs that light to emit light of another wavelength.
- The emitted light can be viewed with a fluorescence microscope.

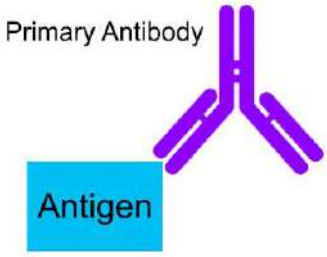


Requirements of Immunofluorescence Techniques

1- **Specific antibodies** that can bind to the antigen to form the Ag-Ab complex. They can be:

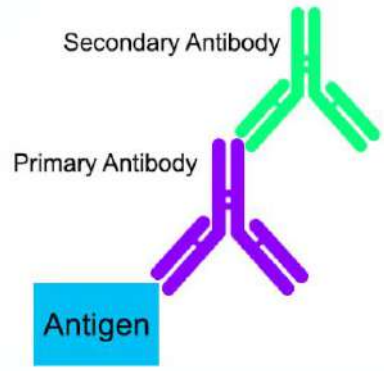
a. Primary Antibody:

The specific antibody which directly binds with antigen.



b. Secondary Antibody:

The antibody which binds to the primary antibody that is already bound with the specific antigen.



2- **Fluorescent dye (Fluorochromes):** which are conjugated to the antibody. Fluorochromes are:

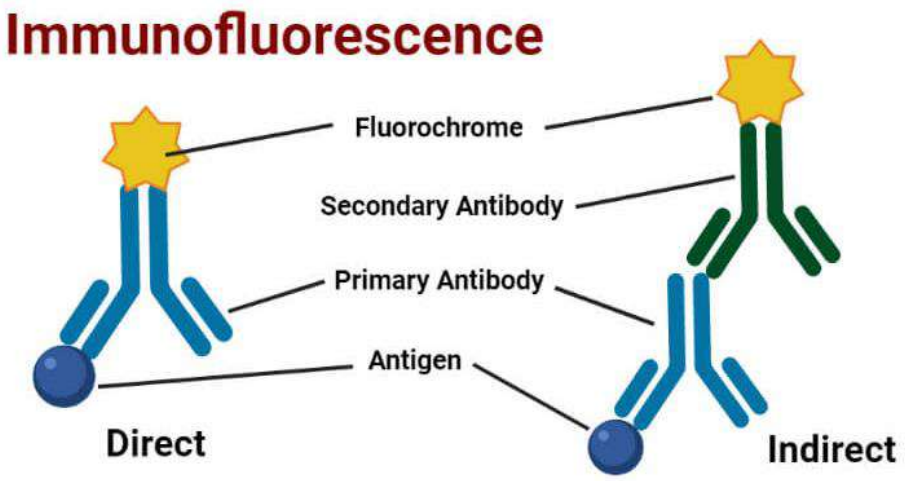
- Fluorescein
- Rhodamine
- Phycoerythrin

3- **Immunofluorescence microscope:** for visualization.

4- **Wash buffers:** such as Phosphate Buffered Saline (PBS).

Types of Immunofluorescences

- ❑ Primary (direct)
- ❑ Secondary (indirect)



Primary (direct) immunofluorescence

Single antibody (primary antibody) is conjugated directly to a fluorescent dye. If the antigen is present, the primary antibody directly reacts with it and fluorescence can be observed under the fluorescent microscope.

Uses of Direct Immunofluorescence Test

- For the detection of rabies virus antigen in the skin smear
- For the detection of N. gonorrhoeae, C. diphtheriae, T. pallidum, etc. directly in appropriate clinical specimens.

Advantages of Direct Immunofluorescence Test

1) Shorter staining time & simple workflow
2) Offer best solution for specific targeting
3) Avoid any cross reactivity between secondary antibodies

Disadvantages of Direct Immunofluorescence Test

1) More expensive.
2) Less sensitive than indirect immunofluorescence

Secondary (indirect) immunofluorescence

Double antibodies are used (The primary antibody is not labeled and a fluorochrome-labeled secondary antibody is used for detection)

Uses of Indirect Immunofluorescence Test

- In detection of specific antibodies for diagnosis of syphilis, amoebiasis, toxoplasmosis, and other diseases.
- Also used in the detection of autoantibodies that cause auto immune disorders.

Advantages of Indirect Immunofluorescence Test

- In case of secondary antibodies, a single fluorochrome-labeled antibody is used for detecting many Ag-Ab interactions.
- More sensitive than direct immunofluorescence test.
- Multiple secondary antibodies can bind to the primary antibody which amplifies the fluorescence signal.

Disadvantages of Indirect Immunofluorescence Test

- It is more complex and time-consuming than the direct IF.
- Cross-reactivity of secondary antibody to other agents can be problematic.

Applications of Immunofluorescence

- Used on tissues or cell sections to determine presence of different biological molecules which also includes proteins, carbohydrates, etc.
- Used in localization of antigen in tissue sections
- Plays a key role in the detection of autoimmune disorders (lupus).

Lab. 1 + 2

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Safety & Principles of Sterilization in Microbiology Lab.

Introduction:

Laboratory techniques Laboratory techniques are the set of procedures that are used in natural sciences like chemistry, biology to conduct an experiment, all of them follow scientific methods; these procedures involve the use of laboratory equipment from (laboratory glassware to electrical devices), and others require more specific or expensive supplies.

Safety Rules in Microbiology laboratory:

⇒ **Treat all microorganisms as potential pathogens** especially unknown cultures as if they were pathogenic. A student who has a compromised immune system or has had a recent extended illness should talk with the instructor before working in the microbiology laboratory.

⇒ **Sterilize equipment and materials.** All materials, media, tubes, plates, loops, needles, pipetes, and other items used for culturing microorganisms should be sterilized by autoclaving. Otherwise, use commercially sterilized products. Understand the operation and safe use of all equipment and materials needed for the laboratory.

⇒ **Disinfect work areas before and after use.** Use a disinfectant, such as a 10% bleach or 70% ethanol solution, to wipe down benches and work areas both before and after working with cultures. Also be aware of the possible dangers of the disinfectant, as 70% ethanol can catch fire around open flame or high heat sources. Bleach, if spilled, can ruin your clothing. Either alcohol or bleach can be dangerous if splashed in the eyes. Students should know where the nearest eyewash station and sink are located.

⇒ **Wash your hands.** Use a disinfectant soap to wash your hands before and after working with microorganisms. Gloves may be worn as extra protection.

⇒ **Never pipette by mouth.** Use pipette bulbs or pipetting devices for the aspiration and dispensing of liquid cultures.

⇒ **Do not eat or drink in the lab, nor store food in areas where microorganisms are stored.** Never eat or drink in the laboratory while working with microorganisms. Keep your fingers out of your mouth,. Cover any cuts on your hands with a bandage.

⇒ **Label everything clearly.** All cultures, chemicals, disinfectants, and media should be clearly labeled with their names and dates. If they are hazardous, label them with proper warning and hazardous information.

⇒ **Autoclave or disinfect all waste material.** All items to be discarded after a class, such as culture tubes, culture plates, swabs, disposable transfer needles, and gloves, should be placed in a biohazard autoclave bag and autoclaved. If no autoclave is available and you are not working with pathogens, the materials can be covered with a 10% bleach solution and allowed to soak for at least 1 to 2 hours.

⇒ **Clean up spills with care.** Cover any spills or broken culture tubes with a 70% ethanol or 10% bleach solution; then cover with paper towels. After allowing the spill to sit with the disinfectant for a short time, carefully clean up and place the materials in a biohazard autoclave bag to be autoclaved.

