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Intended for: Food Science Students – 2nd Year Level

Microbiology

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INTRODUCTION

Introduction

Microbiology (from the Greek "mikros" = small, "bios" = life, "logia" = science) is the science that aims to study microorganisms (from the Greek: mikrós = small, organismós = organism) or microbes. The term microorganisms (or microbe) includes all living organisms invisible to the naked eye (dimensions less than 0.1 mm).

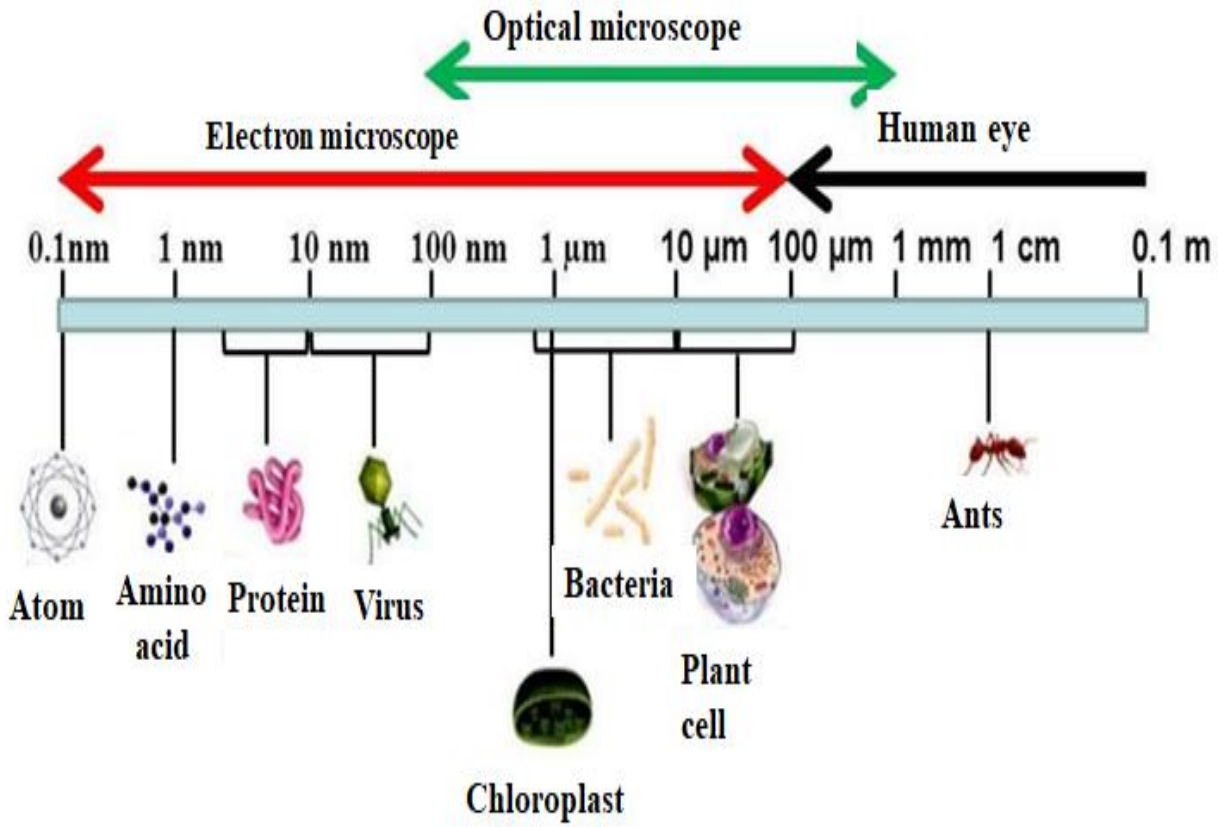


Figure 1: Resolution limits of optical and electron microscopes. Dimensions are indicated on a scale. To the right of the scale are the approximate sizes of cells, bacteria, viruses, molecules, and atoms (Madigan et al., 2015).

Microbiology is a branch of biology dedicated to the analysis of microorganisms, which encompass a wide range of living organisms such as bacteria, fungi, algae, protozoa, and viruses. Microorganisms are found in all ecosystems: they have been isolated from hot springs, the ocean floor, icy regions, and on and inside the human body, among other places. Microorganisms account for approximately 50% of the carbon and 70% of the biological nitrogen present on Earth.

Microorganisms are of paramount importance to the viability and functioning of the biosphere, participating in various biogeochemical cycles such as carbon and nitrogen. They thus constitute the main nutrient supply for all food chains and in each ecosystem.

Some microbial species carry out photosynthesis, which promotes the capture of carbon dioxide and the emission of oxygen into the atmosphere.

Microorganisms are also used in industrial and biotechnology sectors: in the manufacture of bread, cheese, enzymes, antibiotics, etc. However, there are species of microorganisms that are pathogenic and can cause diseases in animals and plants (Michael and Burton, 2011).Microbiology is divided into two main disciplines:

- Fundamental microbiology, which studies microorganisms by examining their morphology, structure, nutrition, etc.
- Applied microbiology, dedicated to the practical applications of microorganisms in various areas of life.

Since microbiology is primarily an experimental science, the use of practical exercises is one of the best teaching methods for acquiring the skills required in this field.

This microbiology practical work handout is intended for second-year food science students at Amar Telidji University in Laghouat. The objective is to create a bridge between the theoretical and practical aspects of the module and to help students acquire the skills necessary for experimental learning of microbiology.

Module Objectives

The student must acquire fundamental knowledge about the microbial world, as well as the techniques used to observe microorganisms, their growth, and bacterial classification.

Prerequisite Knowledge

The student must acquire fundamental knowledge about the microbial world, as well as the techniques used to observe microorganisms, their growth, and bacterial classification.

Evaluation methods

Evaluation method: Continuous assessment and semester exam.

Practical session n° 01:

Introduction to the Microbiology Laboratory

I.1 Objectives

The primary goals of this hands-on session are to give students a basic comprehension of biological risk and to familiarise them with the microbiology laboratory setting, its apparatus, and working protocols. In particular, the students will:

1. Understand the fundamental concepts of biological risk, particularly those related to the handling of microbial strains.
2. Become familiar with the microbiology laboratory, its equipment, and how it operates.

I.2 Overview of the Microbiology Laboratory

The microbiology laboratory must consist of three main parts:

1. The laboratory itself, where the analyses are performed
2. The culture media preparation room
3. The laundry room, which processes the products and materials used for the analyses and provides clean and sterile glassware and equipment.

The analysis laboratory must:

- Have sufficient space with limited traffic.
- Smooth but non-slip, non-absorbent walls, ceilings, and floors that are easy to clean and disinfect.
- Be well lit and protected from drafts.
- Equipped with microbiological safety hoods.
- Have cabinets for storing glassware, disposable equipment, dehydrated media, and certain chemicals.
- Have a refrigerated cabinet for storing ready-to-use media and bacterial strains.

I.3 Safety rules to apply before and after each practical work session

- Clean work surfaces with a suitable disinfectant before and after all handling.
- Wash your hands before and after all handling.
- Place only the necessary equipment in the work area.

- Place equipment in the work area in such a way that you are as comfortable as possible while handling it.
- Be familiar with all the steps before beginning handling.
- Work seated, without talking, and wear a buttoned blouse to avoid contaminating your clothing.
- Never eat or drink.
- Quickly clean and disinfect any spills in the work area.

I.4 Materials used in microbiology

I.4.1 Main Apparatus Used in Microbiology

I.4.1.1 Autoclave

Sterilisation of media, solutions and equipment before use and contaminated items.

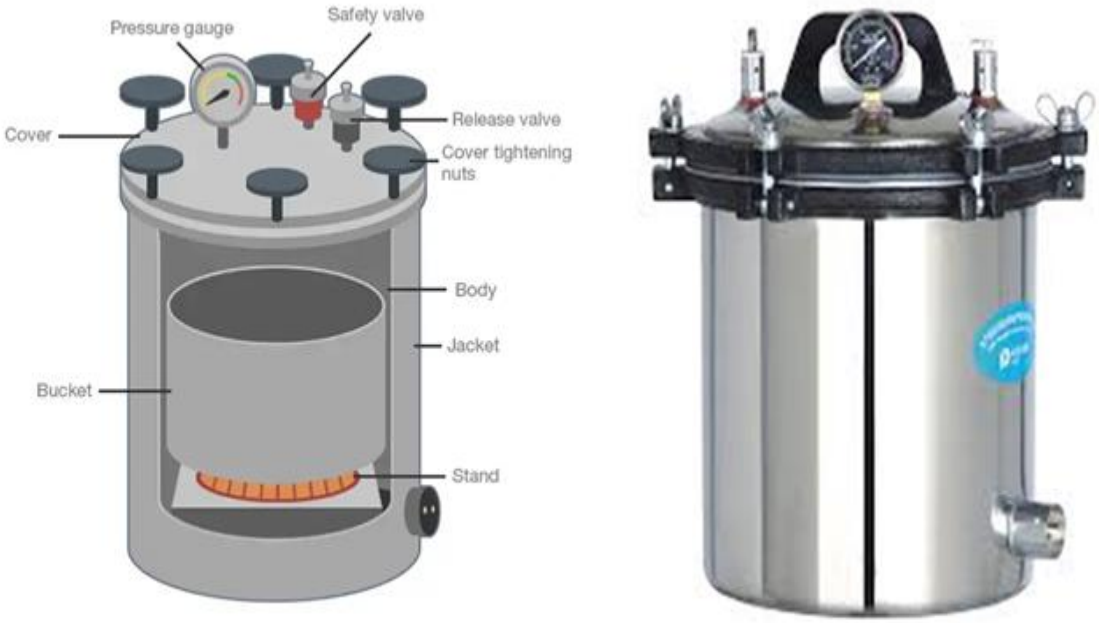


Figure 2: Schematic diagram of an autoclave.

I.4.1.2 Microscopy

To preserve the microscope's functionality and lifetime, proper handling and care are essential. Always use both hands to hold the microscope, one for the arm and the other for the

base. Examine the instrument thoroughly before each usage, noting any oddities or obvious damage. To prevent scratches or residue, keep the condenser lens, objectives, and oculars clean using only dry lens paper. Remove the slide from the stage, clean the oil-immersion objective, and place the low-power objective in the vertical position at the conclusion of each lab session. Lastly, to prevent dust and damage, put the microscope back in its box or designated locker and reinstall the dust cover, if one is available.

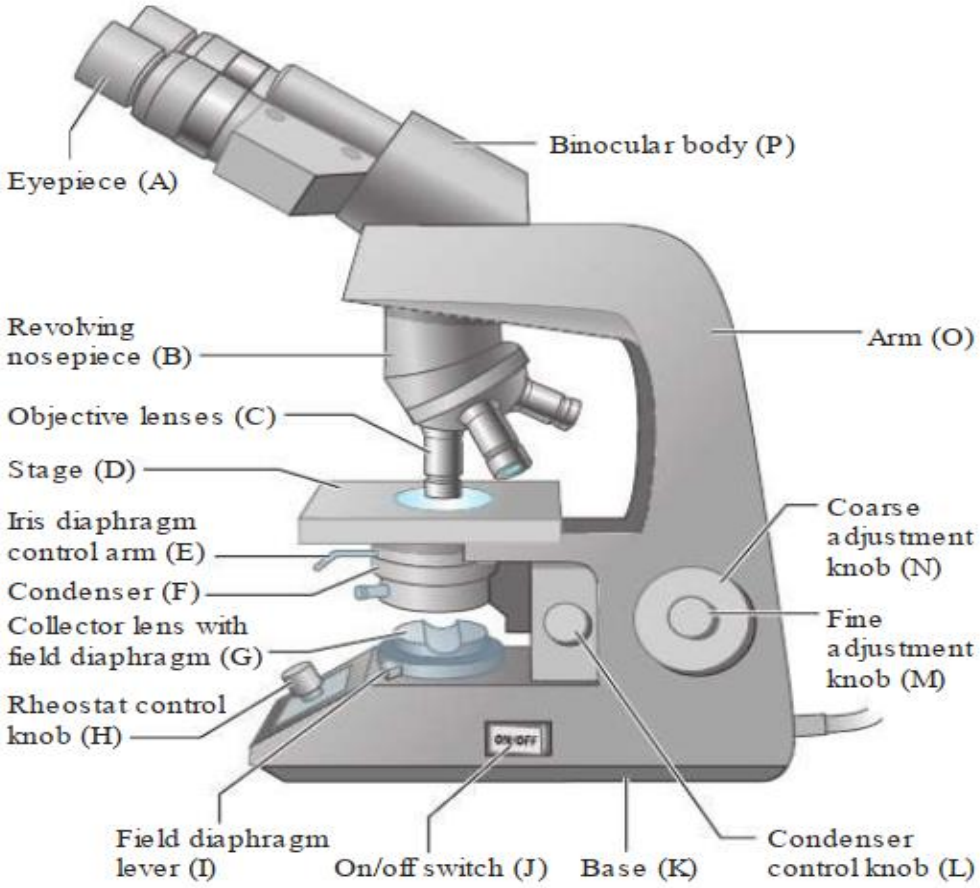


Figure 3: Descriptive diagram of a laboratory microscope.

I.4.1.3 Incubator

An incubator in microbiology is a laboratory apparatus used to promote the growth and multiplication of microorganisms (bacteria, yeasts, molds, etc.) under regulated conditions of temperature, humidity and sometimes a gaseous environment (such as CO_2 for particular cultures).

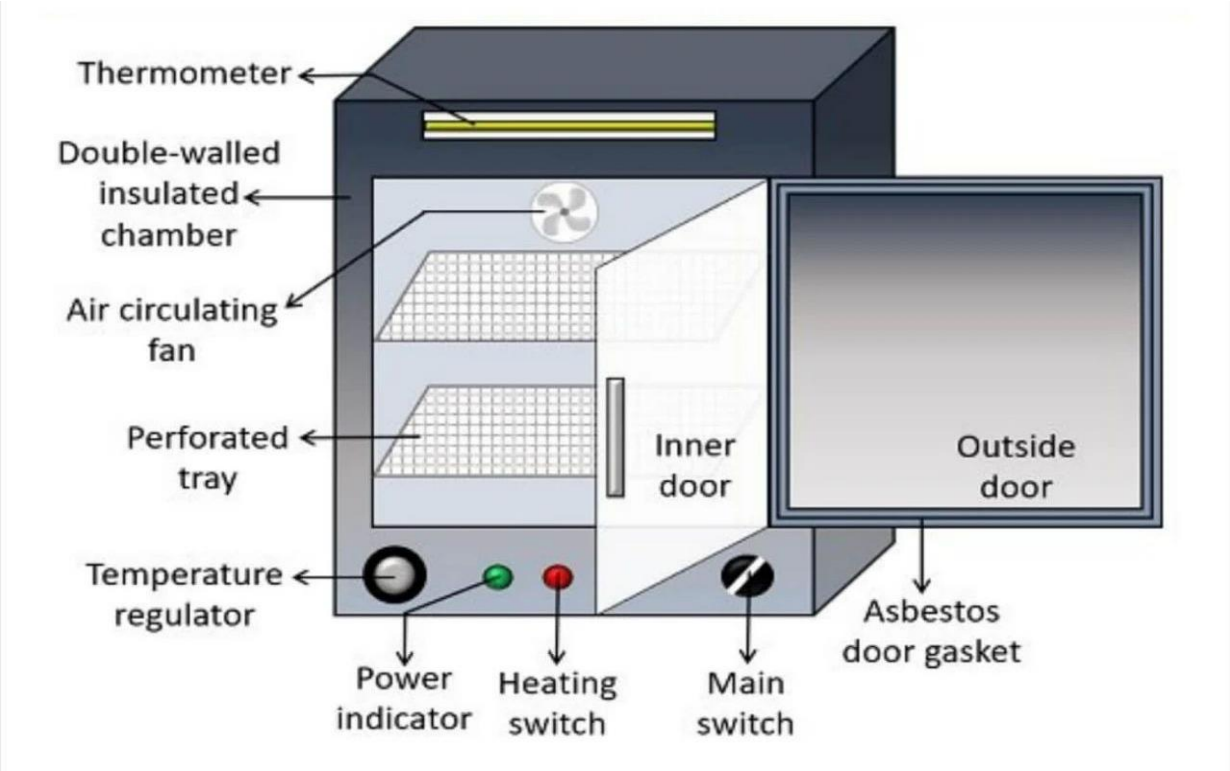


Figure 4: Laboratory Incubator for Microbial Growth.

I.4.1.4 Laminar Air Flow/ Laminar Hood

Laminar Flow Hoods are lab equipment that continuously circulates HEPA-filtered air to create a sterile, particle-free work environment. When working with sterile materials, delicate samples, or conducting studies that require a clean, controlled atmosphere, they are indispensable. Therefore, activities that are extremely susceptible to contamination, such as plant tissue culture and genetic transformation experiments, where maintaining aseptic conditions is essential for effective results, are frequently carried out using a Laminar Flow Hood.



Figure 5: Laminar Air Flow Cabinet: A Sterile Environment for Laboratory Work.

I.4.1.5 Other Apparatus Used in Microbiology

In addition to the main apparatus used in microbiology, several other devices play an important role in laboratory work. These include the magnetic stirrer, vortex mixer, analytical balance, and water bath. Each of these instruments contributes to ensuring accuracy, homogeneity, and controlled conditions during experimental procedures. They are essential for preparing culture media, mixing samples, maintaining constant temperatures, and performing precise measurements, all of which are crucial for reliable microbiological analysis.

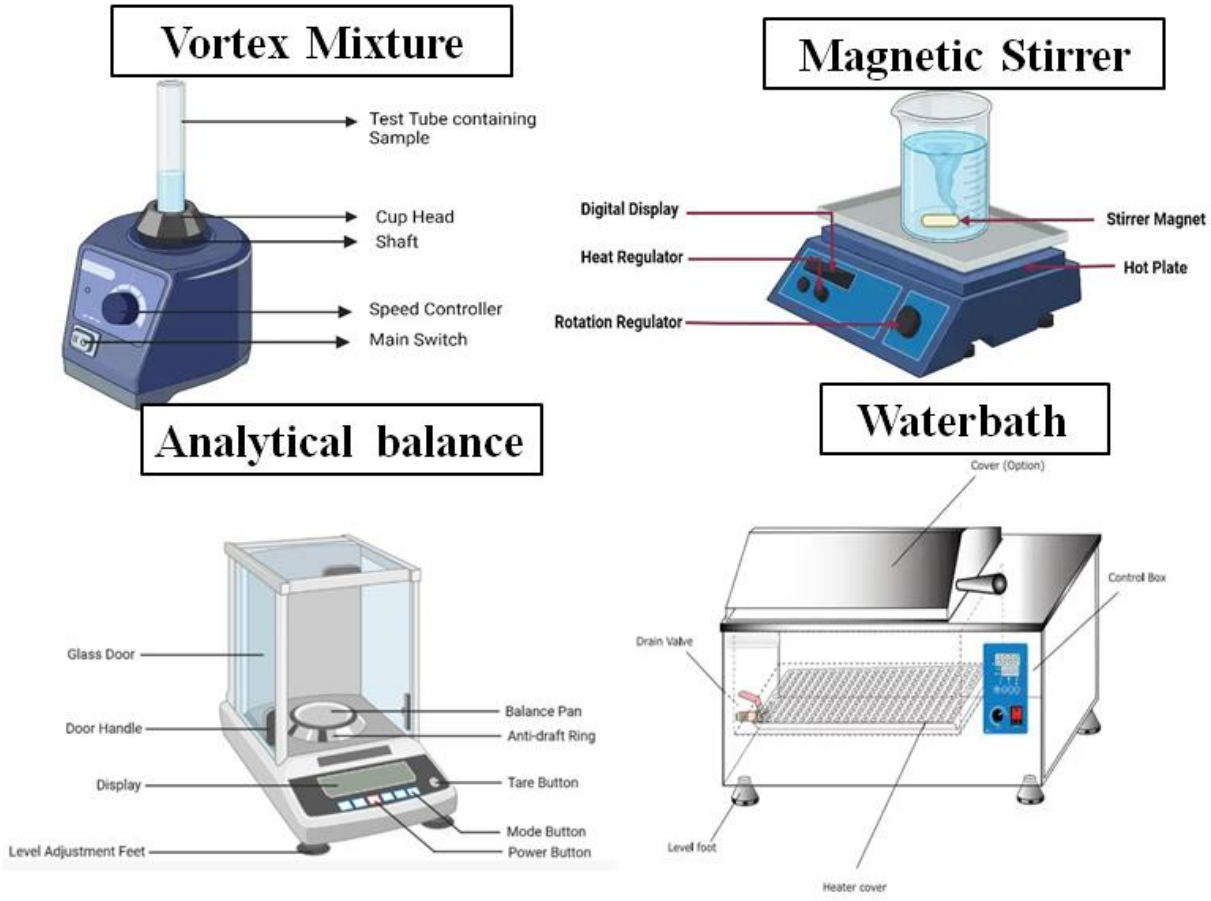


Figure 6: Other Apparatus used in Microbiology.