Wash the area again with disinfectant. **Never** pick up glass fragments with your fingers or stick your fingers into the culture itself; instead, use a brush and dustpan.

Principles of Sterilization in Microbiology Lab.

Sterilization: is defined as a process of complete elimination or destruction of all microorganisms including the most resistant bacteria and spores, which is carried out by various physical, mechanical, and chemical methods.

- ## The aims of sterilization are:

1. Eliminate the possibility of transmission of infectious agents to laboratory workers, the general public, and the environment.

2. Protect the cultures from environmental contaminants.

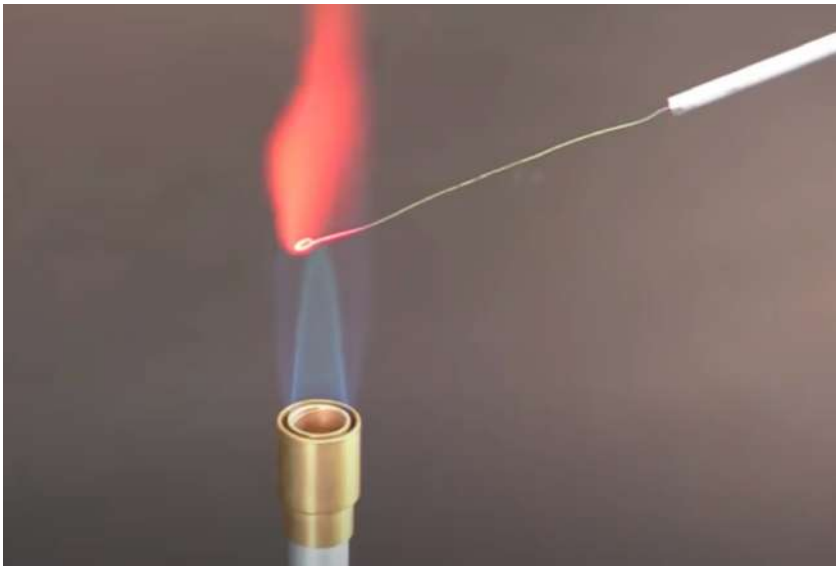
Methods of sterilization

1. Physical Method:

- **Heating:** Is the most rapid and best method of sterilization. which the material to be sterilized is stable enough to withstand the required temperature necessary to kill the microbes. The time needed for sterilization depends on the initial number of organisms present, type of materials to be sterilized (hence washed and cleaned items are easier to sterilize than dirty ones) and also on the temperature used. Spores need higher temperatures while vegetative bacteria can be destroyed at lower temperatures.

i. **Dry Heat:** Dry heat is frequently used for the sterilization of glassware and laboratory equipments. In dry heat sterilization, microbial cells are killed by oxidation of their constituents and protein denaturation (The essential cell constituents are destroyed and the organism dies). The temperature is maintained for almost an hour to kill the most difficult of the resistant spores. Dry heat is applied in the following ways:

a) Red heat: Inoculating wires, points of forceps and searing spatulas are sterilized by holding them in the flame of a bunsen burner until they are seen to be red hot.



b) Flaming: This method is used for sterilizing scalpels, needles, mouths of culture tubes, slides and coverslips. It involves passing the article through the Bunsen flame without allowing it to become red hot.

FLAMING

c) Hot air oven: We can sterilize Glassware (like petri dishes, flasks, pipettes, and test tubes), Powder (like starch, zinc oxide, and sulfadiazine), Materials that contain oils, Metal equipment (like scalpels, scissors, and blades). To destroy microorganisms and bacterial spores, a hot air oven provides extremely high temperatures over several hours.

Temperature (°C)	Time (min)
170	30
160	60
150	150

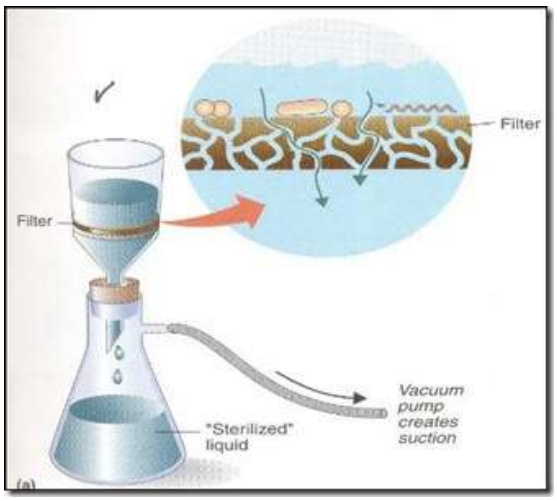
ii. **Moist Heat:** Moist heat kills microorganisms mainly by hydrolysis & coagulation of proteins (denaturation), which is caused by breakage of the hydrogen bonds that hold the proteins in three-dimensional structure. The temperature at which denaturation occurs varies inversely with the amount of water (moist) present. Sterilization in saturated steam thus requires precise control of time, temperature, and pressure. The recommendation for sterilization in an **autoclave** **is 15 minutes at 121°C and 1.5 atmospheric pressure (15 psi) .** In certain cases (e.g. thermolabile substances) Alternative conditions, with different combinations of time and temperature can be used.

Temperature (°C)	Approximate corresponding pressure (kPa)	Minimum sterilization time (min)
126-129	250 (~2.5 atm)	10
134-138	300 (~3.0 atm)	5

Rediation: By Damaging DNA. It can be Ionizing radiation (X rays, gamma rays, electron beams) or Nonionizing radiation (UV). it is used for surface sterilization only. The main difference between radiations is their penetration. UV has limited penetration in air and sterilization occur in a small area around the UV lamp. However, it is relatively safe and useful for sterilizing small areas like hoods. x-ray has more penetrating power which make them more dangerous but very effective for large scale sterilization of plastic items (e.g syringes).

2. Mechanical Method:

Filtration: Is the quickest and effective way to sterilize solutions without heating. This method involves filtering with a pore size that is too small for microbes to pass through. It is particularly useful for solutions containing toxins, enzymes, drug, serum and sugars. Sugar solutions used for the cultivation of microorganisms tend to caramelise during autoclaving and so they are best sterilized by filtration. Filters with known pore sizes which are sufficiently small to hold back bacteria are employed. Recently filters that can remove viruses are also available. Filtration is an excellent way to remove the microbial population from solution containing heat sensitive material.



3. Chemical Method:

Alcohol: Denaturing protein through a process that requires water therefore they should be diluted to 60-90% in water. Alcohol kills germs through a simple chemical process known as denaturation, through a process that requires water therefore they should be diluted to 60-90% in water. Denaturation occurs when alcohol molecules break down the proteins present in the structure of germs. When the proteins break down and lose their structure, the cells can't function properly. For example, alcohol can eliminate common bacteria, such as *E. coli*, *salmonella*, and *Staphylococcus aureus.* Alcohol has also been shown to kill viruses such as herpes, hepatitis B, HIV,

influenza, and coronaviruses, among others. Ethanol is widely used as a disinfectant although isopropanol is a better solvent for fat and therefor it is a better option.

(Lab. 4)

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General Stool Examination (GSE)

What is a GSE?

A stool analysis is a series of tests done on a stool (feces) sample to help diagnose certain conditions affecting the digestive tract. These conditions can include infection (such as from **parasites**, **viruses**, or **bacteria**), **poor nutrient absorption**, or **cancer.**

Precautions:

- Avoids warm temperature (the specimen should reach the lab within 30 min, to prevent death with heat sensitive organisms), like vibrio can die or become unrecognizable after that period.
- Prevent drying of specimen
- Prevent contamination
- Sample should not be collected from bedpans.

Criteria of specimen rejection:

1. Contaminated with urine
2. Dry Specimens
3. Submitted with fixative & additive

Procedure

1- Physical Examination:

A. Colour:

1. **Normal stools** : light to dark brown in colour
2. **Bleeding in the small intestines:** into the intestinal tract the stools become dark tarry in nature
3. **Bleeding in large intestines or rectum:** the blood may be bright red.
4. **Cholera** :the stools have a rice water appearance
5. **Biliary tract obstruction** : clay coloured

B. Consistency

1. **Normal stools**: formed.

2. **Diarrhea and dysentery**: semi solid or watery.
3. **Dehydration**: solid (Hard) and the semi-solid
4. **Taking certain medications**: soft or loose
5. **malabsorption of fats:** pale bulky and semi-solid.

2- Chemical Examination:

pH:

pH of stools is acidic in amoebic dysentery and is alkaline in bacillary dysentery.

- A low pH may be caused by poor absorption of carbohydrates or fat.
- Stool with a high pH may mean inflammation in the intestine (colitis), cancer, or antibiotic use.

3- Macroscopic Examination

⇒ **Direct wet preparation:**

a) Saline specimen preparation

b) Iodine preparation

1. Take a dry microscopic slide, & label it with the name or number of the patient.
2. Put one drop of normal **saline/ iodine** solution
3. Take a small amount of stool and mix well using wooden stick or tung pressure.
4. The smear should be thin.
5. Put cover slide without any air bubble, and see under the microscope 10x, 40x, &100x.

A. Blood:

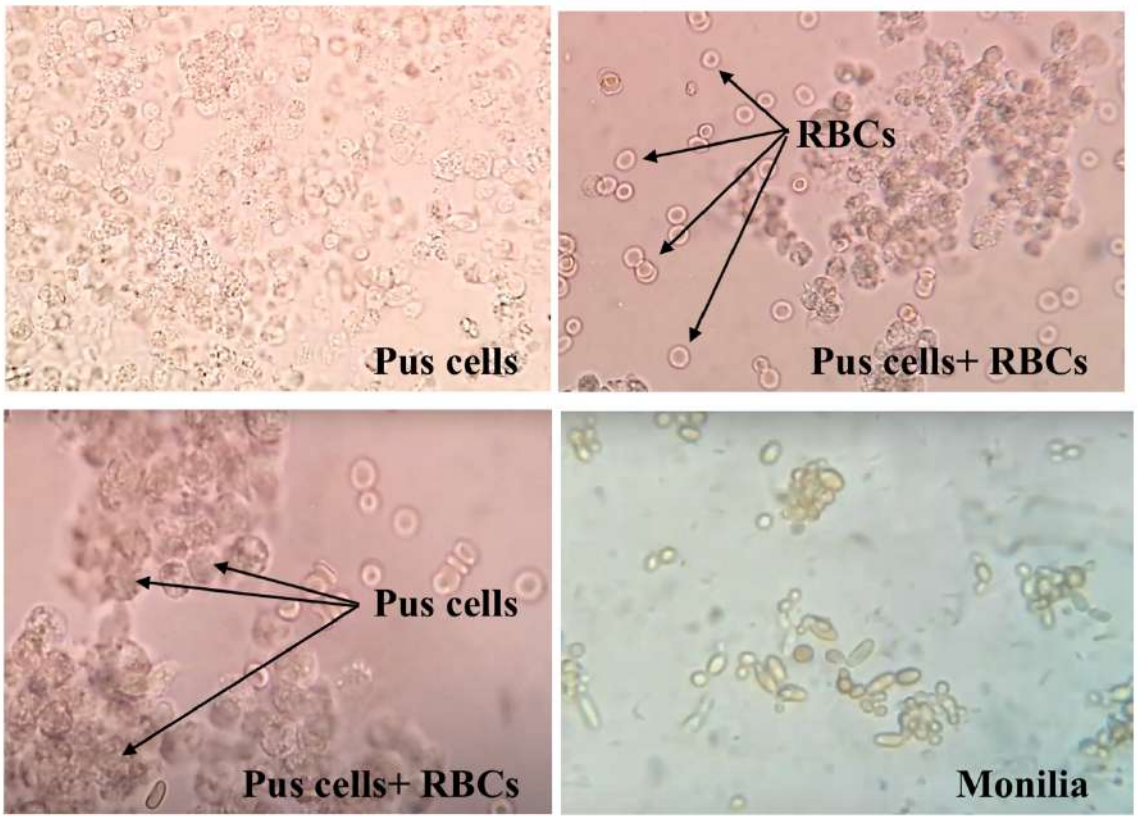
Blood should be noted in stools if present as it is indicative of ulceration or presence of any other pathology like malignancy. It should also be noted if the blood is bright red or is altered in colour as it may be a clue to the site of pathology in the intestinal tract.

B. Mucus:

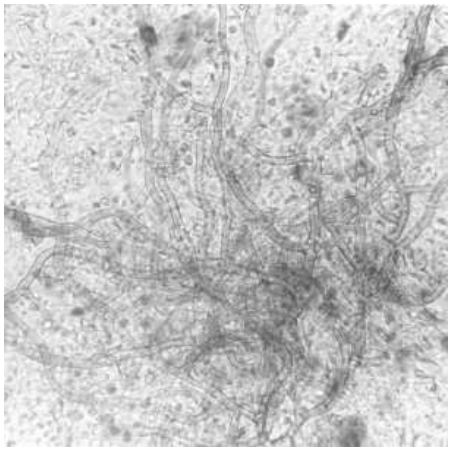
Mucus is present in certain conditions like amoebic or dysentery. White blood cells in the stool may be caused by inflammation of the intestines, such as ulcerative colitis, or a bacterial infection.

C. Parasite: Stools may contain adult helminthes. Nematodes like ascaris are easily visible as their size is large. Hook worms and proglottids of cetodes may also be present

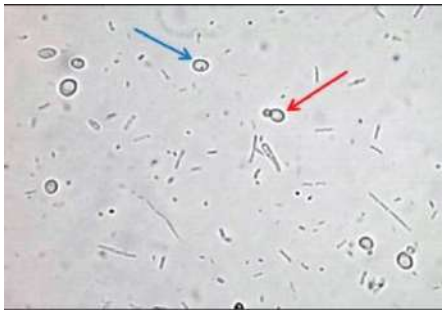
Macroscopic Examination:



Hyphae (long branching filamentous of a fungus



Monilia



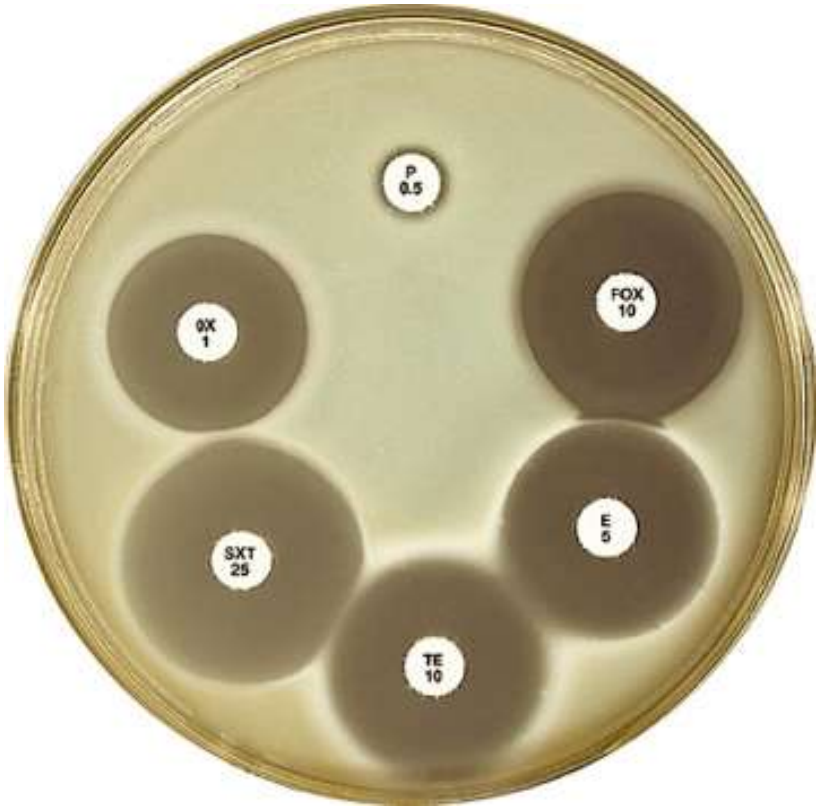
Stool culture:

A stool culture is a test on a stool sample to find germs (such as bacteria or a fungus) that can cause an infection. A sample of stool is added to a substance that promotes the growth of germs. If no germs grow, the culture is negative. If germs that can cause infection grow, the culture is positive. test results usually take 2 to 3 days. But some cultures for fungus and parasites may take weeks to get results.

E. coli on blood agar **E. coli on MacConkey agar**

Antibiotic Sensitivity Test:

An antibiotic sensitivity (or susceptibility) test is done to help choose the antibiotic that will be most effective against the specific types of bacteria or fungus infecting an individual person. Some types of bacteria or fungus are resistant to certain antibiotics because of differences in their genetic material (genes).



LAB. REPOERT

GENERAL STOOL EXAMINATION

Color:

Appearance:

Mucus:

Blood:

pH:

<u>Microscopic Examination:</u>

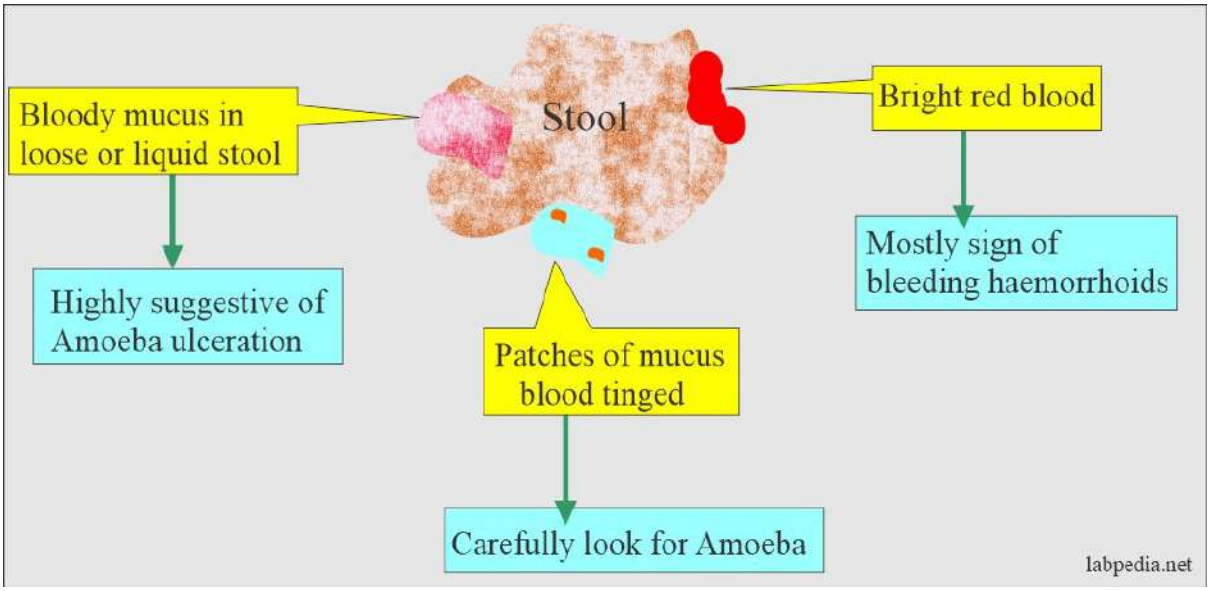
WBC: **/HPF**

RBC: **/HPF**

Ova:

Cyst:

Others:



LAB REPORT

Patient Name	: Baby SRIHITHA	**Organization**	: SRI ADITHYA DI (BEERAMGUDA)
Age / Gender	: 6 MONTHS / Female	Sample ID	: 193100469
Referred By	: SELF	Registered	: Nov 06, 2019, 10
Reg No	: 100027	Collected On	: Nov 07, 2019, 12
Sample Type	: STOOL	Approved On	: Nov 07, 2019, 08

CLINICAL PATHOLOGY

COMPLETE STOOL EXAMINATION (CSE)

TEST NAME(s)	RESULTS	UNITS	REFERANCE RANGE
COLOR	Yellow		Brown
APPEARENCE	Solid		Solid - Semi solid
MUCUS	Absent		Absent
BLOOD	Absent		Nil
PH.	Acidic		
(Method : Methyl Red & Bromothymol Blue)			
MICROSCOPIC EXAMINATION			
WBC	1 - 2		1 - 2
RBCS	Nil	/hpf	1 - 3/HPF
OVA	Not found		Nil
Cyst	Not found	/hpf	Nil
Others	Nil	/hpf	
(Method : Microscopy (Concentration technique))			

Checked By: imran
Senior Biochemistry(Technician)

END OF REPORT

Lab. 5

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Examination of Sputum

Sputum or **phlegm** is the mucousy substance secreted by cells in the lower airways (bronchi and bronchioles of the respiratory tract. It differs from saliva, which is produced in the mouth.

A sputum test can diagnose:

- **Bronchitis**
- **Lung abscess**
- **Pneumonia**
- **Tuberculosis**
- **Chronic obstructive pulmonary disease**
- **Cystic fibrosis**

Sputum color

- **Clear:** This usually means no disease is present, but **large amounts** of **clear sputum** may be a sign of **lung disease.**
- **White or gray:** This may also be normal, but increased amounts may mean **lung disease.**
- **Dark yellow or green:** This often means a **bacterial infection**, such as **pneumonia.**
- **Yellowish-green**: sputum is also common in people with **cystic fibrosis.** Cystic fibrosis is an inherited disease that causes mucus to build up in the lungs and other organs.
- **Brown:** This often shows up in people who **smoke.** It is also a common sign of **black lung disease**. Black lung disease is a serious condition that can happen if you have **long-term exposure to coal dust.**
- **Pink:** This may be a sign of **pulmonary edema**, a condition in which Pulmonary edema is a condition caused by **excess fluid in the lungs.**
- **Bloody**: often found in **tuberculosis** & this may be an early sign of **lung cancer.**
- **Rusty**: colored - usually caused by **pneumococcal bacteria** (in **pneumonia**).
- **Purulent**: containing pus:

Sample collection & Transport:

- At first, the patients need to rinse out the mouth with clear water for 10-15 seconds to eliminate any contaminants in the oral cavity.
- Commonly, the "deep cough" sample of the early morning is collected before eating or drinking anything to avoid bias in interpreting the results.
- After expelling saliva, the patients then breathe in deeply three times to cough at 2-minutes intervals until bringing up some sputum.
- The sputum is then released in a sterile well-closed container

Testing Procedures:

Sputum Staining Tests Procedure

- The sputum specimen is a smear on a microscope slide.
- Different staining dyes are added to the cells, bacteria, or fungi of the sample on the slides (Gram stain or Acid fast bacilli)
- The slides are then diagnosed under a microscope. If the bacteria, fungi, or specific cells are identified in the specimens, the **results are positive.**

Sputum Cytology Examination Procedure

- The smear sputum slide is stained with different dyes according to the instructions.
- Then the pathology specialist examines the stained slide under the microscope to find the **abnormal cells** from the sputum specimen.
- If a **few white blood cells** and **no abnormal cells** have been found in the sputum sample, that means the sputum cytology examination is regular, and other reasons may cause the patient's symptoms.