I.4.2 Basic Microbiological Laboratory Materials

- **Bunsen burner :**In microbiology, the Bunsen burner is a heating tool that creates a controllable flame. In order to avoid contamination while handling, it is used to heat materials, sanitize equipment, and create a sterile area surrounding the flame.
- **Petri dish:**In microbiology, a Petri dish is a flat, round glass or plastic container used to grow, separate, and study microorganisms on an agar media.Under sterile conditions, it permits the growth of bacteria, yeasts, or fungi.The most typical measurements are roughly 15 mm in height and 90 mm in diameter.
- **The inoculation loop:**a wire (platinum, tungsten, or nickel-chromium) mounted on a wire holder. This loop is used for sampling, inoculation, and subculturing. It is sterilized in the blue flame of a Bünsen burner before and after each use.

- **Sterile Pasteur pipettes** with a long tapered end are most often used for the sterile transport of liquid products. They are not graduated and are purchased ready-to-use. They are to be used with a suction bulb. Pasteur pipettes must be sterilized before and after each sample collection.
- **Test tubes:** Glass tubes with metal stoppers. These tubes can be capped with carded cotton (hydrophobic).

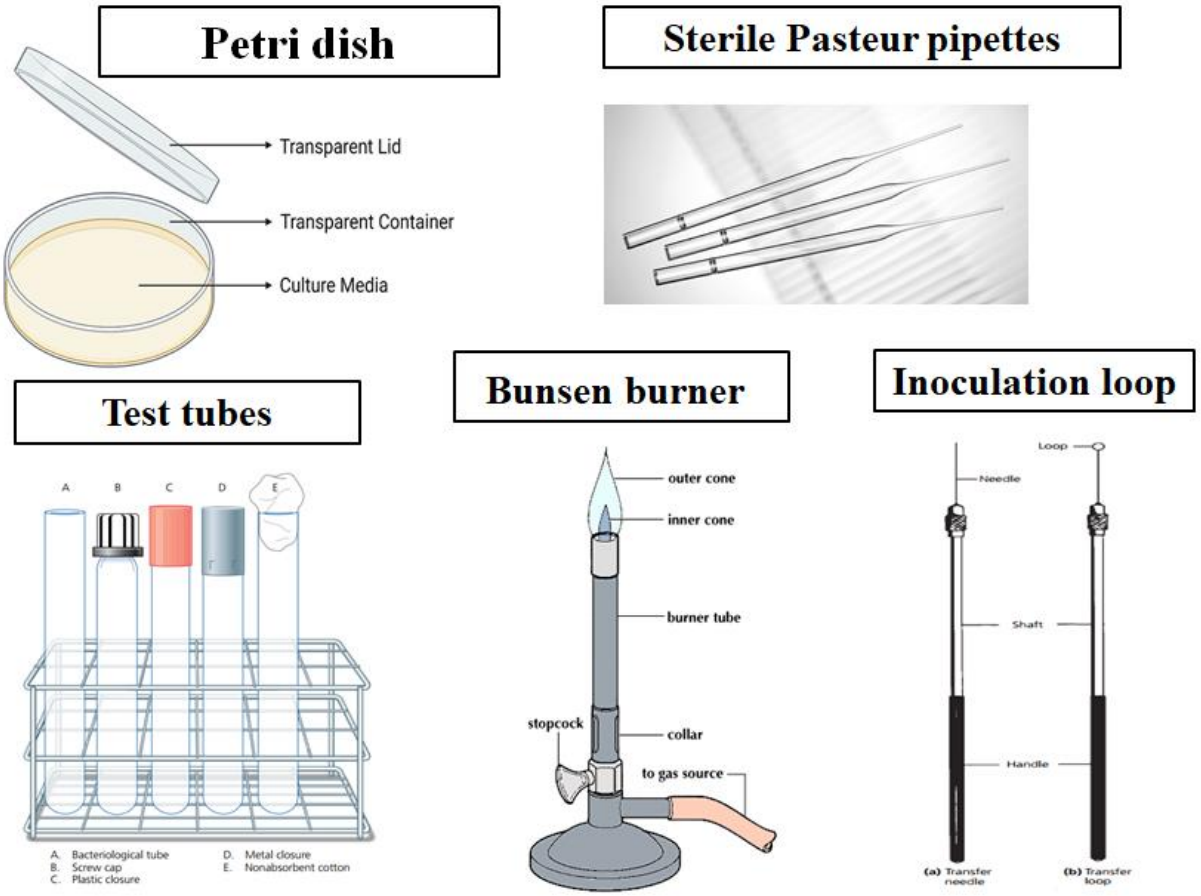


Figure 7: Basic Microbiological Laboratory Materials.

Practical session n° 2:

Method of studying microorganisms and the different sterilization processes

II.1 Objectives

1. Learn about workstation organization, aseptic handling, and sterilizing methods.

II.2 Sterilization

This is the process by which all living cells, viable spores, and viruses are destroyed or eliminated from an object, inert environment, or a location, in a lasting manner. A sterile object is completely free of microorganisms and spores; the chemical or physical agent that enables sterilization is called a sterilizing agent. In microbiology, the goal of sterilization is, on the one hand, to control microorganisms introduced into the study environment, and, on the other, to prevent contamination of the external environment and people.

II.3 Disinfection

Is the process of destroying, inhibiting, or getting rid of potentially dangerous microbes. Chemical compounds are typically applied to things as disinfectants. Spores and other germs may still be present after employing a disinfectant, therefore microorganisms are not always totally eradicated.

II.4 Asepsis and Antisepsis

The goal of asepsis and antisepsis is to stop microorganisms from contaminating a space or piece of equipment by using preventive measures like sterilizing or wearing protective gear.Contrarily, antisepsis involves utilizing antiseptic products to eradicate bacteria that are present on live tissue in order to stop infections.

Both chemical and physical sterilizing techniques are based on these two complementary ideas.

II.5 The Different Methods of Sterilization

II.5.1 Sterilization by physical methods

Table 1: Physical Methods of Sterilization.

	Methods	Effectiveness	Applications
Radiation	Ultraviolet rays	Do not penetrate deeply into objects.	Air and work surfaces, laboratories, inoculation rooms, operating rooms, etc.
	X-rays and gamma (γ) rays	Penetrate deeply into objects.	Pharmaceutical and food products, plastics, bandages, Petri dishes, and the canning industry.
Filtration (through porous substances = filters; pore size: 0.22 µm)		Does not retain viruses.	Solutions of heat-sensitive (thermolabile) substances such as certain culture media, amino acids, vitamins, hormones, antibiotics, etc.
Dry Heat	Flaming with a Bunsen burner (gas flame)	Direct contact with flame.	Used to sterilize metallic, stainless steel, or glass materials such as forceps, Pasteur pipettes, inoculating loops, etc.
	Hot air (Pasteur oven)	Temperature of 180 °C for 1 hour destroys both vegetative forms and spores.	Used for metallic, stainless steel, porcelain, or glass materials (e.g., Pasteur pipettes). Not suitable for culture media or plastic and rubber objects.
Moist Heat	Boiling (steam treatment): 100 °C for 30 minutes	Destroys most bacteria, fungi, and viruses, but not all; ineffective against endospores.	Thermoresistant medical or industrial equipment, needles, syringes, forceps, concentrated sugar solutions, gelatin.
	Pasteurization: 90 °C for 30 seconds	Destroys all pathogens but not necessarily all bacteria; does not destroy endospores.	Protects food products (milk, juice, etc.).
	Tyndallization: three consecutive treatments at 60–90 °C for 1 hour at 24-hour intervals	Vegetative forms are destroyed on the first day; endospores that germinate at room temperature between treatments are destroyed during subsequent heating.	Used for non-autoclavable media (relatively heat-sensitive and non-filterable substances): vaccines, sera, egg yolk emulsions, etc.
	Autoclaving: 120 °C for 20 minutes	Destroys all vegetative cells and endospores.	Culture media, metallic, stainless steel, rubber, or glass materials (forceps, Pasteur pipettes, etc.).

II.5.2 Sterilization by Chemical Methods

Table 2: Chemical Methods of Sterilization.

Disinfectant	Examples	Mode of Action	Advantages	Disadvantages	Application
Phenolic Compounds	Phenol	Protein denaturation and membrane alteration	Active long after application	Unpleasant odor	General antiseptic and disinfectant
Alcohols	Ethanol, Isopropanol 70-80%	Protein denaturation	Widely used	Can dry skin	Disinfection and antisepsis
Halogens	Iodophores (e.g., Betadine, Providine)	Oxidation and disturbance of cellular components	Water-soluble, non-staining, relatively stable	Burns and skin irritation	Preoperative antisepsis, hospital and laboratory disinfection
Chlorine	Gas (Cl_2), Calcium hypochlorite, Sodium hypochlorite	Oxidation of cellular constituents	Highly effective	Irritant, corrosive	Water treatment, premises disinfection
Hydrogen Peroxide (H_2O_2)	Liquid	Oxidizing agent	-	Irritant at high concentration	General disinfection
Quaternary Ammoniums	Soaps, Detergents (cationic and anionic)	Disruption of plasma membrane	Stable, colorless, odorless	-	Local therapeutic use, wetting agents, enhance other disinfectants' action
Sterilizing Gases	Formaldehyde vapors, Ethylene oxide	Protein denaturation, strong bactericidal effect	Suitable for heat-sensitive objects	Highly toxic	Sterilization of thermolabile products or objects, premises disinfection

II.6 Pictograms indicating chemical and biological hazards

In microbiology, operators must use chemicals to prepare culture media or reagents, or for cleaning and disinfection. It is essential to understand the hazards posed by some of these products by reading the warnings on the labels.

Table 3: Chemical and Biological Hazard Symbols.

Symbol	Meaning	Comments
	**Flammable Product, F**	This symbol indicates flammable products, which should be kept away from flames or heat sources. (F = easily flammable, F+ = extremely flammable)
	**Corrosive Product, C**	This symbol indicates corrosive products that can attack biological tissues as well as materials.
	**Explosive Product, E**	This symbol indicates products capable of exploding upon shock or when exposed to a heat source.
	**Environmentally Hazardous Product, N**	This symbol indicates products harmful to the environment, which should be collected after use for proper disposal (e.g., organic solvents).
	**Oxidizing Product, O**	This symbol indicates oxidizing products that facilitate combustion and should be kept away from flames or heat sources.
	**Toxic Product, T**	This symbol indicates toxic products that can cause death even in small doses and must be handled with proper protective measures.
	**Biological Risk Pictogram**	Warns of the presence of infectious or potentially infectious biological material (blood, animals, pathogenic microorganisms, etc.)

II.7 Organization of the bench in the microbiology laboratory

The layout of a microbiological bench is intended to guarantee sanitary, organized, and secure work. The Bunsen burner is positioned 20 cm from the edge in the middle. The instruments, which include forceps, platinum loops, and Pasteur pipettes, are used with the right hand on the right, while the samples and culture media are handled with the left hand on the left. Equipment that cannot be flame-sterilized is decontaminated using a wash bottle

filled with a chemical disinfectant behind the Bunsen burner. This setup reduces the possibility of contamination and makes handling easier.

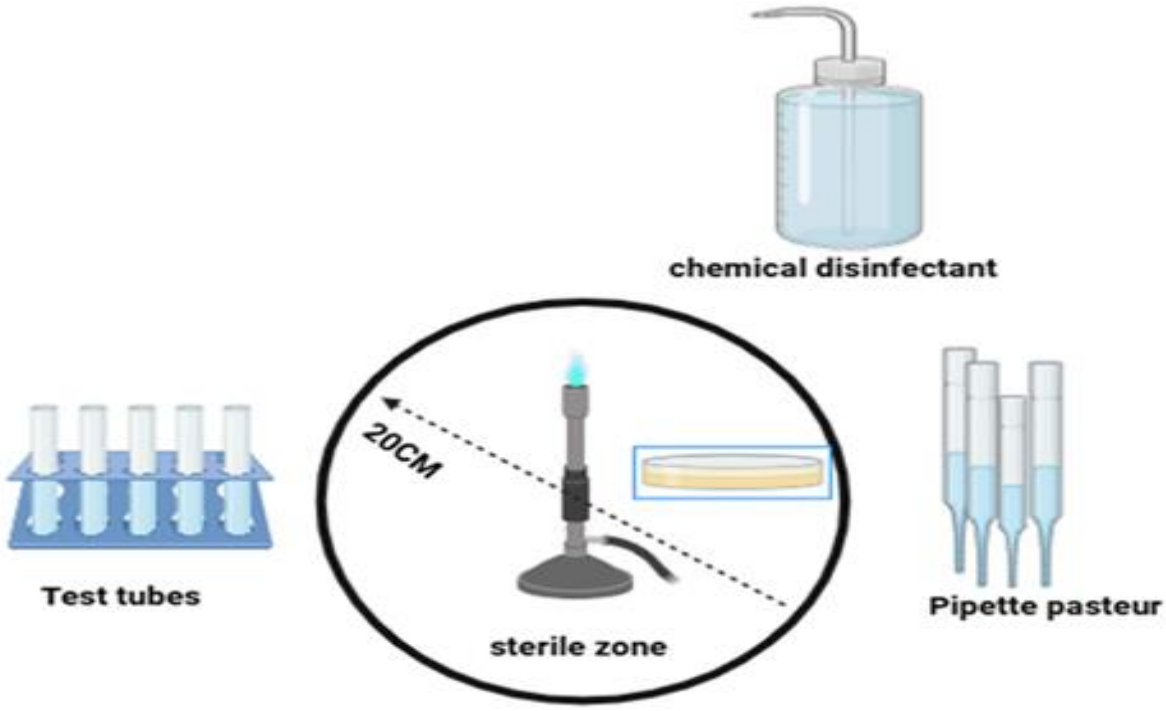


Figure 8: Organization of the bench in the microbiology laboratory.

II.8 Definition of Terms Frequently Used in Microbiology

- **Germs;**
- **Microorganisms:** Microscopic living organisms such as bacteria, fungi, viruses, and protozoa.
- **Colony:** A cluster, visible to the naked eye, composed of billions of descendants from a single bacterial cell.
- **Strain:** A group of microorganisms with the same origin, derived from a single microorganism and maintained as a pure culture.
- **Sterile Material:** Material completely free of microorganisms and spores.
- **Bacterial Culture:** Bacteria grown in a culture medium.
- **Inoculation:** Introduction (transfer) of microorganisms into a culture medium.
- ***In Vitro*:** Performed in the laboratory, outside the natural habitat.
- ***In Vivo*:** Occurring within the natural or living organism.

Practical session n° 03:

Preparation of Culture Media

III.1 Objectives

- Apply the rules and measures of aseptic technique in microbiology.
- Know the different culture media and the preparation of a culture medium.

III.2 Definitions

III.2.1 Culture medium

A culture medium is a preparation used for the development of microorganisms, packaged in a tube or Petri dish. It must therefore meet the microorganism's nutritional requirements, which includes: Covering the microorganism's needs for mineral ions (phosphate, iron, sodium, calcium, potassium, etc.), providing a source of energy, carbon, and nitrogen, as well as water, and possibly specific needs such as vitamins. Culture media are used to isolate bacteria from the environment that contains them, to identify them, to study their properties, and possibly to preserve them.A good culture medium must:

- Be sterile
- Be nutritious
- Have an optimal Ph

Culture media are used either: To isolate bacteria from the medium that contains them, to identify them, to study.

III.2.2 Agar

Agar is a sulfated polymer composed mainly of D-galactose, anhydrous 3,6-L-galactose, and D-glucuronic acid. It is extracted from red algae belonging to the *Rhodophyceae*.Agar's physical characteristics make it a great gelling (or solidifying) agent. It can be dissolved in boiling water (80 to 90°C), cooled to 40 to 45°C, and then solidified to form a stable gel. Because of this gel's limited remelting temperature of 80 to 90°C, agar is especially well-suited for microbiological culture media.Agar also has the advantage of not being hydrolyzed by most microorganisms, ensuring the stability of the medium.

- Thermal properties of agar:
  - Dissolves in hot water or melts at approximately 80–90°C.
  - Remains supercooled at around 45°C before gelling.
  - Becomes solid below 45°C, but is reversibly liquefiable by simple boiling.

III.2.3 Peptones

Peptones are obtained following the chemical (HCl) and/or enzymatic (proteases) hydrolysis of protein-rich biological materials (meat, plants, yeast, etc.). The composition of a peptone therefore depends on the starting protein material and the hydrolytic treatment.

Peptones differ greatly in their composition of amino acids and oligopeptides, nitrogenous bases, vitamins, mineral ions, and carbohydrates.

- **Peptones are of two types:** simple peptones resulting from the hydrolysis of a single protein substrate (peptic meat peptones, tryptic casein peptones, yeast peptones, etc.) and compound peptones resulting from the hydrolysis of several protein substrates or mixtures of simple peptones (polypeptones, tryptones, etc.).
- **Meat extracts** (or meat peptones): These are aqueous extracts of beef. Beef extract containsamino acids, peptides, nucleotides, organic acids, vitamins, and minerals.
- **Yeast extracts:** Yeast extract is an excellent source of vitamins (especially B vitamins) as well asnitrogenous and carbon compounds.

Biofluids: Some media are enriched with biological fluids: blood, cooked blood, serum, milk, etc.

III.3 Classification of culture media

III.3.1 Classification Based on Consistency

A culture medium can be in liquid, solid, or semi-solid form, with the addition of agar.

III.3.1.1 Liquid media

Liquid media do not contain any solidifying substance. The most commonly used liquid medium is broth. All the components required for cultivation are dissolved in water.Bacterial broth culture is carried out in test tubes or flasks. The disadvantage of this medium is that its morphology cannot be directly identified, but its advantage lies in its ability to provide the bacteria with better nutrients.

III.3.1.2 Solid media

Liquid media can be transformed into a solid medium by adding a solidifying agent. This agent must be non-toxic, non-metabolizable by microorganisms, and resistant to high sterilization and storage temperatures.Agar, extracted from red algae, is the most commonly used solidifying agent. It consists mainly of polygalactose and is generally added at a concentration of 15 to 20 g/L.Agar melts at temperatures above 80°C and solidifies when cooled below 45°C. Solid media are widely used to observe the macromorphological characteristics of bacterial colonies, as they allow species to be distinguished based on the appearance of their colonies.

III.3.1.3 Semi solid media

Semi solid media is a type of culture medium with a consistency somewhere between liquid and solid media.It contains a low concentration of gelling agent (usually 0.3 to 0.5% agar), giving it a soft and partially viscous texture. This type of medium does not allow for the formation of well-defined colonies, but it is particularly useful for studying bacterial motility, the growth of fastidious microorganisms, or for cultivation in microaerophilic conditions. It allows for the observation of the diffusion of bacteria or certain metabolites throughout the bulk of the medium.

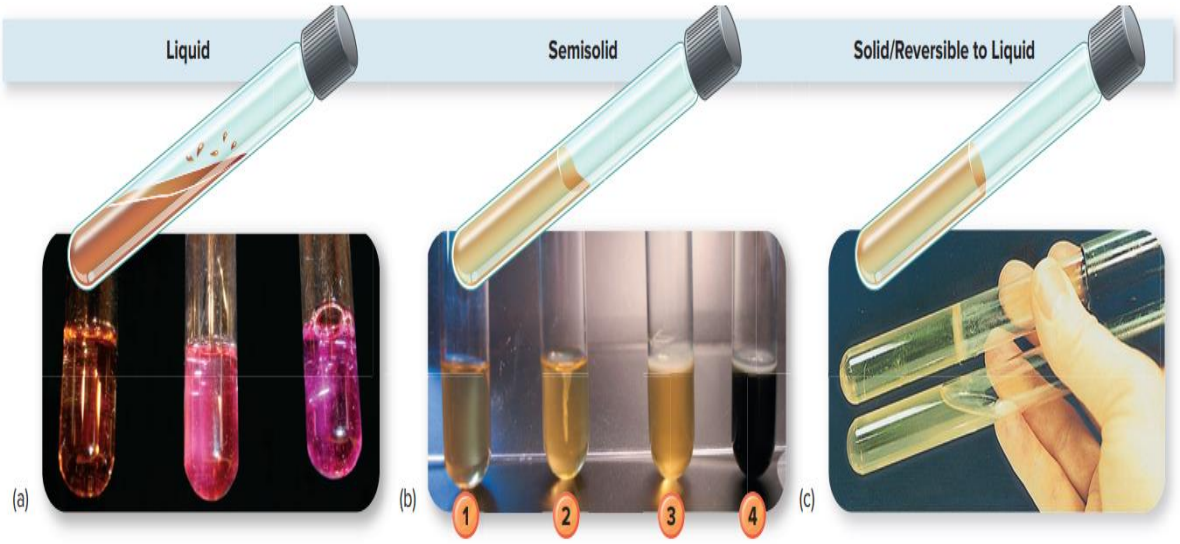


Figure 9: Culture Media Based on Physical Form.