Sputum Culture Procedure

- The sputum sample is added to a culture plate (Blood, Chocolate or MacConkey) agar.
- Cover the lid of the dish and place it in a 37 degree C incubator.
- Check the bacteria or fungi growth in the sputum plate every day. Once the sputum culture is positive, **microscopy, colony morphology, or biochemical tests** of bacterial growth will be performed to identify the **specific type of bacterium or fungus.**

- **If the pathogenicity organisms grow after 24 hours of incubation in the culture dish, the result is positive,** if no bacteria or fungi grow in 6 to 8 weeks for solid culture media or six weeks for liquid culture media, **the result is negative.**

The most common cause **of** **bacterial pneumonia** is ***Streptococcus pneumoniae***.

Other common bacteria include:

- ***Staphylococcus aureus*** (staph)
- ***Klebsiella pneumoniae***
- ***Streptococcus pyogenes***

Lab. 6

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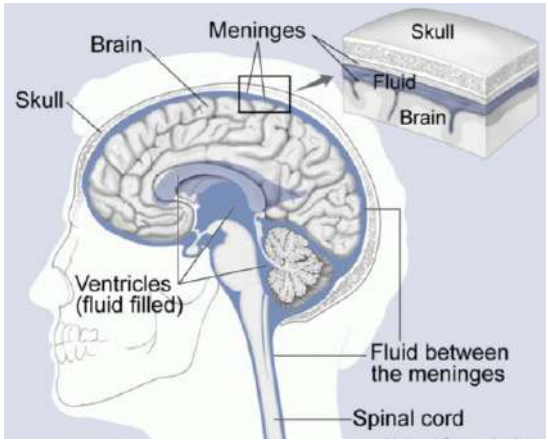
Examination of Cerebrospinal Fluid (CSF)

Cerebrospinal fluid (CSF): is a clear, watery liquid that flows around the brain and spinal cord, surrounding and protecting them. CSF testing is performed to evaluate the level or concentration of different substances and cells in CSF in order to diagnose conditions affecting the brain and spinal cord (central nervous system). CSF is collected from the lower back using a procedure called a **lumbar puncture** or **spinal tap.** CSF can be very valuable in diagnosing a variety of conditions affecting the central nervous system CNS.



CSF examination is used to Diagnose:

- **Bacterial, viral or fungal meningitis** (infection of the layers that cover the brain and spinal cord)
- **Encephalitis** (infection in the brain)
- **Acute leukaemia** (cancers that have spread to the central nervous system)
- **Lymphoma** (cancer of the lymphatic system)
- **Multiple sclerosis** (**Autoimmune diseases** that affect the central nervous system)
- **Subarachnoid haemorrhage** (SAH) is a type of stroke
- **Spinal canal blockage** (Blockage of an artery carrying blood to the spinal cord prevents the cord from getting blood and thus oxygen. As a result, tissues can die.

Normal CSF:

- **Clear and colourless.**
- Cell count: **<5 x 10^6/L** mononuclears (**blood cells that have a single, round nucleus**, such as **lymphocytes** and **monocytes**.
- **no neutrophils** or **red cells**
- **Glucose**: **60-70%** of plasma levels (usually) 2.8-4.4 mmol/L x18 =50-80 mg/dl
- **Lactate**: 1.2-2.8 mmol/L
- **Protein:**
    a) Neonate: 0.1-1.2 g/L x100 = mg/dl
    b) Adult: 0.15-0.45 g/L.

CSF Physical Features (pressure and appearance)

Pressure: of the CSF can be measured when opening (starting) and closing (finishing) the collection.

- **Increased** CSF pressure may be seen with a variety of conditions that increase pressure within the brain or skull and/or obstruct the flow of CSF, such as **tumors, infection, or bleeding.**
- **Decreased** pressure may be due to **dehydration**, **or leakage of CSF through an opening** (e.g., another **lumbar puncture** site or **sinus fracture**).

Appearance: the sample of CSF is usually compared to water.

- **Color**: **normal fluid** is **clear and colorless**. **Changes in the color** of the CSF are **not diagnostic** but may point to additional substances in the fluid.

    ⇒ **Yellow, orange, or pink** CSF may indicate the breakdown of blood cells due to bleeding into the CSF or the presence of **bilirubin (Xanthochromia_The presence of bilirubin in CSF is the main cause of xanthochromia associated with high spinal fluid protein content).**

    ⇒ **Green CSF** may also sometimes be seen with bilirubin or infection.

- **Turbidity**—cloudy or turbid CSF may indicate the **presence of WBCs or RBCs, microbes,** or an **increase in protein levels.**
- **Viscosity**—normal CSF will have the same consistency as water. CSF that is "thicker" may be seen in case of certain types of **cancers** or **meningitis**التهاب السحايا.

CSF Chemical Tests

Chemical tests detect or measure the chemical substances found in spinal fluid. Many of the substances in CSF are also in blood and the relative amounts in CSF and blood are often compared. Normally, levels of certain substances in CSF, such as protein and glucose, mirror the levels in blood.

- **CSF glucose**: The glucose level in the CSF should be 50 to 80 mg/100 mL (mg/dl) (or greater than 2/3 of the blood sugar level). Note: Normal value ranges may vary slightly among different laboratories. **Abnormal results include higher** and lower glucose levels. Abnormal results may be due to:

⇒ **Infection (bacterial or fungus)**
⇒ **Inflammation of the central nervous system**
⇒ **Tumor**

- **CSF protein**: only a small amount is normally present in CSF because proteins are large molecules and do not cross the barrier between the blood and brain easily. **Decreases in CSF protein are not generally considered significant**. **Increases in protein are most commonly seen with:**
  - **Meningitis**
  - **brain abscess**
  - **Brain or spinal cord tumors**
  - **Multiple sclerosis**
  - **Syphilis (Treponema Pallidum)**

Cell Counts, Differential and Microscopic Exam

Normal CSF has no or **very few cells** present and appears **clear**. A small drop of CSF is examined using a microscope, and cells are counted manually (or in some cases counted using an instrument).

⇒ If the number of **WBCs** is **very few** (for example, 5 or less in an adult), the laboratory may or may not identify them or perform a cell differential.

⇒ If **WBCs** are **numerous** (such as greater than 5), a differential will most likely be done to determine the different kinds of white blood cells that are present.

⇒ If **cancer** is suspected or has been previously diagnosed, **a differential is always performed.**

- **CSF total cell counts**
    - **Red blood cell (RBC) count—normally no RBCs are present in the CSF**. The presence of RBCs may indicate **bleeding** into the CSF or may indicate a "**traumatic tap**" – blood that **leaked into the CSF sample during collection.**
    - **White blood cell (WBC) count—normally very few WBCs are present**. A **significant increase in white blood cells in the CSF can be caused by infection or inflammation of the central nervous system.**

- **CSF white blood cell (WBC) differential**—Small numbers of **lymphocytes, monocytes** (in **neonates**, and **neutrophils**) are **normal** in a sample of CSF.
- An increase in **neutrophils** with a **bacterial infection**
- An increase in **lymphocytes** with a **viral or fungal infection**
- Sometimes an increase in **eosinophils** with a **parasitic infection**
- A **slight increase** in **lymphocytes** with **immune disorders** of the **central nervous system,** such as **multiple sclerosis**
- Presence of abnormal WBCs with **leukemia** that has spread to the central nervous system
- Abnormal cells from cancerous tumors present; if they are seen on a differential, CSF **cytology will be performed** .

Cytological Examination:

CSF cytology— **used to look for abnormal cells**. This is often done when a **central nervous system tumor or metastatic cancer** is suspected. The presence of certain abnormal cells, can indicate the type of **cancer present** (**Lymphoma or leukaemia**).

Tests for Infections:

Normally, CSF does not contain any bacteria, fungi, viruses or parasites.

If **meningitis** or **encephalitis** is suspected, **select tests may be performed to detect and identify microbes**. The **selection of testing** is frequently done based on **signs and symptoms, the health of your immune system**, and **possible exposure to certain pathogens**. Some of the more frequently performed tests are listed below.

- **CSF Gram stain**
- **CSF culture**

- **Molecular testing** of the CSF by **polymerase chain reaction (PCR)** assays can be performed to detect nucleic acid from various pathogens that may be present in the sample. This method detects bacterial, viral, fungal or parasitic genetic material (DNA, RNA) and is particularly helpful if the microbe does not grow in routine culture or if the patient has been on antibiotics.

Other CSF tests for infectious diseases that are less commonly ordered include:

- **CSF AFB testing**: used to detect infection with mycobacteria, such as ***Mycobacterium tuberculosis***.

Other laboratory tests that may be ordered along with or following CSF testing include:

⇒ **Blood culture**: to detect and identify bacteria or fungi in the blood culture .
⇒ **Blood glucose (RBS).**
⇒ **Total protein (TSP)**: to compare with CSF glucose and protein levels.
⇒ **CBC (complete blood count)**: to evaluate cell counts in blood.
⇒ **CRP (C-reactive protein)**: to detect inflammation.
⇒ **ESR (erythrocyte sedimentation rate)** may be done instead if CRP is not available.

Lab. 7

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Viruses Diagnosis

A viral test is done to find infection-causing viruses. **Viruses cause disease by:**

1. **Destroying or damaging the cells they infect**
2. **Damaging the body's immune system**
3. **Changing the genetic material (DNA) of the cells they infect**
4. **Causing inflammation that can damage an organ**

Viruses cause many types of diseases, such as:

1. **Human immunodeficiency virus (HIV) - AIDS**
2. **Cold sores**
3. **Chickenpox**
4. **Measles**
5. **Flu (influenza)**
6. **Some types of cancer**

Viral tests may be done for viruses such as:

1. **Herpes simplex (HSV)**
2. Chickenpox
3. Respiratory syncytial virus (RSV)
4. Cytomegalovirus (CMV) .. CMV spreads from person to person through body fluids, such as blood, saliva, urine, semen, and breast milk
5. Rotavirus: causes severe, watery diarrhea and vomiting in infants and young children. Children may become dehydrated and need to be hospitalized and can even die
6. Hepatitis (HAV, HBV, HCV, HDV& HEV)
7. Genital warts (human papillomavirus, or HPV)
8. Influenza (flu)
9. Human immunodeficiency virus (HIV)

Sampling: A wide variety of samples can be used for virological testing:

1. Nasopharyngeal swab

2. Blood
3. Skin
4. Sputum, gargles and bronchial washings
5. Urine
6. Faeces
7. Cerebrospinal fluid
8. Tissues (biopsies or post-mortem)

Methods for Viral diagnosis

1- Viral isolation (culture)

2- Microscopy Based Method

3- Host Antibody Detection

4- Hemagglutination assay

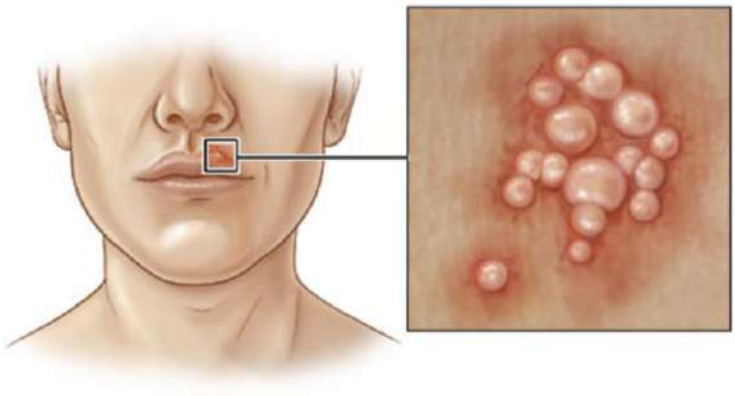
5- Nucleic acid-based methods (PCR)

6- Serology

HSV TEST

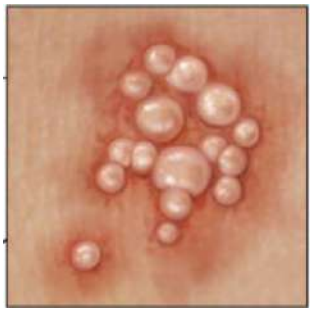
A serum herpes simplex antibodies test: is a blood test that looks for antibodies to the herpes simplex virus (HSV). The two types of herpes infections are HSV-1 & 2 (HSV)

a) HSV-1, commonly known as oral herpes, usually causes cold sores and blisters near the mouth and on the face.



Herpes simplex virus type 1 (HSV-1) can cause groups of small blisters that usually appear on the lip and around or inside the mouth. The groups of blisters are commonly called cold sores. They may be itchy or painful.

b) HSV-2 most often causes genital herpes. It's generally transmitted through sexual contact.



The virus doesn't always cause symptoms, but when it does, you may experience the following symptoms.

The symptoms of HSV-1 are:

- **Small, fluid-filled blisters around the mouth**
- **Tingling or burning sensation around the mouth or nose**
- **Fever**
- **Sore throat**
- **Swollen lymph nodes in the neck**

The symptoms of HSV-2 are:

- **Small blisters or open sores in the genital area**
- **A tingling or burning sensation in the genital area**
- **Abnormal vaginal discharge**
- **Fever**
- **Muscle aches**
- **Headache**
- **Painful urination**

The test is done to find out whether a person has ever been infected with oral or genital herpes. It looks for **antibodies to (HSV-1) & (HSV-2)**. This test does not detect the virus itself.

Different types of tests may be done.

1- **Antigens specific to HSV-1 and HSV-2** can also be readily detected by direct immunofluorescence assay, these methods have been found to lack sensitivity in nonsymptomatic patients.