III.3.2 Classification Based onmedia composition

III.3.2.1 Simple media

It's a general-purpose media that supports the growth of non-fastidious microbes, and it is primarily used for the isolation of microorganisms. Examples are nutrient broth, peptone water, and nutrient agar.

III.3.2.2 Complex media

Complex media consist of components whose exact chemical composition is not known, such as yeast extract, peptone, or spore extracts. These media are the most widely used because they meet the nutritional requirements of a large number of microorganisms. Examples include tryptic soy broth and blood agar. Complex media generally contain a wide variety of growth factors that support the cultivation of unknown bacterial species and fastidious microorganisms.

III.3.2.3 Defined Medium

In a defined medium, the chemical composition of the medium is known with certainty. This type of media usually consists of pure biochemical substances, and is often used to study the minimal nutritional requirements of a microorganism.

III.3.3 Classification of culture media based on application

III.3.3.1 Non-Selective or General-Purpose Media (Basic Media)

Media such as nutrient agar and nutrient broth are called general-purpose media because they allow the growth of many microorganisms, and most of them are complex media.

III.3.3.2 Enriched Media

Media that contain selective agents designed to enrich the medium with the desired organism. Blood and other special nutrients such as growth factors (amino acids and vitamins, etc.) can be added to general-purpose media to promote the growth of fastidious heterotrophs. (Examples include blood agar and Mueller Kaufmann medium, which is used to detect *salmonella.)*

III.3.3.3 Selective Media

Selective Media promote the growth of a single microbial species, which is being sought to the detriment of all others (by inhibiting the growth of other germs). These media contain selective agents called inhibitors, such as antibiotics, certain dyes like basic fuchsin, and bile salts, etc.Examples:

- **MacConkey medium** is a medium widely used for the detection of Gram-negative bacteria (Enterobacteriaceae) such as E. coli, and it contains bile salts that inhibit the growth of Gram-positive bacteria.
- **Sabouraud medium:** used to isolate fungi; it contains an antibiotic (chloramphenicol) as a selective agent to inhibit bacteria.
- **Chapman medium,** which allows the cultivation of Staphylococci, is selective due to its high NaCl concentration. The selection possibilities are endless, and there are dozens of special selective media in use.

III.3.3.4 Differential Medium

These media allow the differentiation of bacterial species of the same genus, as well as bacterial species of different genera growing in the same medium. These media are used to highlight one or more biochemical properties (metabolism, mainly the ability to degrade a substrate) in an isolated microorganism.Example: **Eosin Methylene Blue (EMB) agar** We can distinguish *E. coli* bacteria that produce a greenish glow (1) and *E. aerogens* bacteria which are pink (3) figure.

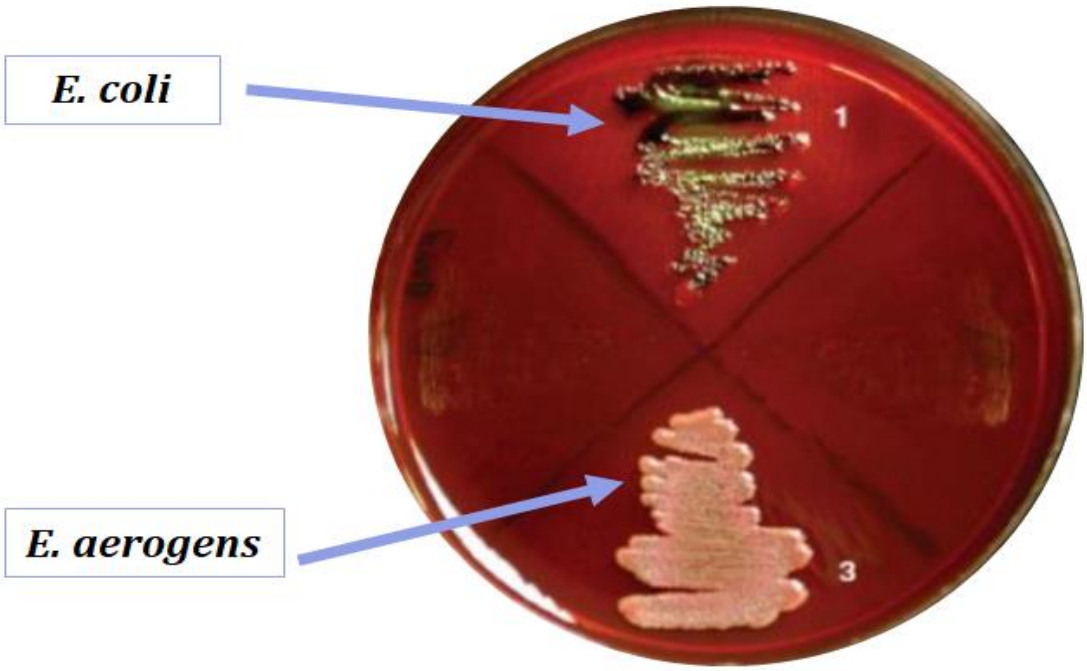


Figure 10: Differentiation of bacterial species on Eosin Methylene Blue (EMB) agar: Escherichia coli colonies showing a green metallic sheen (1) and Enterobacter aerogenes colonies appearing pink (3).

III.3.3.5 Storage media

The most commonly used culture medium is a streak-inoculated agar slant or a centrally inoculated nutrient agar pellet for storage at +4°C. For long-term storage, freezing should be carried out at the lowest temperature (often -80°C) using media containing polyalcohols (glycerol).

III.4 Steps for Preparing a Culture Medium

To prepare a culture medium, the required amount of powdered ingredients is first accurately weighed using a weighing balance and dissolved in an appropriate volume of distilled water. The pH of the solution is then adjusted if necessary using either NaOH or HCl. If a solid medium is desired, agar is added and the mixture is boiled until the agar is completely dissolved. The prepared medium is poured into clean flasks and sterilized by autoclaving at 121°C for 15 minutes under 15 psi pressure to eliminate any microbial contamination. After sterilization, the flasks are placed in a laminar airflow cabinet, which is previously disinfected with 70% alcohol. When the medium cools slightly, it is poured into

sterile Petri dishes and left to solidify. Once solidified, the medium is ready for inoculation using either a spreader or an inoculating loop, depending on whether the objective is identification or isolation of microorganisms. The Petri plates are then sealed with parafilm, properly labeled, and incubated in an inverted position at 37°C for 24 hours. After incubation, the plates are examined for visible colony formation, indicating successful microbial growth on the culture medium.

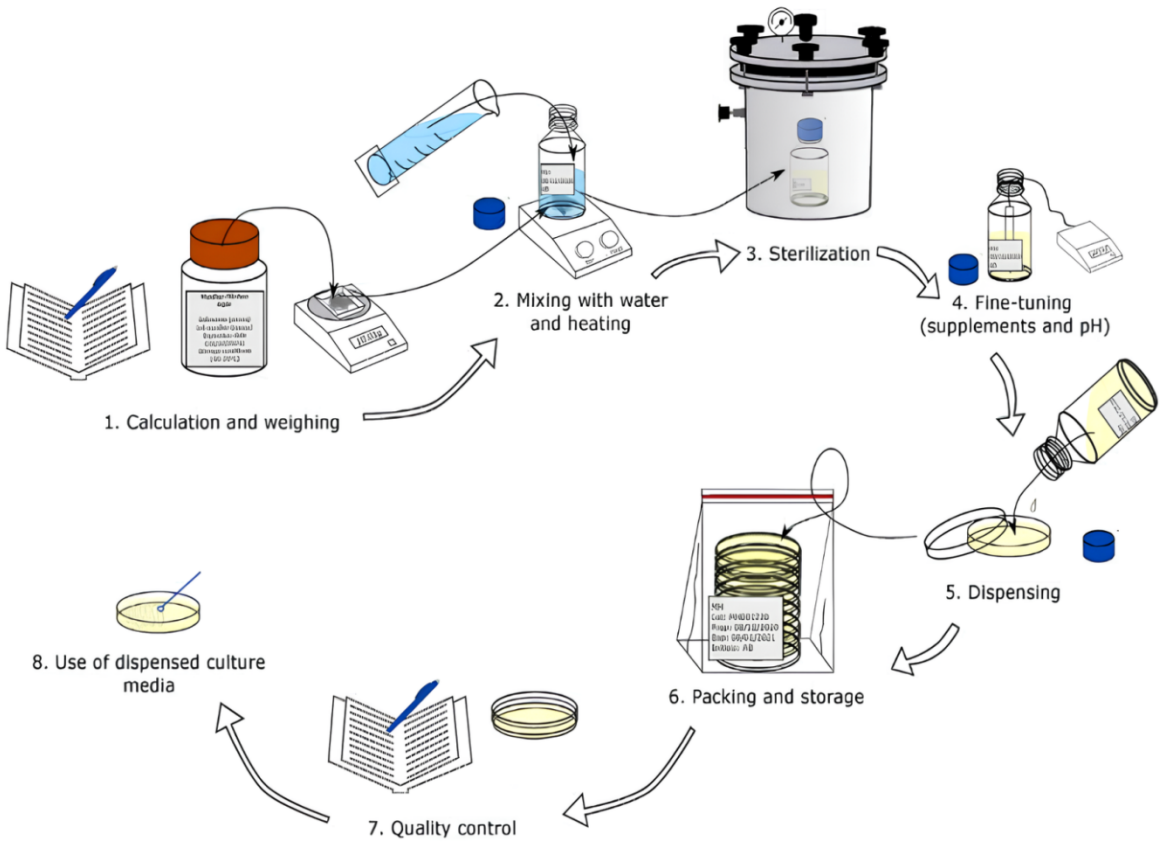


Figure 11: Steps for Preparing a Culture Medium (Kassamali F. et al. (2021).

Practical session n° 04:

Inoculation Methods

IV.1 Objectives

1. Apply the rules and measures of asepsis in microbiology.
2. Learn how to inoculate a broth or nutrient agar using several aseptic methods.

IV.2 Inoculation Method on Pre-poured Nutrient Agar Plates

- **First experiment:** Hand and hair contamination test

This experiment aims to identify the microbial flora present on hands and hair. A Petri dish containing pre-poured nutrient agar is divided into three sections by drawing three lines on the bottom (not the lid) using a permanent marker. A fingerprint is applied to the first section. The same process is repeated on the second section after washing the hands with bleach. Finally, a hair is placed on the third section using a pair of flambéed forceps.

- **Second Experiment:** Water and Surface Contamination Test

This experiment aims to study the presence of microorganisms in water and on work surfaces. A new Petri dish is divided into three sections. Using two sterile swabs, samples are taken from the tap water and the workbench surface, then inoculated onto the corresponding sections, using all available space. An object is placed on the third section, then removed using sterile forceps.

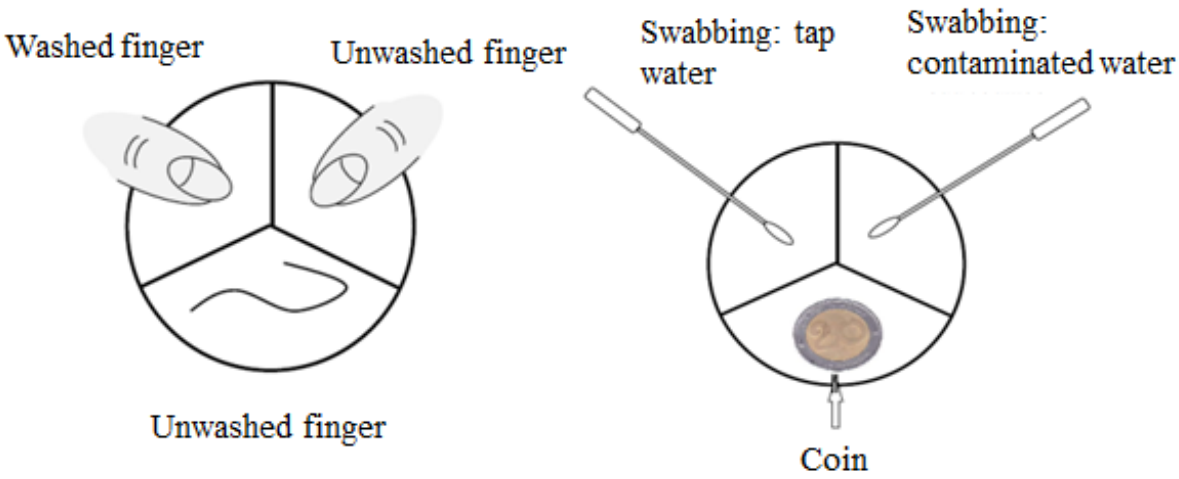


Figure 12. Illustration of Inoculation Methods According to Sample Sources.

IV.3 Mass and Surface Plating

The process begins by mixing the bacterial culture with the liquid agar medium. Once homogenized, the mixture is poured into sterilized Petri dishes and allowed to solidify.Plating can then be done in two ways:

1. Mass plating, where the culture is spread throughout the medium before solidifying.
2. Surface plating, where the culture is spread onto the agar using a spreader.

After incubation, colonies appear. They are then well isolated, easily identifiable, and countable.

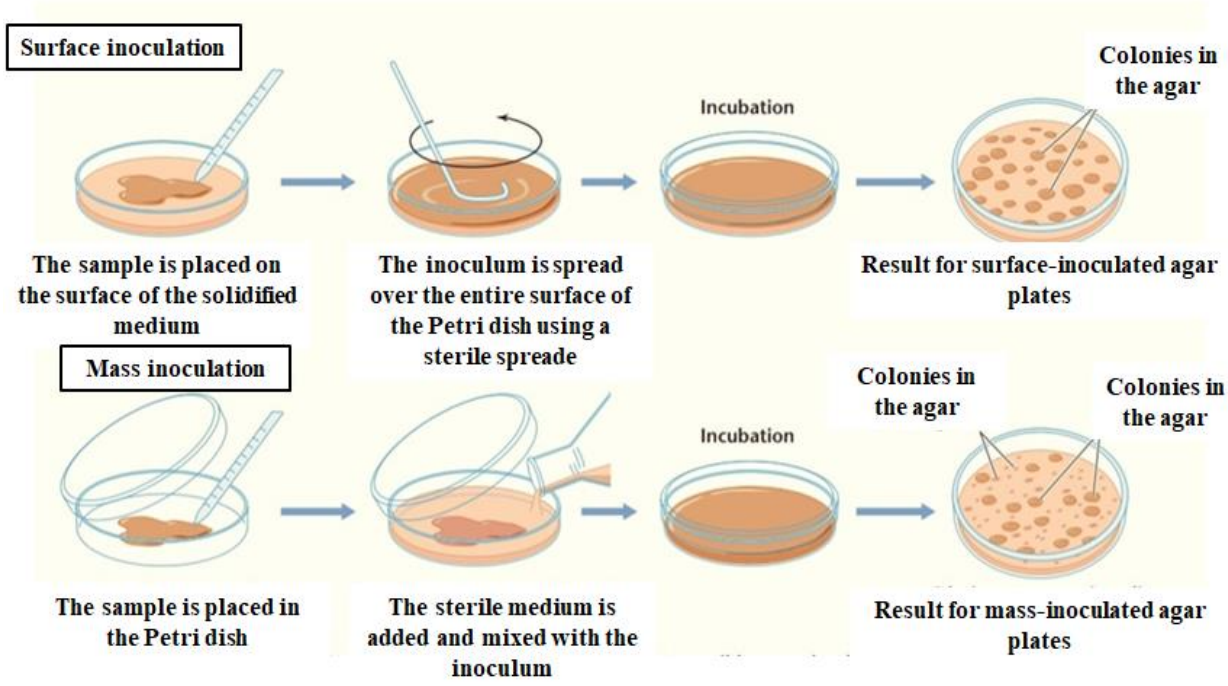


Figure 13: Illustration of Surface and Mass Inoculation Methods.

IV.4 Streak plating

Streak plating is a microbiological technique used to isolate bacteria as pure colonies on a solid medium (often agar). It involves gradually spreading a bacterial sample on the surface of the medium using a sterile loop to dilute the cells and obtain isolated colonies after incubation.

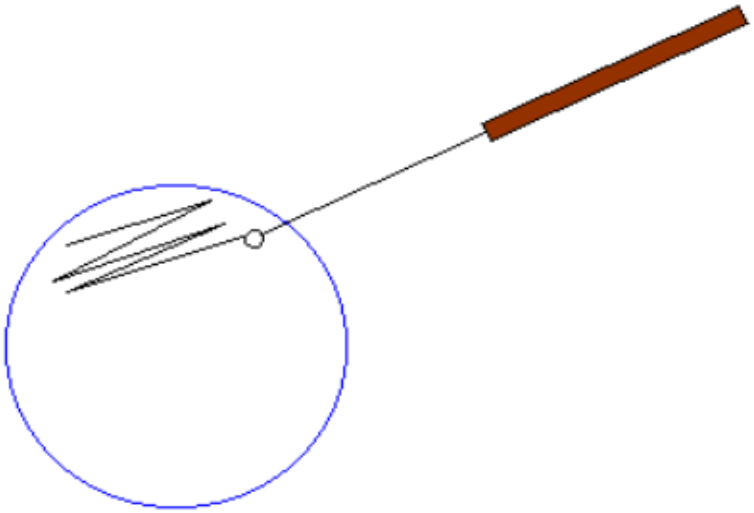


Figure 14: Streak plate method on agar for the isolation of bacterial colonies.

IV.5 Quadrant Streaking

This approach, which involves streaking four equal-sized portions of the agar plate, is the most popular and widely utilized. The "four-quadrant streak," "four sectors," or "fourway streak" methods are other names for it. Each plate is separated into four equal sectors using this procedure, and each neighboring sector is streaked.in order.

The first sector, also known as the first quadrant, is the one that is streaked initially and has the maximum inoculum concentration. The inoculum will gradually get diluted in the second, third, and fourth quadrants. After incubation, solitary colonies form because the inoculum is greatly diluted by the time the fourth quadrant is streaked.

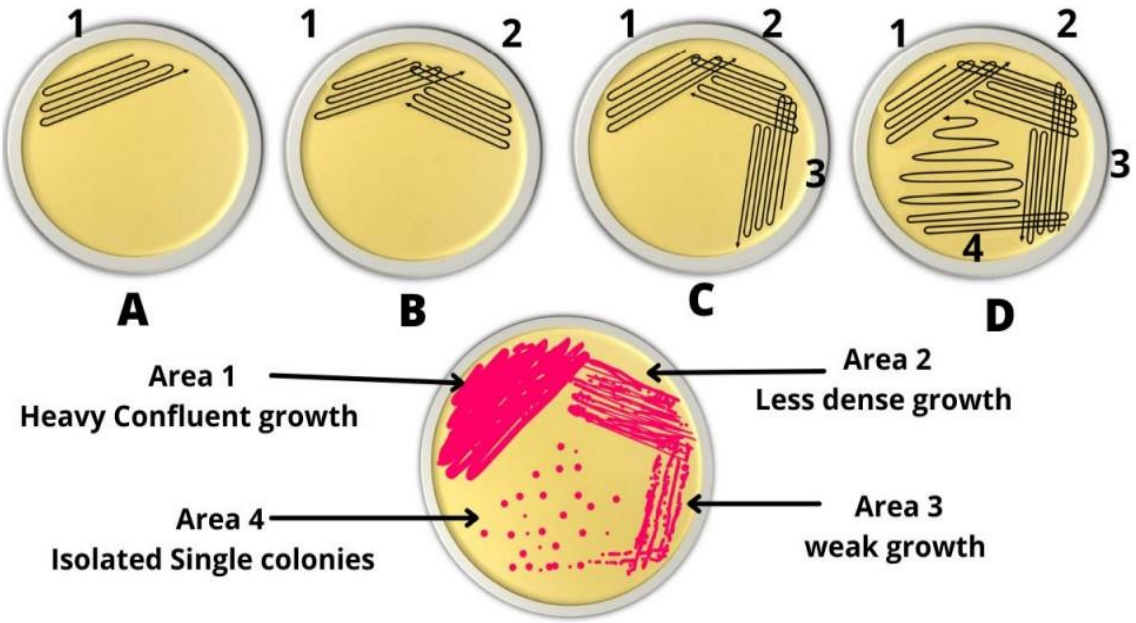


Figure 15. Quadrant Streak Inoculation Method.