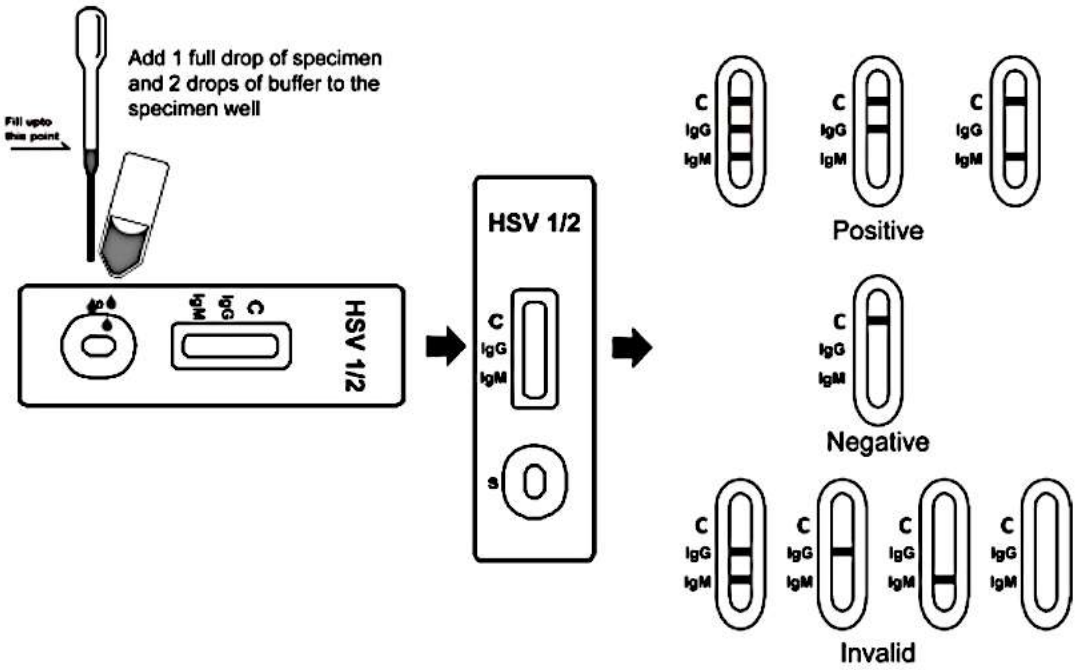
2- **Serological tests**, which use blood sample to check for the disease *antibodies*. These tests can take between 1 day and 3 weeks.

Serological tests are suitable to

⇒ **Detect the subtype of the HSV virus (HSV-1 or HSV-2)**

⇒ **Detect a symptomatic patients**

⇒ **Confirm a clinical diagnosis of HSV because it is a more reliable method than clinical diagnosis**



- **If you test positive for IgG and IgM, or just IgM**, it's likely that you **have a new infection.**
- **If the test is positive for IgG**, it's likely that you have an existing infection that has been around for at **least two months.**
- IgG results may be considered alongside IgM results. IgG antibodies take longer to produce, but last a lifetime. IgM antibodies are detectable after a few days, but disappear within a few weeks.

3- **polymerase chain reaction (PCR).** These methods use molecular-based assays and are widely used for the detection of infectious diseases. Such methods have been found to be more sensitive and rapid and require less stringent conditions in terms of collection data.

Negative/Normal: The herpes virus was not found. You may still have an HSV infection if your results were normal. It may mean the sample didn't have enough of the virus to be detected. If you still have symptoms of herpes, you may need to get tested again.

Positive/Abnormal: HSV was found in your sample. It may mean you have an active infection (you currently have sores), or were infected in the past (you have no sores)

Lec.8 / Lab. 8

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Glucose Test

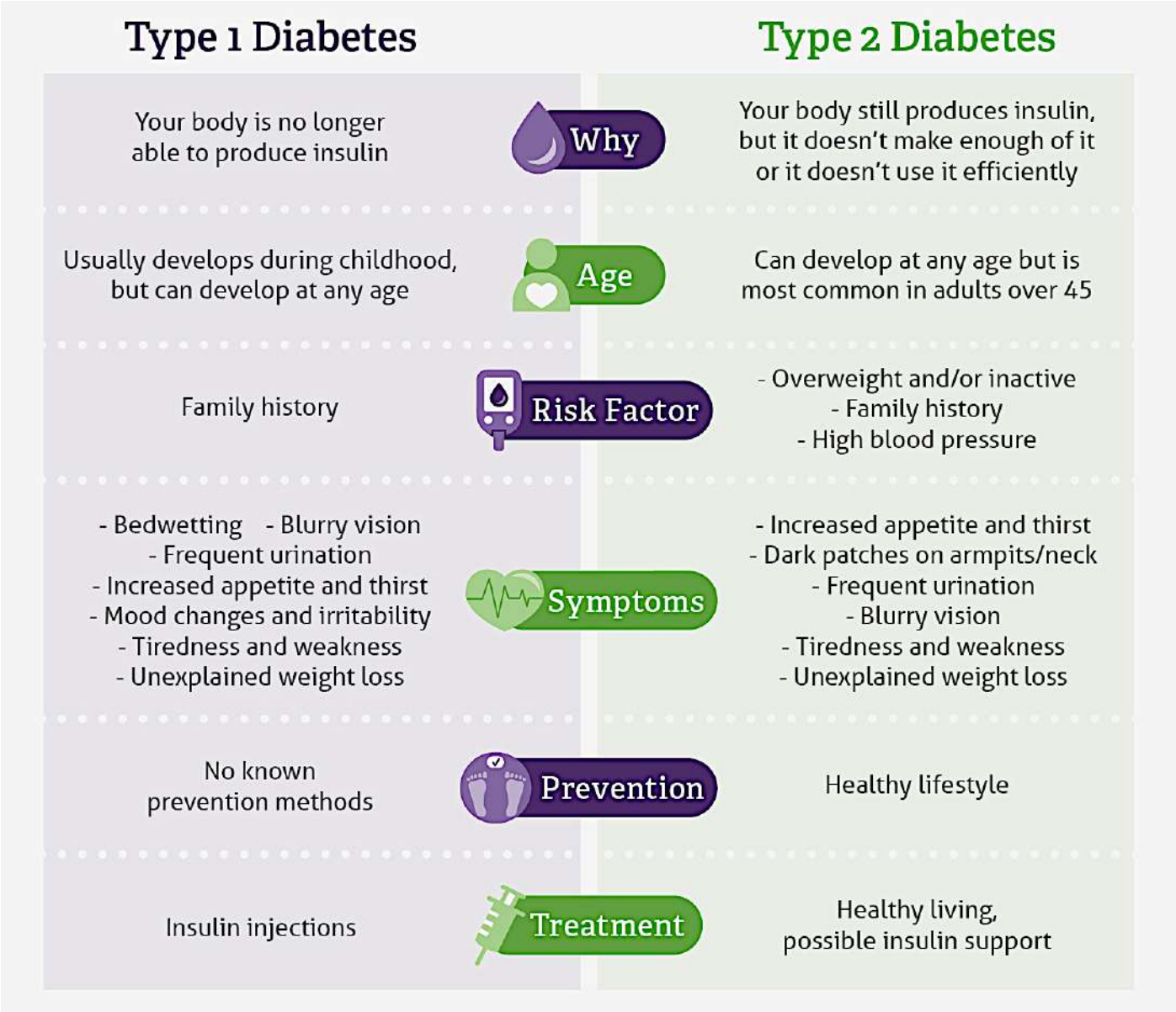
Blood glucose level is the concentration of glucose in the blood at a precise point in time. As this test only provides an indicator of blood glucose, increased levels of glucose are found in diabetes mellitus, hyperthyroidism, pancreatitis, and renal failure. Decreased levels are found in insulinoma, hypothyroidism, hypopituitarism, and liver disease. In contrast, the HbA1c test provides an overall marker of an individual's average blood glucose over the last 3 months. This provides a better reflection of how the levels of sugar in the blood are being controlled over time.

Type 1 Diabetes

The main difference between the two types of diabetes is that type 1 diabetes is a genetic disorder that often shows up early in life, and type 2 is largely diet-related and develops over time. If you have type 1 diabetes, your immune system is attacking and destroying the insulin-producing cells in your pancreas.

Type 2 Diabetes

With type 2 diabetes, your body still produces a small amount of insulin, but it isn't effective enough. The pancreas can't keep up with the high blood sugar levels resulting from poor diet and lack of exercise. Some people with type 2 diabetes have "insulin resistance," which means the pancreas produces insulin but the body does not recognize it (this is different than type 1, in which the insulin-producing cells are being attacked by the immune system).



Glucose Test

Procedure:

	Standard	Sample
Reagent	**1000 µL (1ml)**	**1000 µL (1ml)**
Standard	**10µL**	**-**
Sample	**-**	**10 µL**
Mix well & incubate at 37C for 5 - 10 min.		
Record the absorbance at 505 nm		

Calculation:

$$\frac{\text{Sample}}{\text{Standard (St.)}} * 100$$

(N: Fasting blood sugar :70-120)
(N: 2hr. post prandial BS: Less than 180)

Conversion factor:
mg/dl * 0.0555 = mmol/L

Oral Glucose Tolerance Test (OGTT)

An oral glucose tolerance test (OGTT) is performed to exclude/confirm the diagnosis of diabetes mellitus. In patients with characteristic symptoms of diabetes (e.g. weight loss, thirst, polyuria), a single random glucose concentration often confirms the diagnosis. For individuals not presenting with these classical symptoms, measurement of fasting plasma glucose concentration is essential. If the fasting glucose concentration is equivocal (suspected) , an oral glucose tolerance test may then be performed, to assess the ability of the individual to handle a glucose load or to measure how well the body can process معالجة a larger amount of sugar.

If the blood sugar measured in the test is above a certain level, this could be a sign that sugar is not being absorbed enough by the body's cells. Diabetes or gestational diabetes might be at the root of this problem. The **OGTT** is a very important method to diagnose type 2 diabetes mellitus. If the patient has normal glucose tolerance, blood glucose levels return to normal within 2–3 hours after ingesting the glucose. Since people with diabetes cannot respond to an increase in blood glucose via the release of enough insulin to cause storage, their levels of blood glucose rise above what is observed in normal individuals, and can stay elevated for a longer time.

However, there are some people who are not suitable candidates for OGTT, they include:

- ⇒ People who have a fasting blood glucose level exceed 126 mg/dL
- ⇒ People who already exhibit the symptoms of diabetes like polyuria, & polydipsia
- ⇒ People who have ketonuria (presence of ketones in urine)
- ⇒ OGTT is also not recommended for people who underwen a surgery recently, have had some trauma infection or even extreme psychological stress

Procedure:

1. Blood is taken to determine your baseline blood sugar level. (Fasting blood sugar FBS)
2. 75 g of glucose are dissolved in 250 to 300 ml of water. The amount given to children is based on their body weight.
3. Blood is drawn again for two hours (30 min, 60 min, 90 min, and 120 min)
4. Determine the concentration of glucose every 30 min.

Reagent	**1000 µL (1ml)**
Sample (Serum)	**10 µL**
Mix well & incubate at 37C for 5- 10 min. / Or at 20-25 C° (room temperature) for 15 min.	
Record the absorbance at 505 nm	

Calculation:

$$\frac{\text{(T) Sample}}{\text{St.}} * 100$$

The OGTT normal range :

⇒ **Fasting:**

100 – 125 mg/dL for prediabetes
126 mg/dL or greater for diabetes
greater than 92 mg/dL for gestational diabetes

⇒ **After 2 hour test results :**

140 – 199 mg/dL for pre diabetes

200 mg/dL or greater for diabetes

greater than 153 mg/dL for gestational diabetes

Lec. 9 / Lab. 9

كلية المأمون الجامعة / قسم تقنيات المختبرات الطبية
التقنيات المختبرية المتقدمة
المرحلة الثالثة / صباحي + مسائي

Cell and Tissue Culture

The culture of animal cells and tissues is a generally and widely used technique that involves isolation of cells, tissues and organs from animals and growing them in an in vitro or artificial environment. The term culture means to keep alive and grow in an appropriate medium.

The list of different cell types which can now be grown in culture include **connective tissues such as fibroblasts, skeletal, cardiac and smooth muscle, epithelial tissues, neural cells, endocrine cells and many different types of tumor cells. In vitro culture has been proven to be the most valuable method to study the functions and mechanism of operations of many cells.**

APPLICATIONS OF ANIMAL CELL CULTURE

1. Cancer research
2. Vaccine manufacturing
3. Recombinant protein production
4. Drug selection and development
5. Gene therapy
6. Stem cell biology
7. In vitro fertilization technology
8. Genetic engineering

9. Toxicity testing

10. Virology

LIST OF MAIN COMPONENTS OF FOR CELL CULTURE

1. Bio Safety Cabinets: It offers protection from contaminants during culture.
2. CO 2 Incubators: Cells are grown in an atmosphere of 5%-10% CO2. It keeps constant level of humidity
3. Microscopes: Inverted microscopes are used for this purpose.
4. Culture vessels: These consist of petri dishes, multi-well plates, microtiter plates, roller bottles, screw cap flasksT-25, T-75, T150.
5. Centrifuges: Cells are centrifuged at low temperature and low speed.
6. Freezer: For freezing and short-term storage
7. Hemocytometer: To determine the cell counts before or after culture.
8. Water bath with shaker: For cell dissociation and trypsinization
9. Liquid N2 Cylinder: For long-term cryopreservation
10. pH meter: To determine the pH of the medium.

Primary culture:

Freshly isolated cultures are known as primary cultures until they are passaged or subculture. The first step in obtaining the primary culture is isolation of tissues from the whole part or organ, followed by disaggregation of cells from the tissues. This is done by addition of trypsin to the tissue for disintegration and isolation of cells. The cells obtained after trypsin digestion are incubated in a medium.

Subculture culture: A subculture is a new culture taken from a primary culture and grown separately in the culture medium. Subculture allows the expansion of the culture (it is now known as a cell line).

Procdure:

https://www.youtube.com/watch?v=Qv-Lo2bMjQc

https://www.youtube.com/watch?v=pP0xERLUhyc

Lab. 11

كلية المأمون الجامعة / قسم تقنيات المختبرات الطبية
التقنيات المختبرية المتقدمة
المرحلة الثالثة / صباحي + مسائي

Urea & Creatinine

Urea

Procedure:

	Blank	Standard	Sample
Reagent 1 (R1)	1000 µL	1000 µL	1000 µL
standard	-	10	-
Sample	-	-	10
Mix & incubate for 5 min. at (20-25) °C OR 3 min. at 37°C			
Reagent 2 (R2)	1000 µL	1000 µL	1000 µL
Mix & incubate for 10 min. at (20-25) °C OR 5 min. at 37°C			
Measure the absorbance of the sample & the standard against of the reagent blank at 578 nm			

Calculation:

$$\text{Urea conc. (mg/dl)} = \frac{\text{sample} - \text{blank}}{\text{st.} - \text{blank}} \times 80$$

*** For higher value dilute sample with 1:1 with distilled water, repeat assay & multiple the results by 2.

Normal range: (10-50 mg/dl) OR (1.6 – 8.3 mmol/L)

Creatinine

Procedure:

	Standard	Sample
Working Reagent	1000 µL	1000 µL
standard	100 µL	-
Sample	-	100 µL
Mix & insert the cuvette in the spectrophotometer		
Record the absorbance at 500 nm after 30 sec. (A1), & after 90 sec. (A2)		

Calculation:

$$\text{Cr. conc. (mg/dl)} = \frac{\text{(A2-A1) sample}}{\text{(A2-A1) standard}} \times \text{C standard} \times \text{sample dilution factor} - \text{corrective factor}$$

$$\text{Cr. conc. (mg/dl)} = \frac{\text{(A2-A1) sample}}{\text{(A2-A1) standard}} \times 2 - 0.37$$

Normal range:

Men: (0.9 – 1.3 mg/dl = 80 - 115 µmol/L)

Women: (0.6 – 1.1 mg/dl = 53 - 97 µmol/L)