IV.6 Tube inoculation method

IV.6.1 Methods of Inoculation on Solid Medium in Tubes

The medium is inoculated by a central puncture along the agar plate using a straight-wire platinum loop or a Pasteur pipette. This method allows the culture to be concentrated in the center of the tube and growth to be observed along the inoculation path.Agar SlantsIn this type of tube, inoculation is performed at two distinct locations:

- The base: This is inoculated by a central puncture in the lower part of the tube.
- The slope: This is inoculated by zigzag lines from bottom to top using a Pasteur pipette or a platinum loop.

This method allows growth to be studied simultaneously on the slanted surface and in the deep part of the medium.

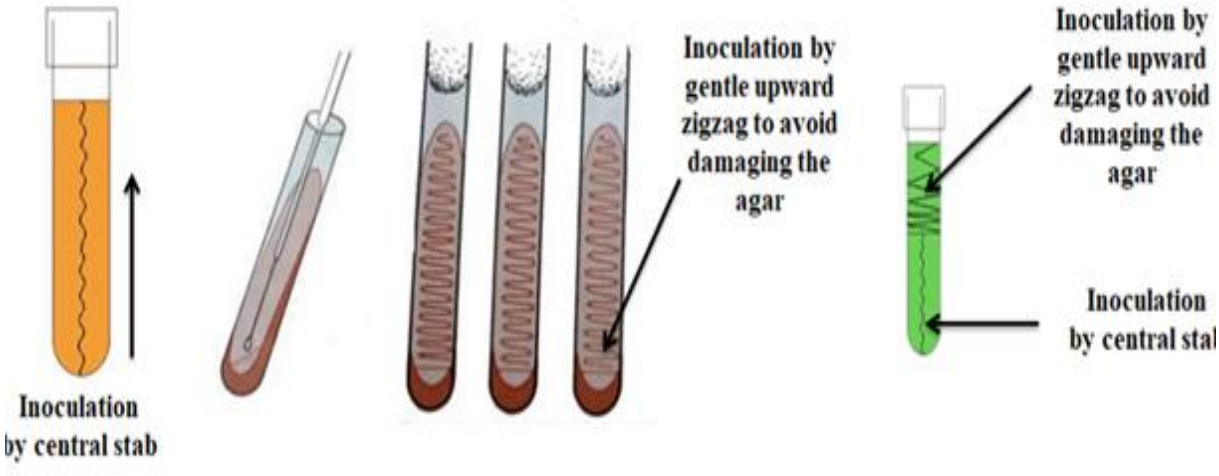


Figure 16: Tube inoculation method.

IV.6.2 Broth Inoculation Method

Bacteria can multiply quickly and uniformly when broth is used. But there are several disadvantages to this kind of media. Since the cells are in a liquid media and mix, it is in fact impossible to distinguish between various species of bacteria in a sample that contains numerous of them or if there is external contamination.However, the method for inoculating broth is still quite straightforward. It entails first collecting the strain to be injected and then sterilizing the tool used to collect the original bacterial strain (pipette for broth, loop for a colony). Before adding the sample to the broth, the sterile broth bottle's opening needs to be disinfected. Both the equipment and the bottle's aperture must be re-sterilized following inoculation.

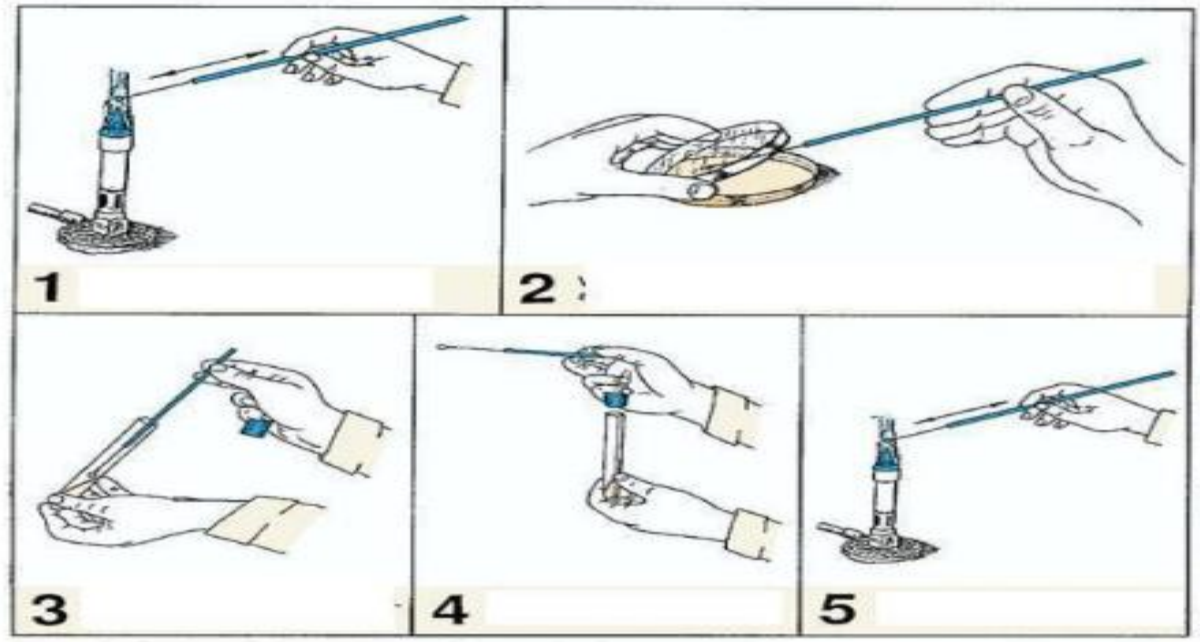


Figure 17: Broth Inoculation Procedure.

Practical session n° 05:

Isolation of the Total and Specific flora from certain products

V.1 Objectives

1. Properly carry out the preparation of a dilution series.
2. Isolate microorganisms from certain products (water, milk, etc.).

V.2 Inoculation from a Series of Decimal Dilutions (Milk)

V.2.1 Materials

- Fresh or treated milk sample
- Sterile test tubes or flasks
- Sterile dilution medium (e.g., 0.1% peptone or sterile saline)
- Sterile pipettes or micropipettes with sterile tips
- Sterile loops or inoculating needles for colony transfer
- Nutrient or selective agar plates depending on the target microorganisms
- Sterile spreader
- Vortex or shaker for homogenizing dilutions
- Incubator at the appropriate temperature (e.g., 30–37 °C)
- Autoclave or sterilization equipment

V.2.2 Detailed Protocol

- **Preparation of the stock solution:**

  Take a precise volume of milk (e.g., 10 mL).

  Add it to a tube or flask containing 90 mL of sterile dilution medium.

  Mix thoroughly using a vortex or by shaking to obtain a uniform suspension. This constitutes the stock solution.

- **Preparation of decimal dilutions:**

  Prepare a series of tubes, each containing 9 mL of sterile medium.

  Transfer 1 mL of the stock solution to the first tube to make the 10^{-1} dilution. Mix thoroughly.

  Transfer 1 mL from the first dilution to the second tube to make the 10^{-2} dilution. Repeat the process for further dilutions (10^{-3}, 10^{-4}, etc.) as needed.

- **Inoculation on agar plates:**

  Take a precise volume (e.g., 0.1 mL) from each dilution.

Inoculate the agar surface using a sterile spreader (surface streak method) or by mass inoculation as required.

Spread the inoculum carefully to obtain isolated colonies.

- **Incubation:**

  Incubate the plates at the appropriate temperature for the target microorganisms (e.g., 30–37 °C) for 24–48 hours

- **Observation and counting:**

After incubation, observe isolated colonies .

Count colonies on plates with a suitable number (30–300 colonies per plate recommended).

Calculate the microbial concentration in the stock solution and in the milk, taking the dilution factor into account.

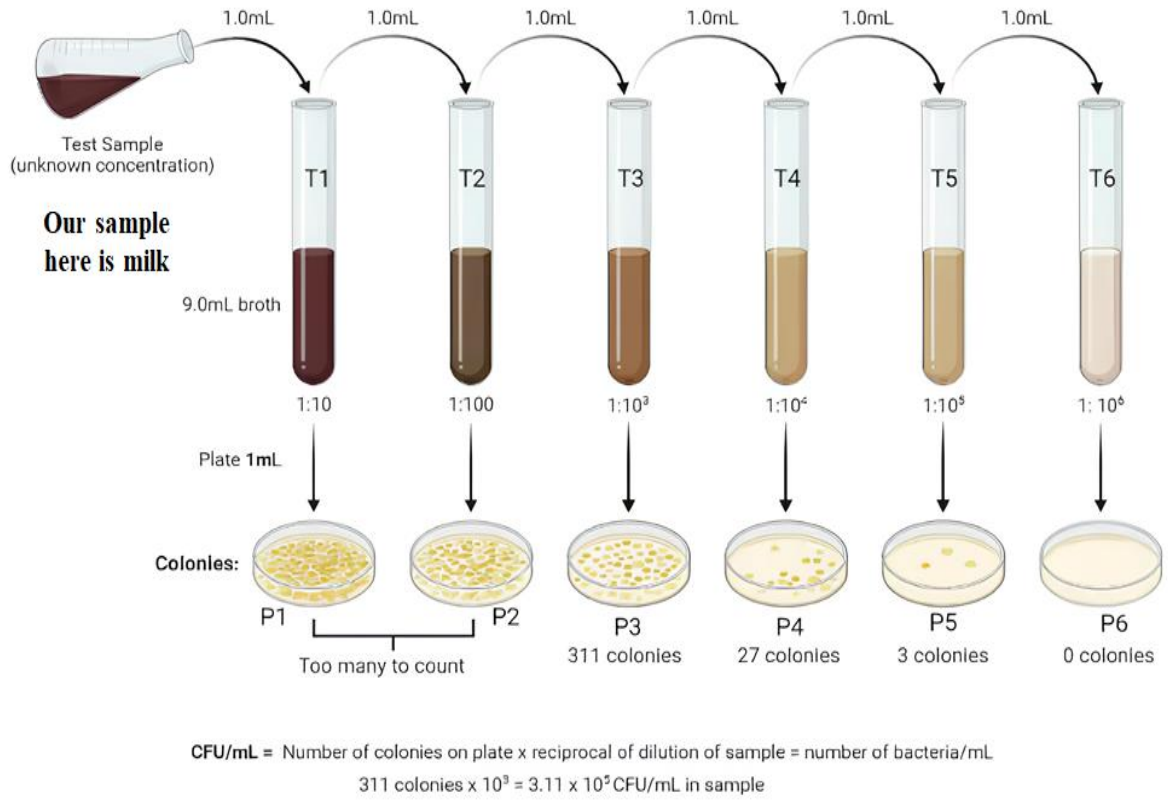


Figure 18: Isolation of Microorganisms from Milk Using Decimal Dilutions.

Practical Session n° 06:

Microscopic study of bacteria, simple staining

VI.1 Objectives

1. Learn and master the simple staining technique to visualize bacteria under a light microscope.
2. Observe and identify bacterial morphology (shape, size, and cell arrangement) from the stained preparation.

VI.2 Microscopic observations (Fresh state and mobility)

Examining microbiological morphology involves using a photonic microscope to determine the shape of the bacteria and how they gather together. These details can be provided by observing a colored frottis or by a straightforward preparation in the state of lame and lamelle, where the bacteria are then seen to be living. Microscopical examinations of samples can yield very useful information for microbiological identification, such as:

- Cell shape and grouping pattern;
- mobility (if present in bacteria).

VI.2.1 The method for preparing microscopy at the state of free (liquid and solid)

Bacteria are regarded as mobile when they exhibit highly varied trajectories (deplacement in all directions). Veiller should observe a significant portion of the lame, but not for more than three to ten minutes at a time.

Table 4: The method for preparing microscopy at the state of free (liquid and solid).

Liquid meduim	Solid meduim
- Place a drop of liquid culture in the center of a clean slide using a Pasteur pipette. - Cover the drop with a coverslip, avoiding air bubbles. (The culture must not overflow the coverslip edges; if it does, discard the slide in a disinfectant solution and start again). - Observe immediately under the microscope (objective ×10 then ×40), with maximum light and the diaphragm closed. - After observation, discard the wet mount in a container with a broad-spectrum disinfectant, as the bacteria are alive.	- Place a small drop of sterile water in the center of a clean slide, then collect a small portion of the colony from the agar using a platinum loop, preferably from the edges, and mix it in the drop of sterile water placed beforehand. - Make a homogeneous suspension in the drop by gently mixing the inoculum (to avoid breaking the flagella). - The remaining steps are the same as those used for the liquid medium.

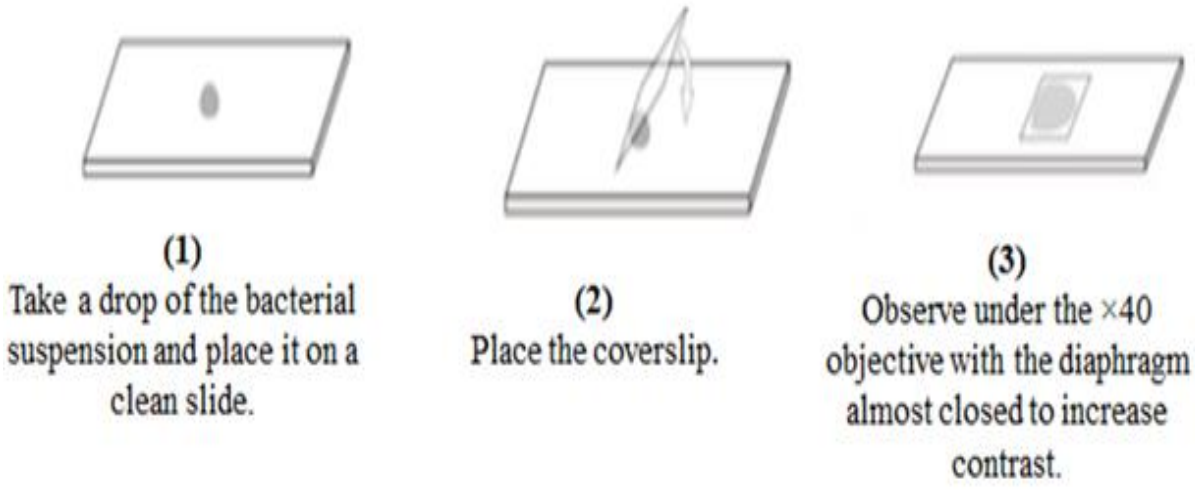


Figure 19: Technique of microscopic preparation in a fresh state.

VI.2.2 Stained smear technique

Smear staining can be either simple or differential. Before any staining, a smear must be prepared — that is, a thin, preferably single-layer spread of bacteria on a slide. The smear technique is simple:

- Place a drop of sterile water on a clean slide if the culture sample is solid, then use a platinum loop to remove a portion of the culture. Mix to obtain a homogeneous suspension. Alternatively, place a drop of incubated liquid medium or a drop of suspension.
- Take the smear with a ground slide (Figure 1) to obtain a thin, homogeneous spread over at least two-thirds of the slide.
- Reduce the burner flame to a minimum.
- Dry and fix the smear over the Bunsen burner flame without overheating it.
- The safest technique is to pass the smear through the flame 3 or 4 times for half a second, with the bacteria-free side facing the flame.
- Once the smear is fixed, the slide no longer presents any risk of contamination.
- The desired coloring can then be carried out

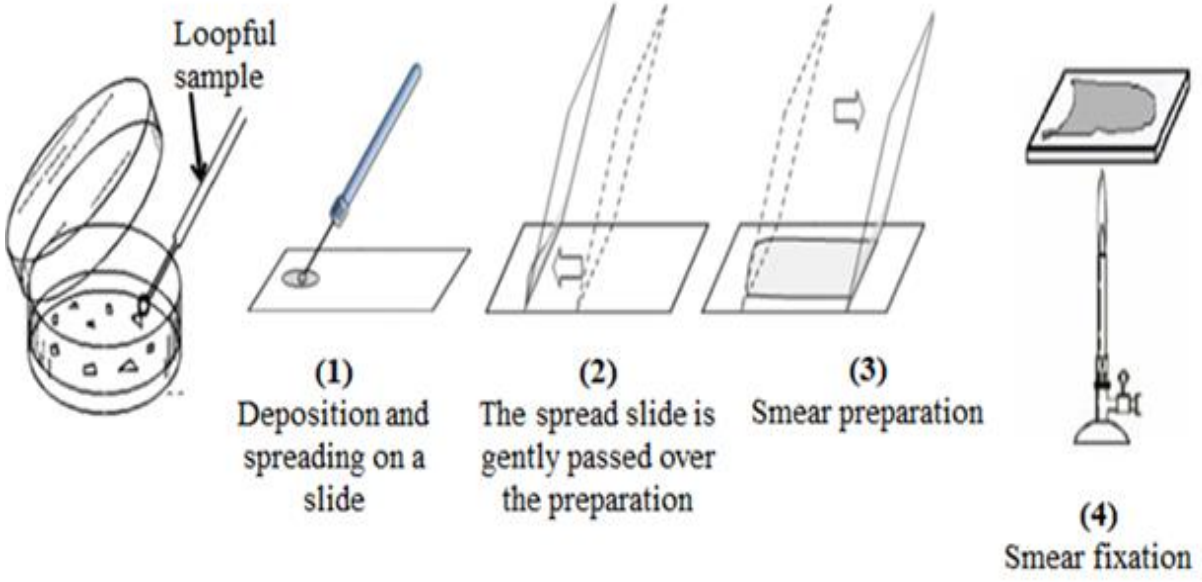


Figure 20: Technique for preparing a bacterial smear.

VI.2.2.1 Simple staining

The most commonly used is methylene blue staining.

- Take a smear and fix it.
- Cover the slide with carbol methylene blue for 1 minute.
- Rinse with tap water.
- Dry between two sheets of absorbent paper and observe using a 100° water immersion objective.

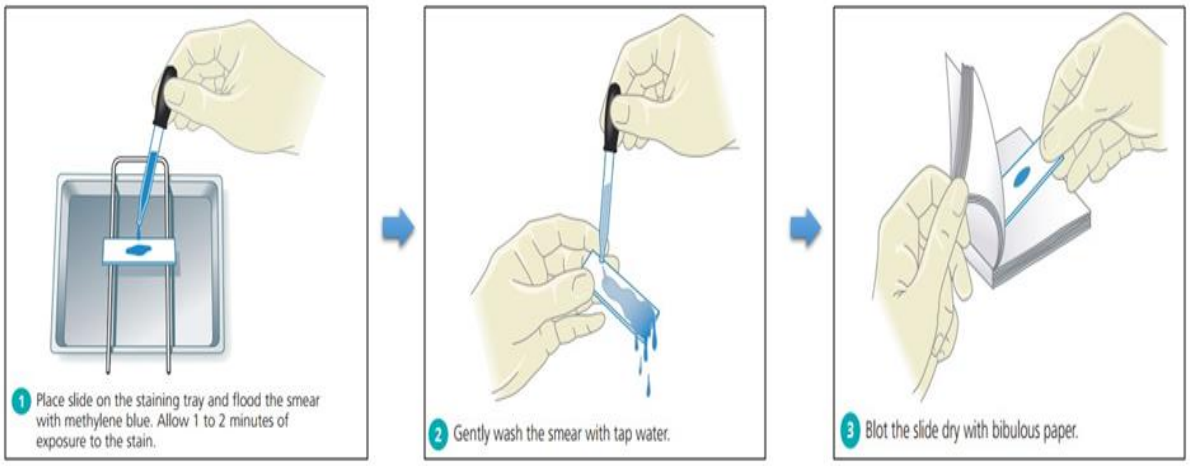


Figure 21: Simple staining procedure.

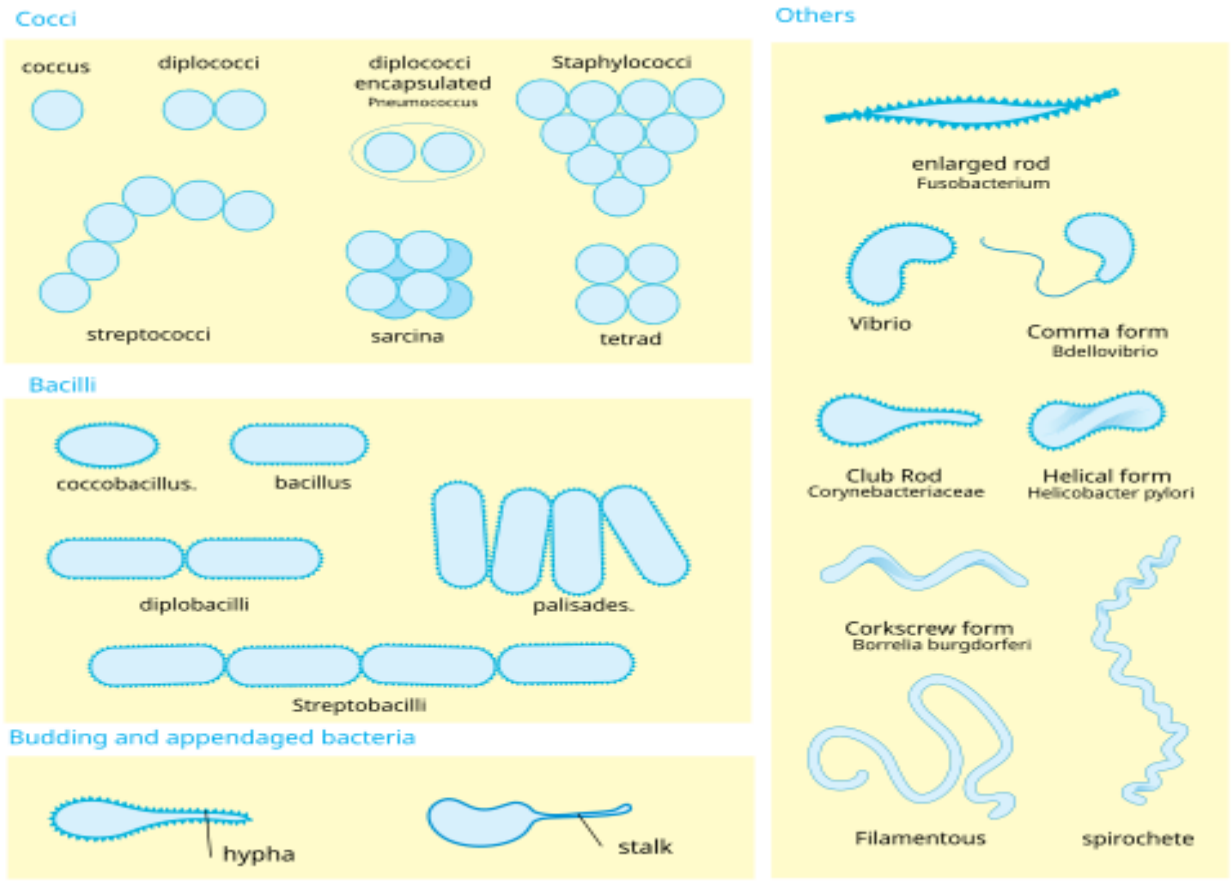


Figure 22: The shape of cells and their grouping mode.

Practical session n° 07:

Morphological study of different bacterial colonies on culture medium

VII.1 Objectives

1. Observe the appearance of the colonies.
2. Describe the colonies obtained.

VII.2 Morphological study of colonies:

The macroscopic examination of cultures is the first examination carried out from isolation after incubation. The appearance of the colonies depends on the medium used, the duration and temperature of incubation. It can only be described properly from well-isolated colonies: the colonies are smaller the closer they are. The characteristics of a colony are as follows:

VII.2.1 The size of a bacterial colony

It can vary between 1 and 10 mm in diameter. It can be measured using a graduated ruler for large colonies. For small colonies, it is also possible to use a microscope at low magnification, using an eyepiece micrometer to measure them.

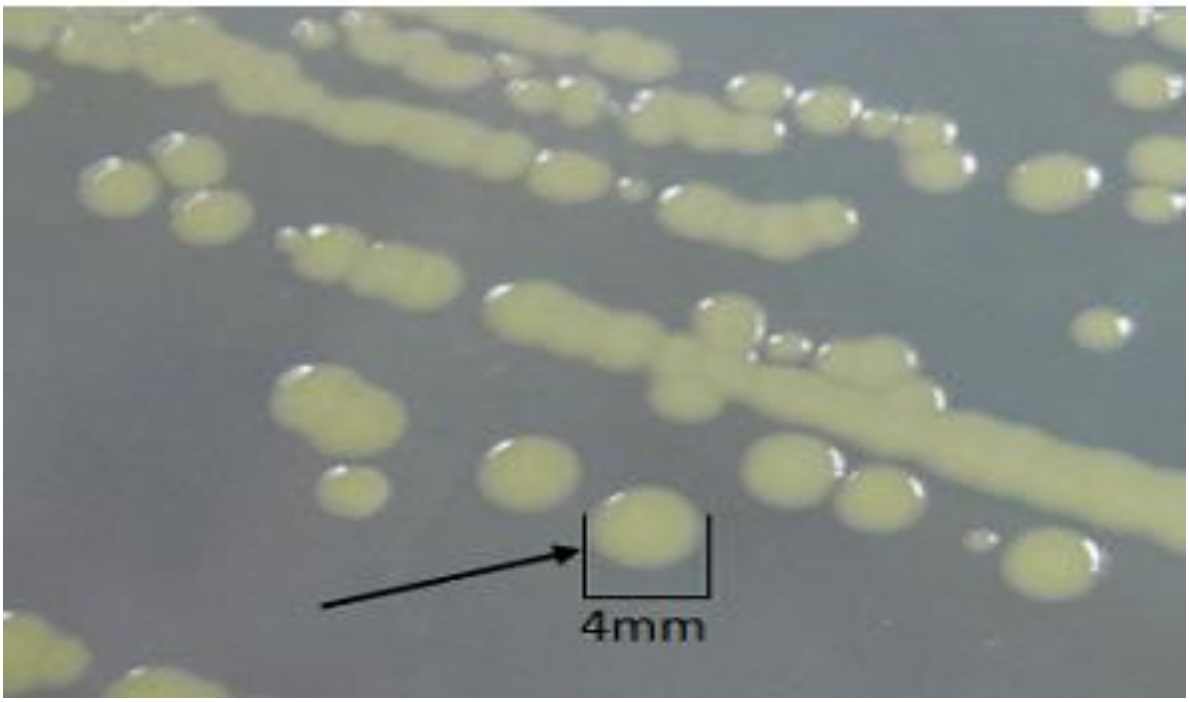


Figure 23: The size of a bacterial colony.

VII.2.2 The shape of a bacterial colony is characterized by:

- The appearance of the contours: smooth, regular, irregular, jagged, or jagged.
- The relief (surface): domed, semi-domed, or flat.
- The center of the colony: sometimes raised or sometimes umbilicated (hollow).

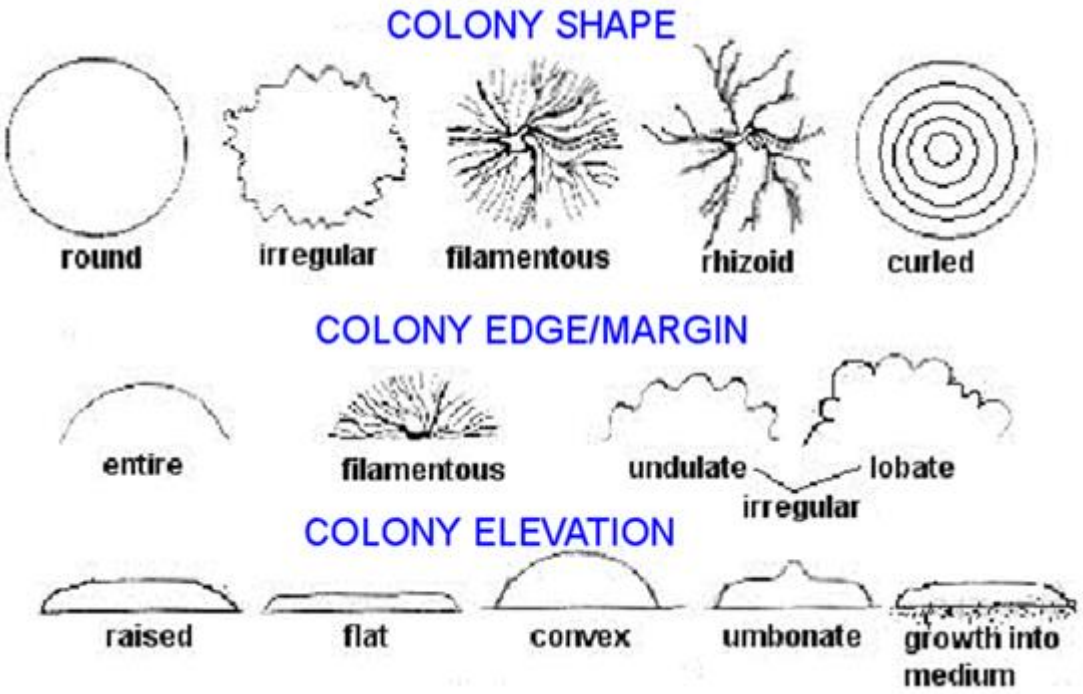


Figure 24: The shape of a bacterial colony.

VII.2.3 Surface appearance

The surface of a bacterial colony can be smooth, rough, or reflect light in a way that gives it a metallic sheen or an iridescent appearance.

VII.2.4 Consistency

This can usually only be assessed at the time of sampling. A distinction is made between:

- Non-viscous fatty and creamy colonies.
- Viscous (stringy) fatty and creamy colonies.

- Dry colonies.

VII.2.5 Opacity

Colonies are described as:

- Opaque (do not allow light to pass through).
- Translucent (allow light to pass through but shapes cannot be seen through it, like frosted glass).
- Transparent (allow light to pass through and shapes to be seen through it, like glass; we speak of dewdrops).

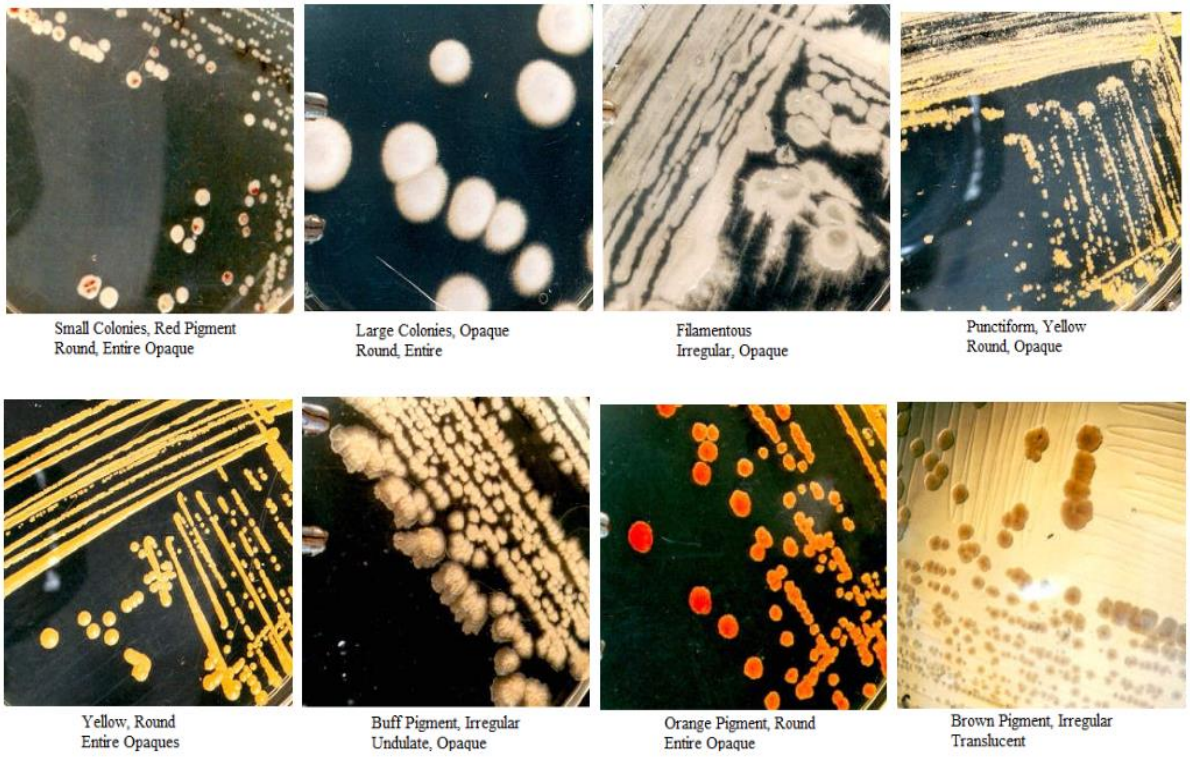
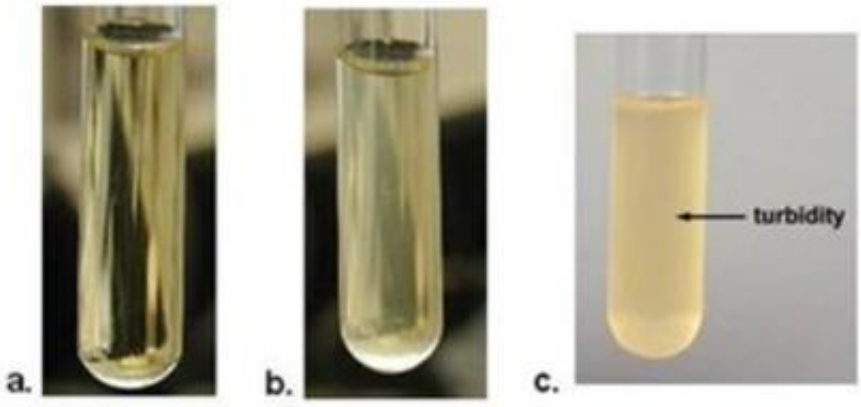


Figure 25: Bacterial Colony Morphology.

VII.3 Appearance of growth in a liquid medium

Nutrient broths are also used to cultivate bacteria. In a broth, microbial growth results in the appearance of cloudiness or opalescence, but the appearance varies depending on the species.



a. Sterile (uninoculated broth) - note how clear the media is
b. Broth showing slight turbidity (some bacterial growth)
c. Broth showing significant turbidity (a lot of bacterial growth)

Figure 26: Bacterial culture in broth medium.

Practical session n° 08:

Gram staining

VIII.1 Objectives

1. Observe and identify stained bacteria under a microscope to determine their Gram type

VIII.2 Differential staining or Gram staining

The best known is the Gram stain, which was developed in 1884 by Hans Christian Gram, a Danish bacteriologist. Bacteria can be grouped into two categories using the Gram staining method. After Gram staining, Gram (+) bacteria turn purple, while Gram (-) bacteria appear pink. Gram staining is an important systematic criterion for classifying bacteria. The technique involves taking a smear (**Practical Session No. 8)** and the following staining steps:

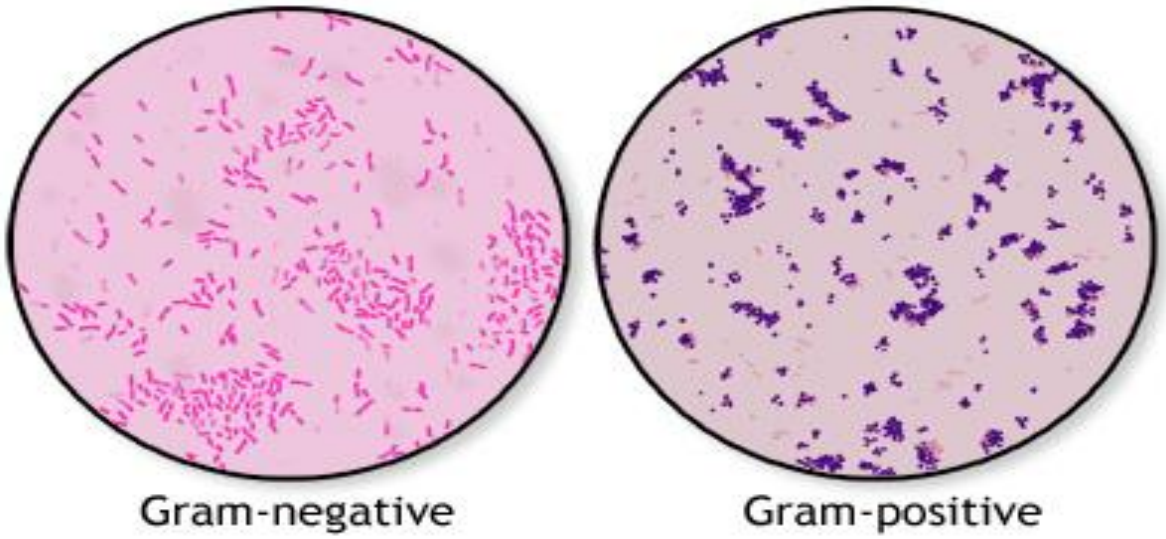


Figure 27: Differences between Gram-positive and Gram-negative bacteria.

Before staining, all bacteria are colorless.Gram staining is used to differentiate bacteria based on the structure of their cell wall:

- **Gram-positive** bacteria appear violet,
- While **Gram-negative** bacteria appear pink after counterstaining.

1.1.Primary Staining: Crystal Violet (Gentian Violet) (1 minute)

VIII.2.1 Primary Staining: Crystal Violet (Gentian Violet) (1 minute)

The smear is first covered with crystal violet, which penetrates the bacterial wall and membranes, staining the cytoplasm purple.

- **Gram (+):** the bacterium is stained violet.
- **Gram (–):** the bacterium is also stained violet.After one minute, rinse gently with tap water.

VIII.2.2 Mordant (Fixation) – Lugol's Iodine (1 minute)

Next, the slide is flooded with **Lugol's iodine solution**, which acts as a mordant. It forms a chemical complex between crystal violet and bacterial cytoplasmic components, fixing the stain inside the cytoplasm.

- **Gram (+):** the bacterium remains violet.
- **Gram (–):** the bacterium remains violet.
  Rinse again with tap water.

VIII.2.3 Decolorization – Alcohol Treatment (30 seconds)

This step is crucial, as alcohol acts differently depending on the bacterial cell wall composition.

- **Gram (+):** the bacterium remains violet because its thick peptidoglycan layer and low lipid content prevent alcohol penetration. These bacteria are called alcohol-resistant.
- **Gram (–):** the bacterium becomes decolorized, since its thin peptidoglycan layer and high lipid content allow alcohol to dissolve lipids and wash out the violet dye. These bacteria are alcohol-sensitive.
  Rinse immediately with tap water.

VIII.2.4 Counterstaining – Diluted Fuchsin (1/20) or Safranin (1 minute)

The slide is then covered with the counterstain (fuchsin or safranin), which penetrates both Gram-positive and Gram-negative cells.

- **Gram (+):** the bacterium remains violet, as the intense violet color masks the fuchsin or safranin.
- **Gram (–):** the bacterium is restained pink, since it was previously decolorized and now takes up the counterstain.

  Rinse with tap water, dry between absorbent papers, and observe under the 100× oil immersion objective with full illumination.

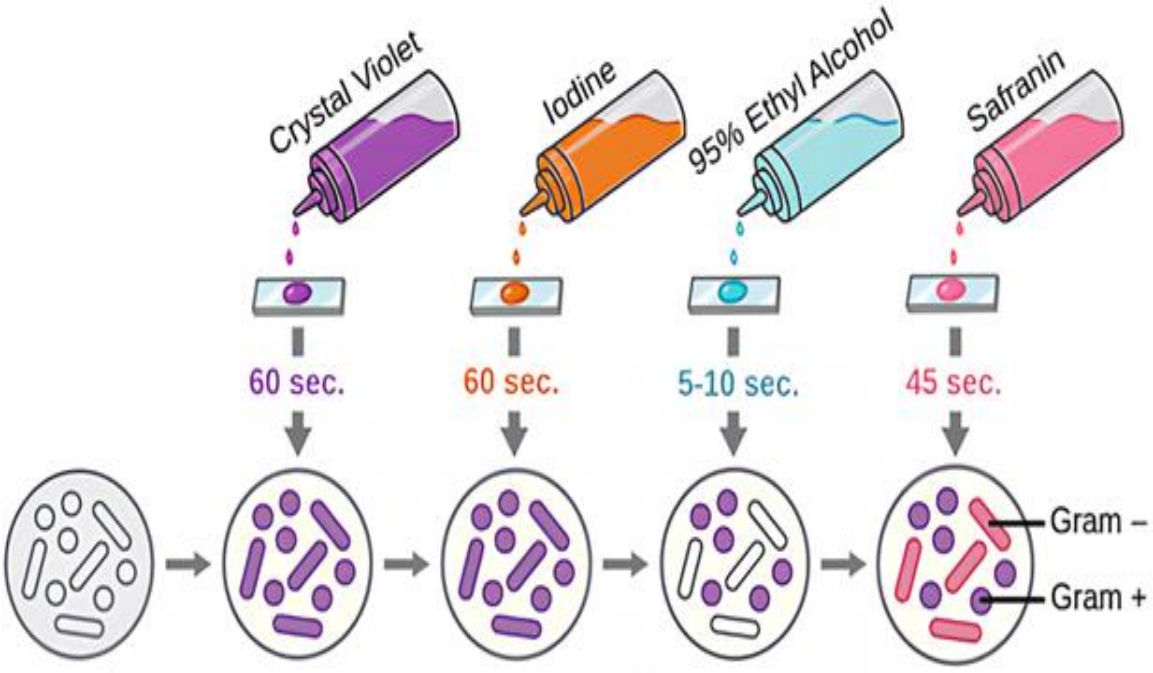


Figure 28: Schematic representation of the Gram staining steps and color differences between Gram-positive (violet) and Gram-negative (pink) bacteria.

Practical session n° 09:

Study of bacterial growth

IX.1 Objectives

1. Understand the concept of bacterial growth and its different phases.
2. Learn and apply different methods used to measure bacterial growth, including cell mass and cell number techniques.

IX.2 Introduction

Bacterial growth may be determined based on the increase in the number of cells. Reproduction, and therefore growth, leads to an increase in cell number and consequently an increase in the population of the bacterial culture. Bacterial growth follows specific patterns that can be represented by bacterial growth curves. The time required for a bacterial population to double in number is referred to as the generation time. Bacterial growth can be measured using either cell mass methods or cell number methods.

IX.3 Standard Plate Count

Bacteria can be present in thousands or millions in a sample, making it difficult to count their numbers. However, serially diluting the cultures makes it easier to determine the count.After serial dilution, the aliquots of the diluted sample are plated on an appropriate culture media. Then, the plates are incubated, after which the number of colonies formed is counted. This technique is also known as plate count or colony counts.

The techniques that assist in standard plate count include the streak plate and pour plate methods. It's essential to ensure that the plate is not crowded with bacterial colonies because, in such conditions, some cells might either not form colonies or fuse with each other resulting in erroneous results. Statistically, it is most valid to count colonies only on plates with 30 to 300 colonies.

In this method, accurate determination of total cell numbers is only possible if each colony is formed of a single cell. However, it's difficult for one to ensure such a case; that's why the total numbers of cells reported using this method are termed Colony Forming Units (CFUs) rather than cell numbers. It's calculated as:

The number of CFUs per ml of sample = The number of colonies (30-300 plate) X The dilution factor of the plate counted

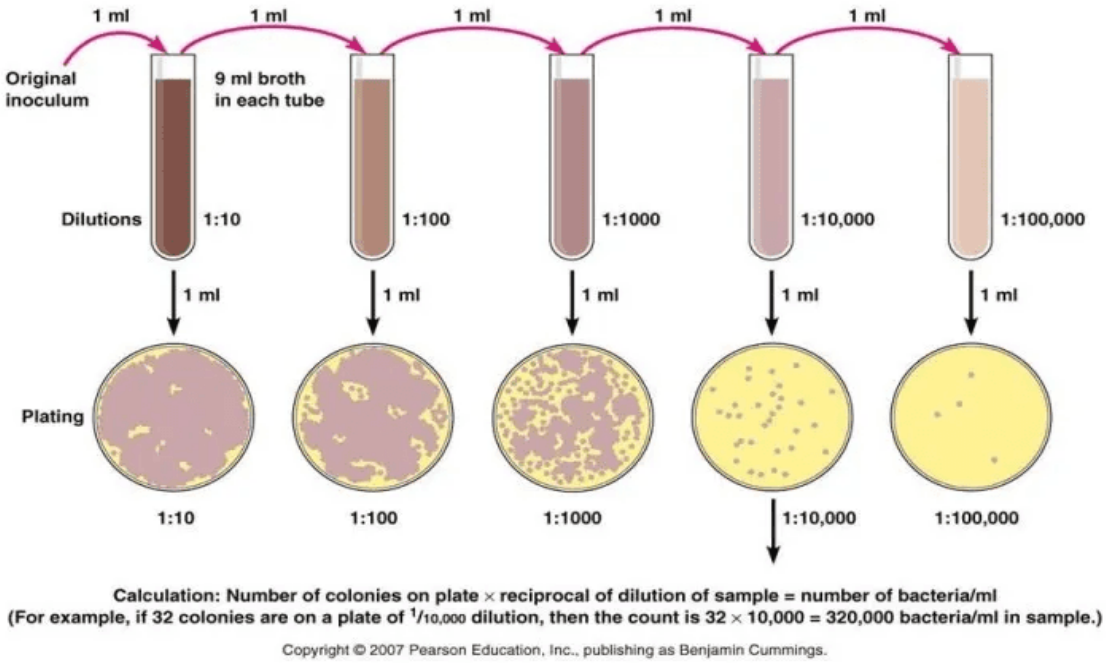


Figure 29: Standard Plate Count.

IX.4 Turbidimetric Measurement

The turbidimetric method is a quick and efficient technique used to measure and estimate the number of bacteria in a given sample. This method is particularly preferred when a large number of cultures need to be counted. When bacteria are suspended in a liquid medium, they create a colloidal suspension that scatters and absorbs light as it passes through the culture. By measuring the amount of light absorbed by the bacterial suspension, it is possible to estimate the concentration of bacteria present in the sample. It is important to note that the amount of light absorbed by the culture is directly proportional to the concentration of bacterial cells.

The method utilize instruments such as a spectrophotometer or colorimeter to measure the turbidity in the given sample. They contain a light source and a light detector separated from the sample compartment.

The cultures are poured into a cuvette and kept in the instrument. When light passes through the culture sample, cells interfere with them, which provides an output value on the machine that helps to calculate the bacteria numbers in the cultures.

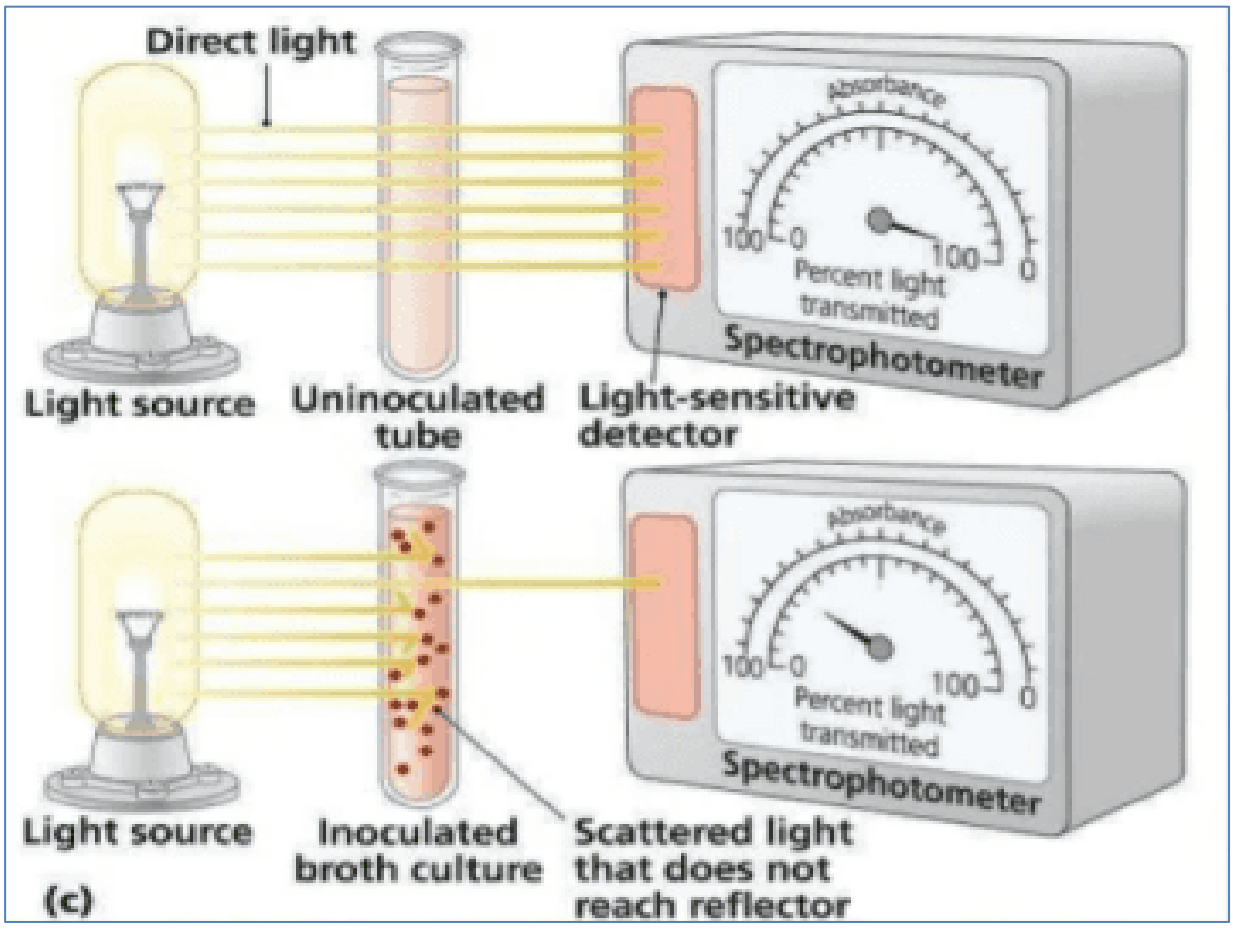


Figure 30: Schematic representation of the turbidimetric method for measuring bacterial growth using a spectrophotometer.

Before experimenting, it's essential to adjust the spectrophotometer at 100% transmittance (which means 0% absorbance), which is done using a sample of the uninoculated medium.

At last, the percentage of transmittance of different bacterial culture dilutions is measured, and the obtained values are converted to optical density using the formula:

Absorbance (O.D.) = 2 – log % Transmittance

The wavelength is determined based on the color of the solution. For example, if it's white, 420 nm is used, 540 nm if the color is yellow, and 600-625 nm if the solution is in the range of yellow to brown color.

Practical session n° 10:

Biochemical identification criteria for bacteria

X.1 Objectives

To identify and differentiate bacterial species based on their biochemical characteristics, by performing a series of biochemical tests that reveal the metabolic activities and enzymatic capabilities specific to each microorganism.

X.2 Concept of Metabolism

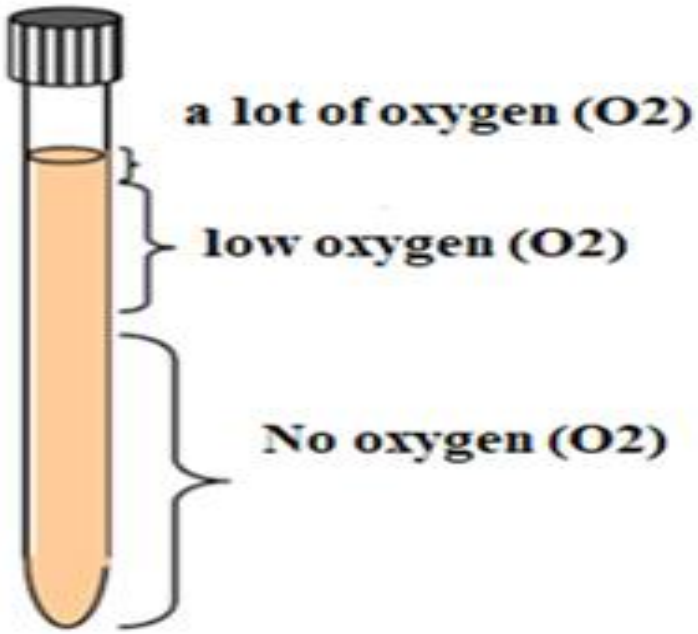
The growth of a microorganism involves the synthesis of numerous components necessary for the synthesis of cellular elements (plasma membrane, cell wall, DNA, etc.). All of these synthesis reactions are called anabolism. These reactions require energy, which is provided by the degradation reactions of organic substrates, most often taken from the environment. All of these degradation reactions are called catabolism.

X.3 Study of the respiratory type

Not all bacteria have the same requirements for O2. Indeed, only bacteria that possess aerobic respiration (and no other alternative pathway) require the presence of O2 to grow (strictly aerobic bacteria). Studying the respiratory type of a bacterium means defining its relationship with oxygen; some can only live in its presence, others only in its absence, and still others are indifferent to it.

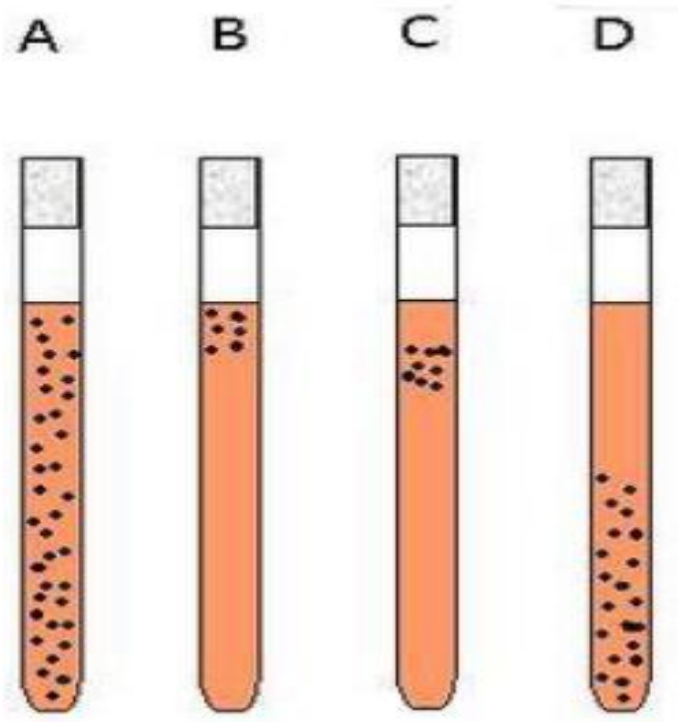
X.3.1 Principle

We use a rich culture medium: meat-liver (MLA) agar in which an O2 concentration gradient (partial pressure) is created.To do this, the agar is placed in a very thin tube and regenerated by boiling, which eliminates dissolved gases (including O2). The agar is then allowed to cool by aerating the tube (with the cap partially unscrewed). This results in a gradual penetration of O2, but this is slowed down by the presence of the agar and the thinness of the tube. This creates an oxygen concentration gradient.



X.3.2 Technique

At the time of use, place the tubes in a boiling water bath for 20 minutes (medium regeneration). Keep the media supercooled in a water bath at 45-50°C. Dip the tip of a buttoned, flame-sterilized Pasteur pipette into a suspension of the bacteria to be studied. Transfer the inoculum to the bottom of the tube and then raise the pipette by making very tight turns, taking care not to aerate the medium. Incubate at 37°C for 18 to 24 hours.



X.3.3 Reading

After incubation, four respiratory types can be recognized:

1. Aerobic-anaerobic bacteria (facultative aerobic-anaerobic): growth over the entire height of the agar plate.
2. Aerobic bacteria (strict aerobic): growth only in the surface zone of the agar plate.
3. Microaerophilic bacteria: growth in an agar cylinder approximately 0.5 cm high and located approximately 1 to 2 cm from the surface.
4. Anaerobic bacteria (strict anaerobic): growth only in the deep zone of the agar plate.

X.4 Enzymes linked to aerobic life (respiratory enzymes)

X.4.1 Catalase Research

Some bacterial metabolic reactions result in aerobic conditions, producing hydrogen peroxide (hydrogen peroxide). Hydrogen peroxide must be eliminated because it is highly toxic to bacterial cells (it is also used as an antiseptic). Its decomposition within the microbial organism can be achieved by catalase. In the absence of a destructive enzyme system, aerobic life generally becomes impossible: microorganisms are then strictly anaerobes. Therefore, catalase is produced in abundance by bacteria with respiratory metabolism (strict aerobic and facultative aerobic-anaerobic), and it catalyzes.

$$2\ H_2O_2 \longrightarrow 2\ H_2O + O_2$$

X.4.1.1 Technique

Place one or two drops of 10-volume hydrogen peroxide on a glass slide. Using the tip of a Pasteur pipette, remove a colony fragment and dissociate the culture in hydrogen peroxide.

X.4.1.2 Reading

The presence of catalase results, within a few seconds, in the formation of oxygen bubbles (Effervescence).

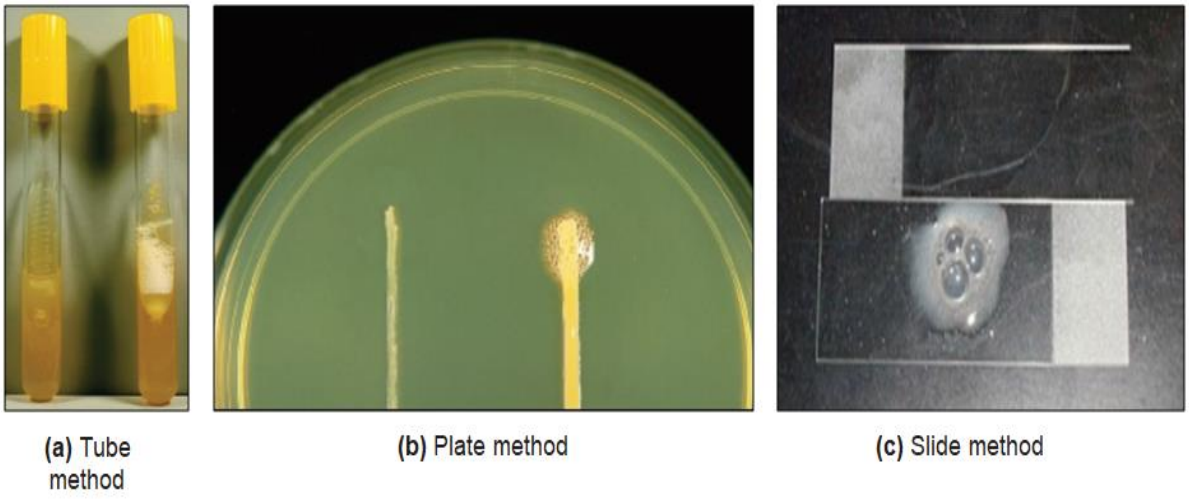


Figure 31: Catalase test.

Negative results are shown on the left and positive results on the right in the **(a)** tube method and **(b)** plate method. Negative results are shown on the top and positive results on the bottom in the **(c)** slide method.

X.4.2 Oxidase Testing

X.4.2.1 Reminders

Cytochromes are proteins that belong to the respiratory chain, composed of a series of electron carriers, particularly cytochromes carrying heme coenzymes. Oxidase testing is one of the most discriminating and widely used criteria for identifying bacteria, especially Gram-negative bacilli. This test involves demonstrating the ability of the tested bacterium to oxidize the colorless reduced form of methylated paraphenylenediamine derivatives to their purplish-pink semiquinone oxidized form.

1. Principle

This test detects an enzyme: phenylenediamine oxidase in bacteria from their agar culture. This enzyme is capable of oxidizing a reagent: N-dimethylparaphenylenediamine. This reagent is colorless and in the presence of the enzyme, it releases a pink-red compound, which turns black in air.

2. Technique

Prepare a bacterial suspension in physiological saline solution, then add an oxidase disk (N-dimethylparaphenylenediamine oxidase).

3. Reading

- **Positive reaction:** Pink color (for a few moments, then blackening).
- **Negative reaction:** Color of the medium unchanged.

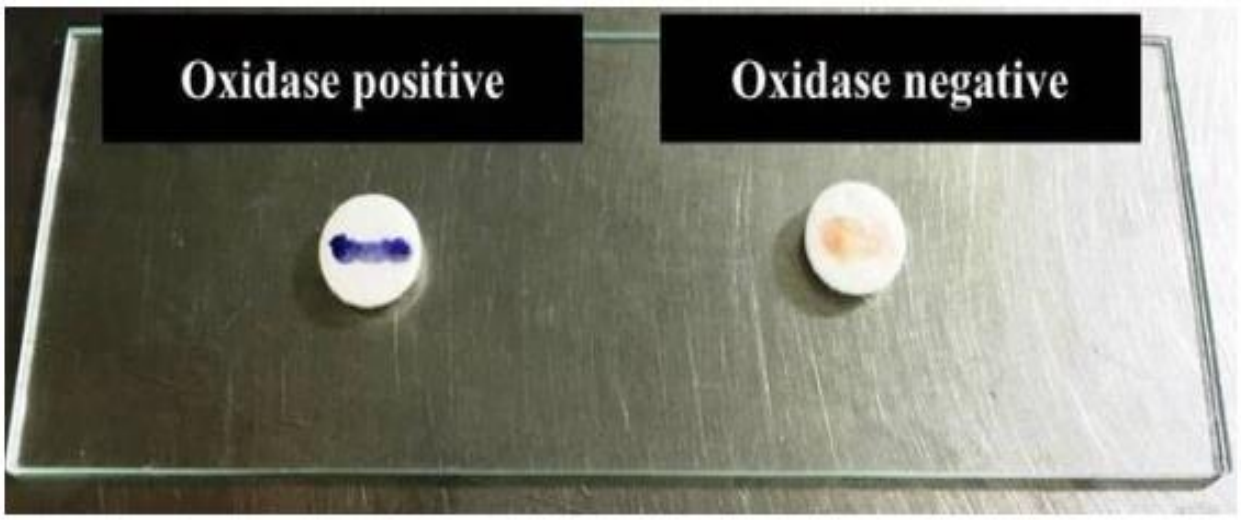


Figure 32: Oxidase Test.

X.5 Nitrate metabolism

X.5.1 Nitrate Reduction Test

The reduction of nitrates by some aerobic and facultative anaerobic microorganisms occurs in the absence of molecular oxygen, an anaerobic process. In these organisms, anaerobic respiration is an oxidative process whereby the cell uses inorganic substances such as nitrates (NO_3^-) or sulfates (SO_4^{2-}) to supply oxygen that is subsequently utilized as a final hydrogen acceptor during energy formation.

X.5.1.1 Technique

- Preparation of Trypticase nitrate broth
- Using aseptic technique, inoculate each experimental organism into its appropriately labeled tube by means of a loop inoculation.
- Incubate all cultures for 24 to 48 hours at 37°C.
- Add five drops of Solution A and then five drops of Solution B to all nitrate broth cultures. Observewhether a red coloration develops in each of the cultures.
- Add a minute quantity of zinc to the cultures in which no red color developed. Observe whether red coloration develops in each of the cultures.

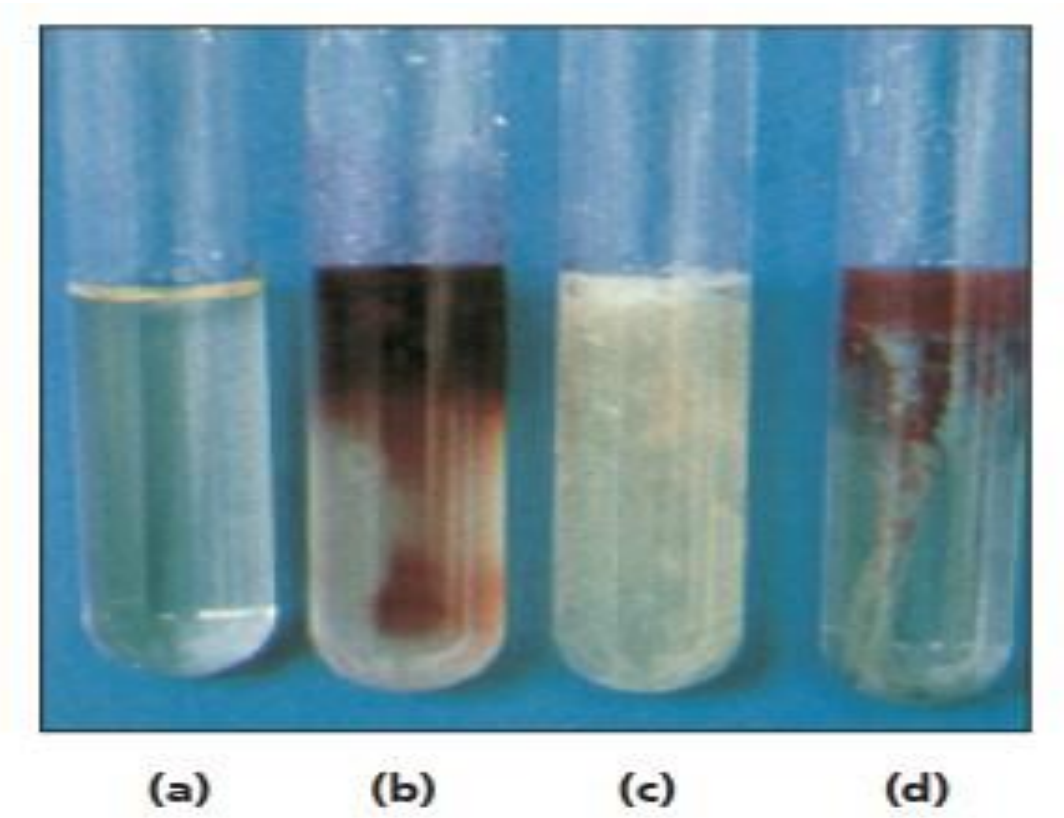


Figure 33: Nitrate reduction tests.

(a) Uninoculated, **(b)** positive with Solutions A + B, **(c)** positive with Solutions A + B + zinc powder,

X.6 Study of carbohydrate metabolism

X.6.1 Fermentation with or without gas, use of Lactose and Sucrose and production of H2S

This reaction is carried out on TSI (Tri-Sugar-Agar) medium: This medium is presented as an agar slant containing 1 g/milliliter of glucose and 2 disaccharides at 10 g/milliliter, along with phenol red as an indicator and an iron salt.

- **In the first stage,** the glucose is fermented, allowing the bacteria to produce the energy necessary for the synthesis of enzymes specific to disaccharides, resulting in an acidification of the medium, which turns yellow at the pellet.
- **In the second stage,** the bacteria begin to degrade the peptones contained in the medium and the disaccharides if they possess the enzymes.

The degradation releases the amino acids that alkalize the medium, and the degradation of the disaccharides releases the acids that acidify the medium. The balance between these two reactions results in:

- Alkalization if the bacteria are disaccharides -: the medium turns red. - Acidification if the bacteria is disaccharide-positive: Yellow color.
- Gas production: Most bacterial fermentations are accompanied by gas production, either CO2, H2, or both.
- Blackening due to the formation of iron sulfide indicates the production of H2S, which means that the bacteria possesses desaccharide-positive enzyme: S2O3 H2S FeS black.

X.6.1.1 Technique

Using a sterile Pasteur pipette, inoculate the TSI medium from the bacterial suspension by making streaks along the slope and puncturing the center of the base.

- Do not completely tighten the tube.
- Incubate the medium at 37°C for 24 hours.

X.6.1.2 Reading

- If the base is yellow: the bacteria is glucose-positive.
- If the slope is yellow: the bacteria is disaccharide-positive. - If there is blackening in the middle of the tube: the bacteria is H2S+.
- If there is one or more pockets of gas: the bacteria is Gas+.

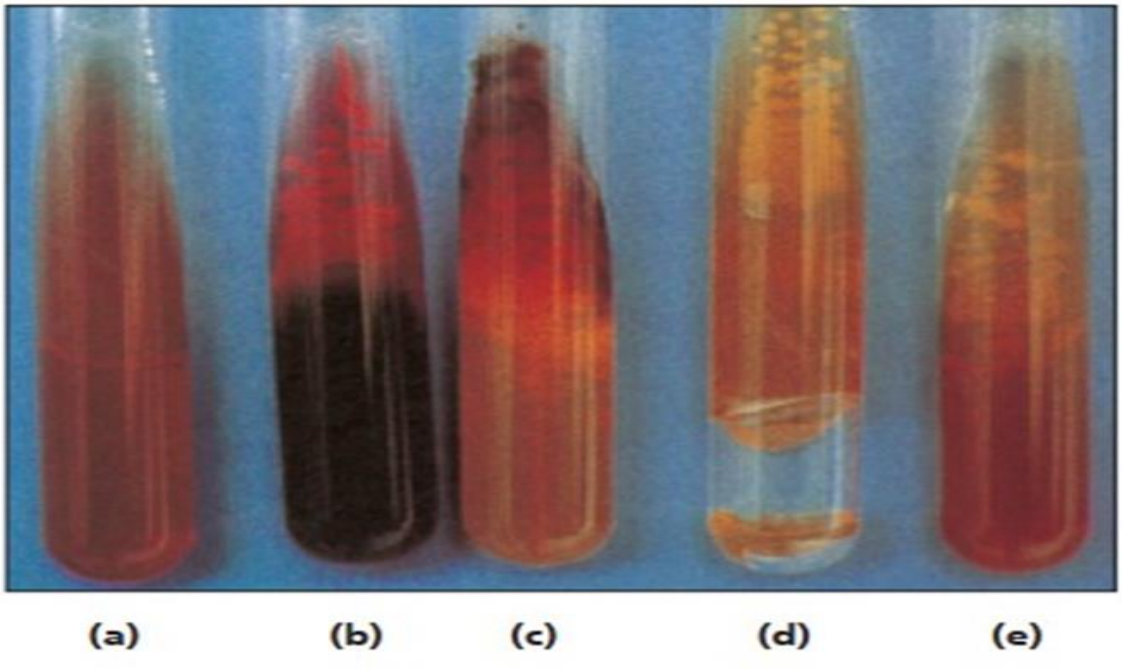


Figure 34: Reactions in triple sugar–iron agar.

(a) Un inoculated; **(b)** alkaline slant/acid butt, H2S; **(c)** alkaline slant/acid butt; **(d)** acid slant/acid butt, gas; and **(e)** acid slant/acid butt.

Practical session n° 11:

Yeasts and cyanobacteria

XI.1 Objectives

1. Recognize the distinctive morphological characteristics of yeasts and cyanobacteria.

XI.2 Cyanobacteria

Cyanobacteria also called cyanophytes or blue-green algae, belong to the domain Bacteria (phylum *Cyanobacteriota*). They are photosynthetic microorganisms without a nucleus and were long mistaken for algae. Their blue-green color comes from the pigment phycocyanin. They include about 2,000 species grouped into 150 genera and are among the oldest living organisms on Earth, with fossils dating back 3.5 billion years.

They played a major role in forming the oxidizing atmosphere through oxygen production during photosynthesis, thus promoting the evolution of life.

XI.2.1 Habitat and Forms

Cyanobacteria live in aquatic environments:

- Planktonic/pelagic: floating freely in the water;
- Benthic: attached to a substrate.

Some, like spirulina, are used for their nutritional properties.

XI.2.2 Characteristics

They exhibit great morphological diversity and remarkable genetic plasticity, allowing them to adapt to various environments. Some species, known as chrococcoid forms (e.g.,*Chroococcus*), are spherical cells surrounded by a mucilaginous envelope. Cyanobacteria are photoautotrophic, using light, water, CO_2, and nutrients to produce their own organic matter. Some species can enter dormancy in the absence of light.

Recently, cyanobacteria (*Cyanobacteria*) have been classified as a subclass of bacteria due to their prokaryotic nature, even thoughthey are still commonly referred to as blue-green algae.



Figure 35: Microscopic observation of the cyanobacterium *Dolichospermum sp.*

XI.2.3 Cyanobacteria Culture Protocol

- **Culture Medium**

Cyanobacteria grow on a synthetic mineral medium, rich in mineral salts but devoid of organic matter. The reference medium is BG-11, suitable for most species.

Table 5: Composition for 1 liter of distilled water.

Compound	Chemical formula	Quantity
Sodium nitrate	$NaNO_3$	1.5 g
Dipotassiumhydrogen phosphate	K_2HPO_4	31 mg
Magnesium sulfate heptahydrate	$MgSO_4 \cdot 7H_2O$	75 mg
Calcium chloridedihydrate	$CaCl_2 \cdot 2H_2O$	36 mg
Citricacid monohydrate	—	7 mg
Disodium EDTA	—	1 mg
Sodium carbonate	Na_2CO_3	20 mg
Ammonium ferric citrate	—	6 mg
Trace metal mixture	—	1 mL

Table 6: Composition of the trace metal mixture (for 250 mL of distilled water).

Compound	Chemical formula	Quantity
Boricacid	H_3BO_3	715 mg
Manganesechloridetetrahydrate	$MnCl_2 \cdot 4H_2O$	452 mg
Zinc sulfate heptahydrate	$ZnSO_4 \cdot 7H_2O$	56 mg
Sodium molybdate dihydrate	$Na_2MoO_4 \cdot 2H_2O$	97 mg
Copper sulfate pentahydrate	$CuSO_4 \cdot 5H_2O$	20 mg
Cobalt nitrate hexahydrate	$Co(NO_3)_2 \cdot 6H_2O$	12.5 mg

To prepare a solid medium, add7 g of agar per liter. The medium should be sterilized for 20 minutes at 130 °C before use.

- **Culture conditions of cyanobacteria**

Cyanobacteria can be grown in transparent, sterilizable containers, such as test tubes, Erlenmeyer flasks sealed with a gauze pad covered with aluminum foil, or small autoclavable culture jars. It is important not to fill the containers completely to ensure good ventilation and allow gas exchange (O_2 and CO_2), and the closures must remain permeable to air to promote growth. These microorganisms necessarily require light for their development, as they carry out photosynthesis; however, excessively intense light can be harmful. Therefore, direct exposure to sunlight, which could destroy the cells, should be avoided and soft, constant artificial lighting, such as a horticultural fluorescent lamp or a white LED lamp, should be used. Cultures can be placed under continuous light (24 hoursaday), but if the intensity is too high, it can be reduced using parchment paper. An ambient temperature of around 25°C is generally sufficient to ensure good development. Strains must be sub cultured regularly, generally every 3 to 6 months depending on the species and culture conditions, because their grow this slow and it is often necessary to wait 5 to 6 weeks to observe visible development after subculture. During this period, cultures must be observed regularly under a microscope to check their purity, color and physiological state. A healthy culture has a uniform blue-green color, without deposits or contamination, and successive subcultures allow the strains to be kept active while preventing aging of the cultures.



Figure 36: Culture of cyanobacteria in BG11 0 media.

XI.3 Yeasts

Yeasts are microscopic unicellular fungi belonging to the group of eukaryotes, as they possess a true nucleus and organized genetic material. A single cubic centimeter of fresh yeast (about 1 g) can contain up to 10 billion living cells.

Because of their biochemical composition — including enzymes, beta-glucans, mannoproteins, and lipids — yeasts play a major role in many bio chemical processes, particularly fermentation, which transforms organic matter into useful substances such as gas, alcohol, and aromatic compounds. From a nutritionals tandpoint, yeasts are a rich source of essential nutrients, including vitamins, minerals, and amino acids, making them a true treasure for health.

XI.3.1 Yeasts exhibit a dual metabolism

- **Under aerobic conditions (with oxygen),** they respire, breaking down glucose into CO_2 and water, producing a large amount of energy that supports their growth.
- **Under anaerobic conditions (without oxygen),**they ferment glucose, producing alcohol, CO_2, and aromatic compounds, which are exploited in the production of bread, wine, and beer.

For optimal growth, yeasts require oxygen, glucose, and sources of nitrogen, phosphate, vitamins, and minerals.

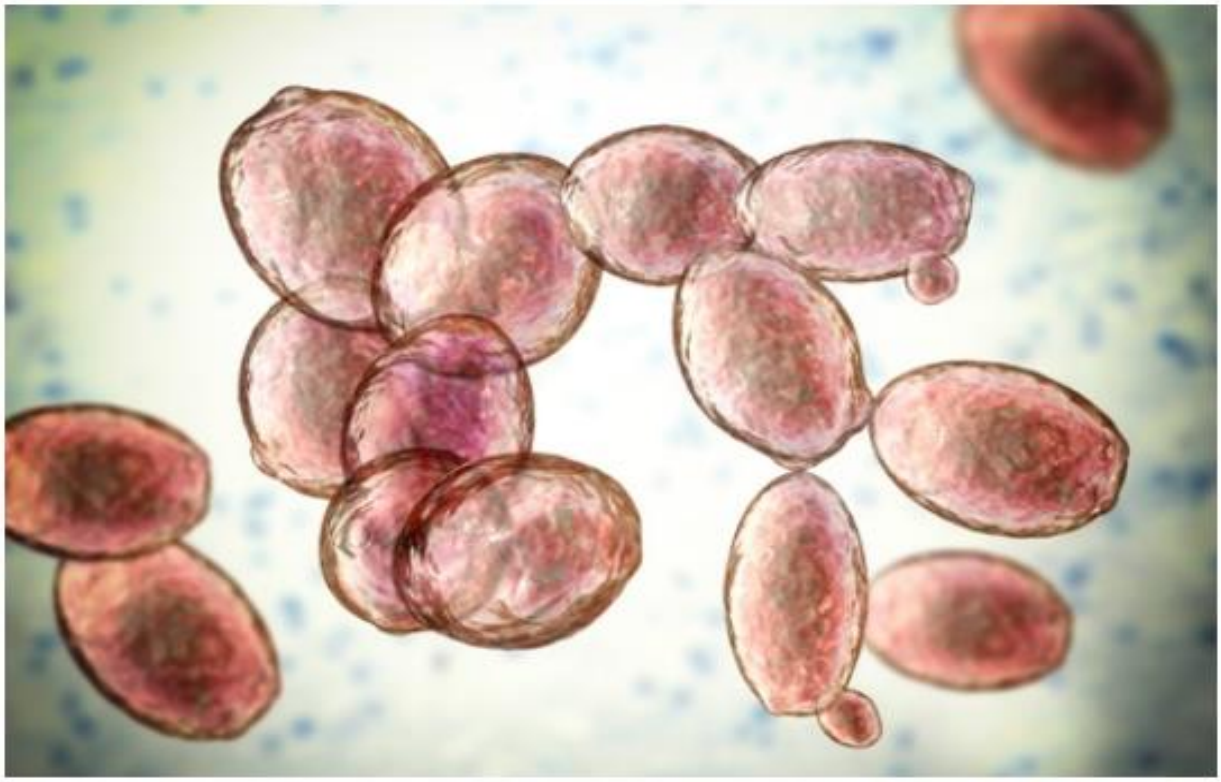


Figure 37: Microscopic observation of yeast cells.

XI.3.2 Observation and cultivation of yeasts

XI.3.2.1 Equipment

- Microscope, slides, coverslips, crystallizing dish with bleach, distilled water, and iodine solution.
- Sterile Petr idish, watch glass, lancet needle, loop, and spreader.
- Tube containing Sabouraud glucose agar, Bunsen burner, and brewer's yeast.

XI.3.2.2 Main Steps

- **Observation in the Raw State**

- Prepare a yeast suspension in a few drops of water on a watch glass.
- Place a drop between the slide and the coverslip and observe under high magnification.

- **Iodine Staining**

- Add a drop of iodine solution (Lugol's) to the edge of the coverslip for 2–3 minutes.

➢ **Inoculation on Agar**

- Melt the glucose agar and pour it into a sterile Petri dish, keeping the lid opening to a minimum.
- Spread the yeast suspension over the solidified surface with a spreader.
- Incubate the dish upside down at 37°C to observe the colonies.

➢ **Colony Collection and Observation**

- Sterilize the loop with a flame and let it cool.
- Colony samples are collected and diluted in a watch glass with water.
- Place a drop on a slide and coverslip, observe under a microscope, and stain with iodine if necessary.

Be careful with contaminating colonies: do not touch them; they must be destroyed by incineration and bleach.

XI.3.2.3 Observations and results

1. Cell Morphology

• Non-motile, spherical cells, measuring 6–8 µm, with a thick membrane and cytoplasm lacking chlorophyll → unicellular fungi (Ascomycetes), known as yeasts.

2. Intracellular Inclusions

• After 2–3 minutes of staining with iodine, brown granules appear: glycogen, a storage substance produced from glucose.

3. Budding and Reproduction

• Yeasts reproduce by budding, an asexual reproduction process: the mother cell forms one or more buds that grow and then detach.

• Fermentation allows them to utilize glucose in the absence of oxygen, while respiration occurs in the presence of oxygen.

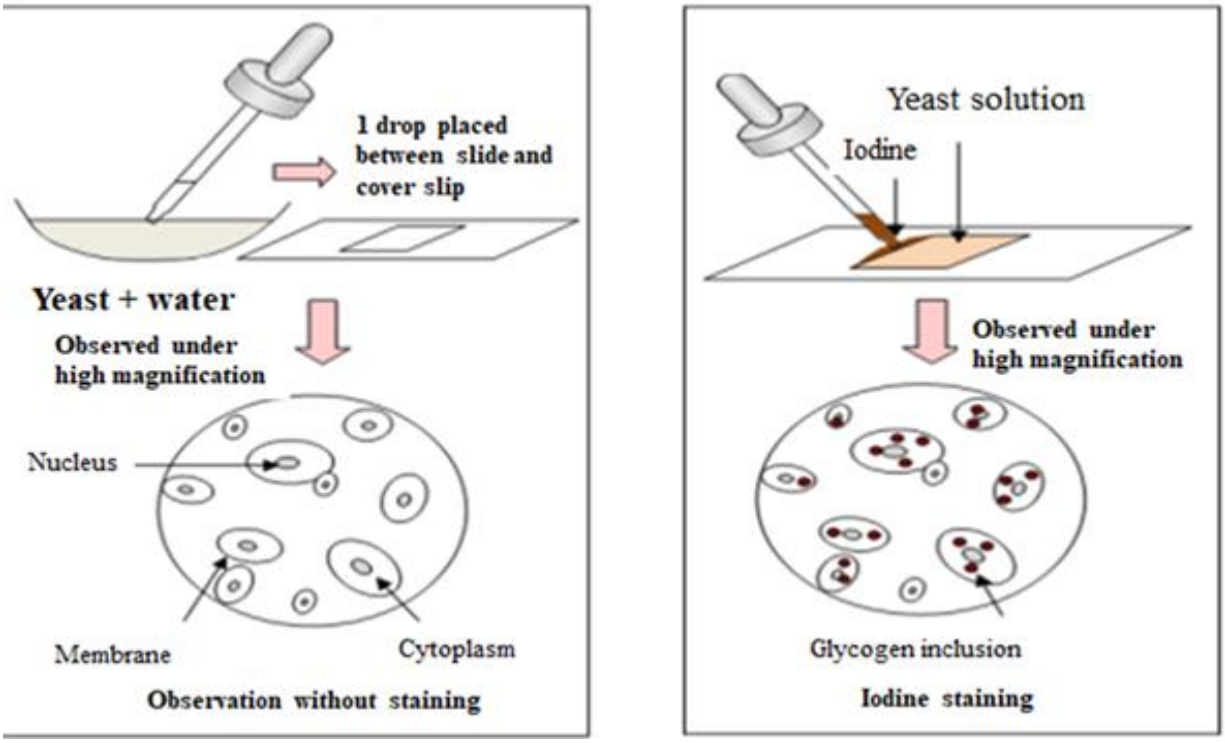


Figure 38: Steps for microscopic observation and cultivation of yeast cells.

Practical session n° 12:

Growth inhibitors, antibiogram

XII.1 Objectives

1. Understand the concept of antibiogram and its value
2. perform a standard antibiogram

XII.2 The antibiogram

The antibiogram is a laboratory test used to evaluate the sensitivity of a pathogenic bacterium to various antibiotics. Antibiotics are chemical compounds produced either naturally by microorganisms or synthetically, and their specific activity is expressed at low concentrations where they act selectively on other microorganisms. Determining the susceptibility of bacteria to different antibiotics is of great importance in microbiology, as it allows the development of selective isolation media, assists in the control and treatment of infections through appropriate chemotherapeutic agents, and can also serve as a valuable tool for bacterial characterization and identification. The antibiotic susceptibility of a bacterial strain can be determined by two main approaches: in liquid medium, through the determination of the Minimum Inhibitory Concentration (MIC), which corresponds to the lowest concentration of an antibiotic that inhibits visible bacterial growth; or on solid medium, by the disk diffusion method ,where antibiotic-impregnated disks are placed on an agar surface inoculated with the test organism, and the resulting inhibition zones are measured after incubation to assess the degree of sensitivity or resistance of the microorganism.

XII.2.1 Materials

- Petri dishes
- A bacterial suspension (test tube)
- Disinfectant
- Bunsen burner
- Sterile forceps
- Graduated pipettes
- Antibiotic test discs
- Sterile swabs
- Vernier caliper (for measuring inhibition zones)
- Mueller–Hinton agar medium used for antibiotic susceptibility testing

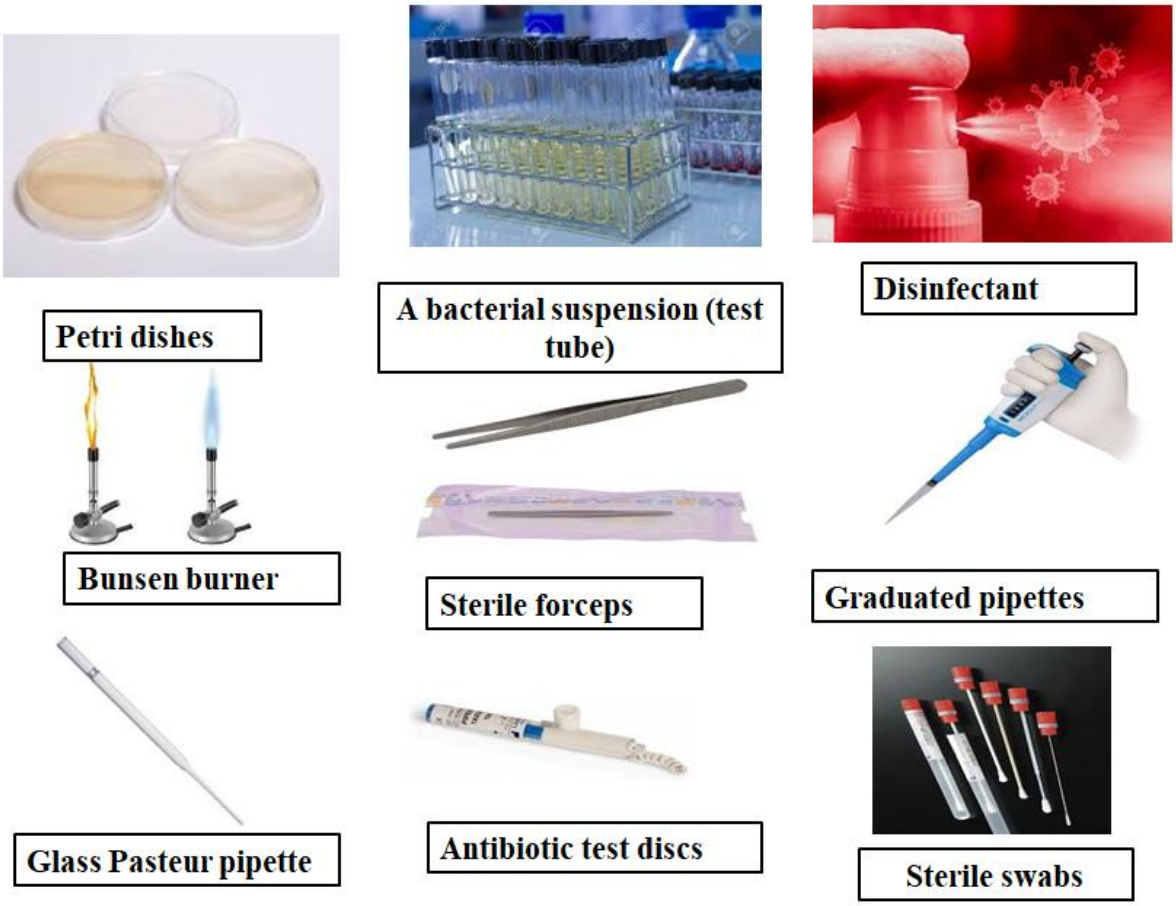


Figure 39: Materials used for the antibiogram.

XII.2.2 Technique

Thoroughly clean the bench with a disinfectant to minimize the risk of external contamination during handling. Light the Bunsen burner to create an upward airflow that sterilizes the immediate environment and destroys microorganisms that could alter the results. All handling must be carried out within a radius of 20 to 25 cm around the flame. Inoculation is performed by swabbing Petri dishes containing Mueller-Hinton medium. A sterile swab is dipped in the bacterial suspension and then wrung out by pressing it firmly against the inside of the tube. The swab is then rubbed over the entire agar surface, making tight, top-to-bottom streaks. This process is repeated four times, rotating the dish 90° with each pass, to obtain a

uniform inoculation. Inoculation is completed by swabbing the entire agar surface one last time.

Using sterile forceps, gently remove an antibiotic disc and place it on the surface of the inoculated agar, pressing it gently to anchor it firmly in the medium. Repeat this process for the four antibiotics tested, taking care to change or resterilize the forceps between each disc to avoid cross-contamination.

Then place the antibiogram plates in an incubator at 37°C for 24 hours to allow for bacterial growth.

After incubation, observe the results and measure the diameters of the zones of inhibition around each disc using a caliper. These measurements will help determine the sensitivity or resistance of the bacterial strain to the antibiotics tested.

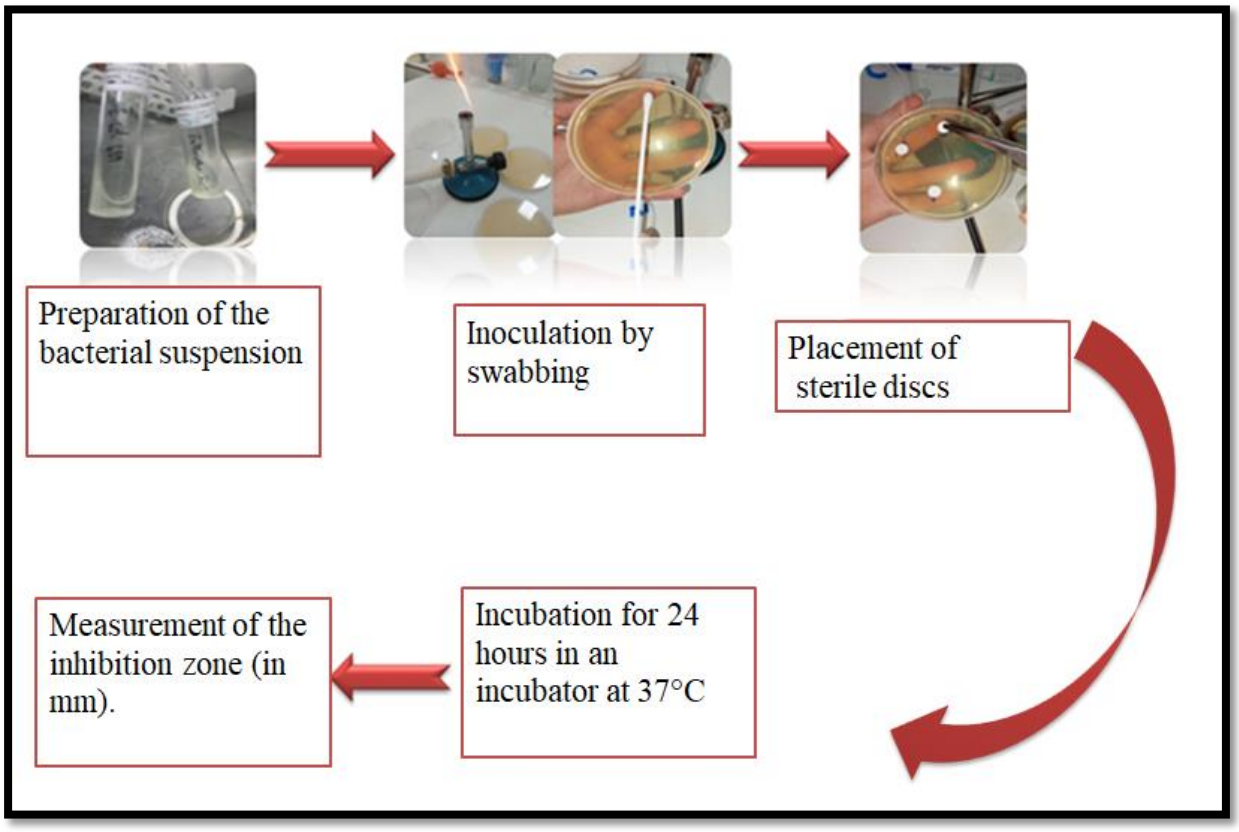


Figure 40: Steps of the Antibiogram Test.

Table 7: Sensibilité et degré d'activité selon le diamètre d'inhibition.

Degree of disc sensitivity	Diameter of the inhibition zone (mm)
Resistant strain	D = 6
Sensitive strain	7≤ D ≤ 11
Highly sensitive	12 ≤ D ≤ 16
Extremely sensitive	D ≤ 17

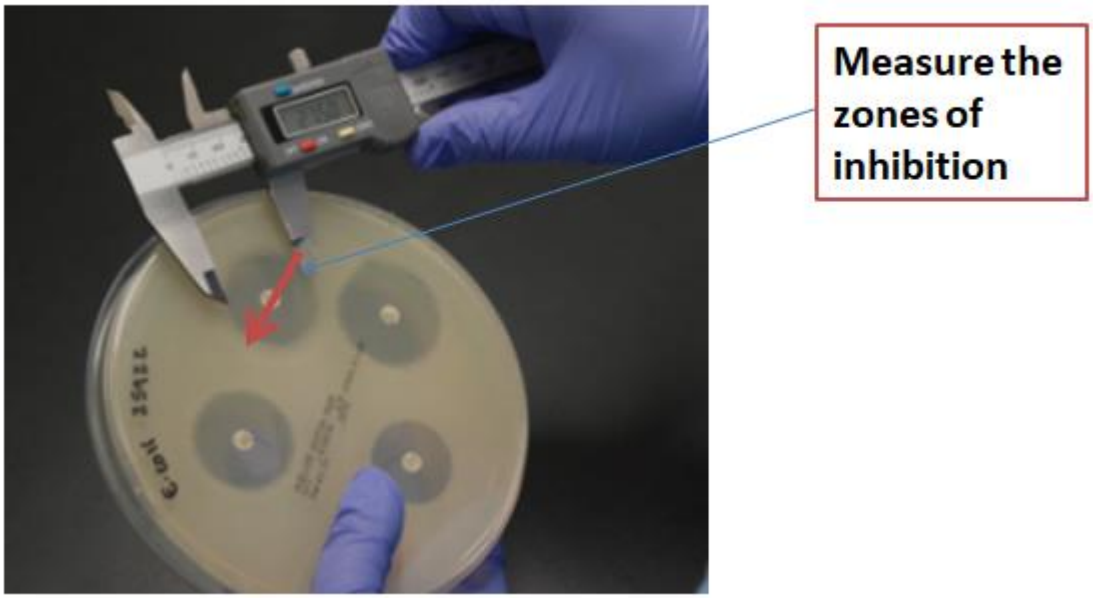


Figure 41: Measure the zones of inhibitio

Bilingual Microbiology Glossary

English Term	Français (Définition)
Inoculation	**Inoculation :** consiste à transférer des micro-organismes d'un milieu de culture initial (échantillon) vers un nouveau milieu stérile. Le transfert se fait généralement à l'aide d'une anse de platine ou d'une pipette de Pasteur.
Isolation	**Isolement :** consiste à déposer des cellules microbiennes à la surface d'un milieu nutritif solide afin qu'après incubation, elles forment des colonies bien séparées.
Subculturing (or Transfer)	**Repiquage (ou Transfert) :** correspond au transfert d'une culture pure vers un nouveau milieu de culture.
Pure Culture	**Culture pure :** constituée de colonies identiques correspondant au même micro-organisme. On suppose que toutes les colonies bien isolées d'une culture pure sont identiques et présentent le même aspect.
Disinfectant	**Désinfectant :** agent chimique capable de détruire les micro-organismes (ou d'arrêter leur croissance) sur des surfaces inertes.
Sterile Material	**Matériel stérile** : totalement exempt de micro-organismes et de spores.
Germs	**Germes :** micro-organismes.
Colony	**Colonie :** masse visible de cellules formée par des milliards de descendants issus d'une seule cellule bactérienne.
Septic	**Septique :** qualifie un milieu contenant des micro-organismes.
Aseptic	**Aseptique :** qualifie un milieu exempt de micro-organismes.
Asepsis	**Asepsie :** ensemble des mesures destinées à empêcher l'introduction de micro-organismes provenant de l'environnement extérieur.
Bactericidal	**Bactéricide :** se dit d'un agent antimicrobien qui tue les bactéries (action létale).
Bacteriostatic	**Bactériostatique :** se dit d'un agent antimicrobien qui inhibe la croissance bactérienne (action inhibitrice).
Fungicidal	**Fongicide :** se dit d'un agent antimicrobien qui tue les champignons (action létale).
Virucidal	**Virucide :** se dit d'un agent antimicrobien qui détruit les virus (action létale).

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QUIZ

Amar Telidji University – Laghouat
Faculty of Science
Department of Agronomic Sciences

Food Science Students – 2nd Year Level
Duration: 1 hour 30 minutes

Last Name and First Name: …… **Mark:**

First Microbiology Module Quiz

Q1 : Choose the correct answer MCQ(10 pts)

1. **Microbiology studies:** a) Plants and animals b) Microorganisms invisible to the naked eye c) Stars and planets d) Rocks and minerals	**6. The microscope should be handled:** a) With one hand b) With both hands, one on the arm and one under the base c) With fingers on the lenses d) With eyes closed
2. **Microorganisms contribute to:** a) Biogeochemical cycles (carbon and nitrogen) b) Production of sunlight c) Mountain erosion d) Plastic production	**7. Agar is used:** a) As a stain for bacteria b) As a gelling agent for solid media c) As the main nutrient for bacteria d) As a disinfectant
3. **What is the first step before starting a** microbiological experiment? a) Put on gloves b) Disinfect the workbench c) Turn on the microscope d) Prepare the culture medium	**8. A semi-solid medium usually contains:** a) 0% agar b) 0.3–0.5% agar c) 15–20% agar d) More than 50% agar
4. **Which of the following safety rules is correct?** a) Eating while handling bacteria b) Talking while handling bacteria to concentrate better c) Work seated, with lab coat buttoned, and hands washed d) Place all equipment randomly on the bench	9**. The quadrant streak method is used to:** a) Count bacteria in a solution b) Isolate pure colonies c) Sterilize the medium d) Prepare a liquid medium
5. **The role of the autoclave is:** a) Observe bacteria b) Incubate bacteria at controlled temperature c) Sterilize media, solutions, and materials d) Mix samples	**10 . Gram staining is used to:** a) Determine bacterial motility b) Differentiate Gram-positive and Gram-negative bacteria c) Sterilize the preparation d) Observe only fungi

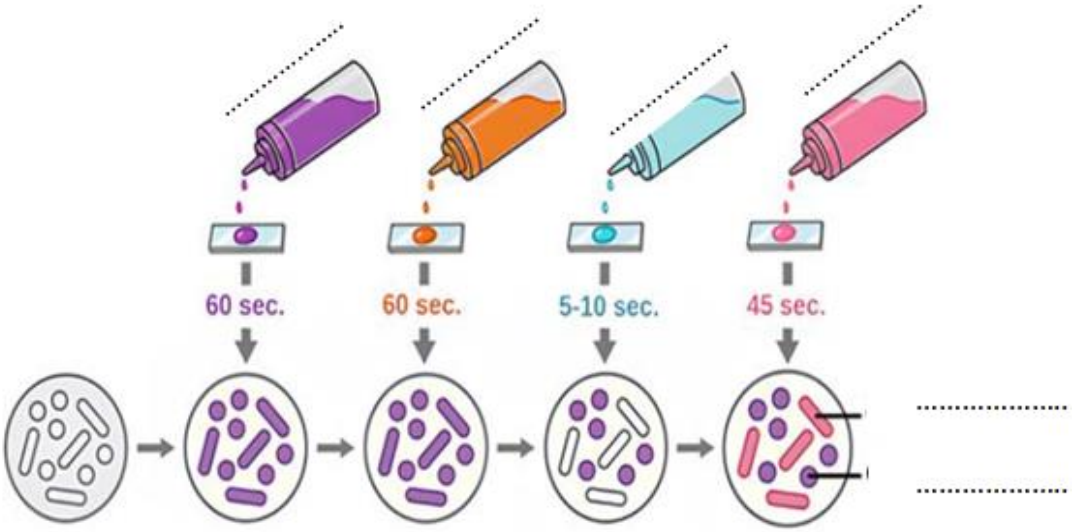
Amar Telidji University – Laghouat
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Food Science Students – 2nd Year Level
Duration: 1 hour 30 minutes

Last Name and First Name: …… **Mark:**

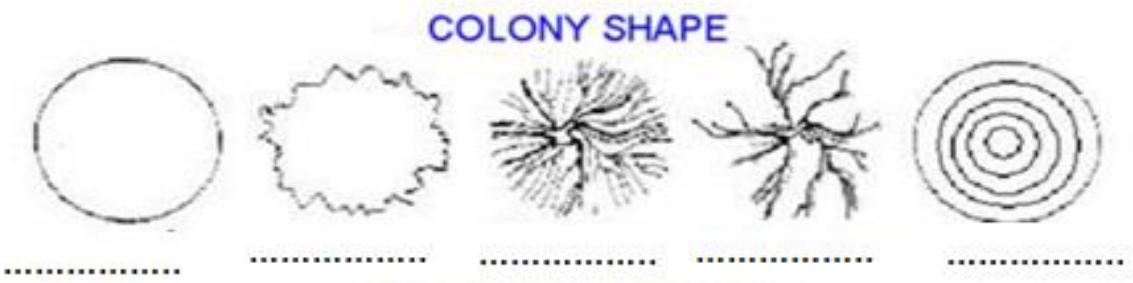
Second Microbiology Module Quiz

Q1 : Give a title and complete the labels of this diagram (5 pts)



Figue :…….

Q1 : Complete the labels of this diagram (2.5 pts)



Q1 : Answer with True or False and correct the false statement (2.5pts)

1. The simple staining technique is used only to observe bacterial motility

   ……

2. To prepare a wet mount from a liquid medium, the drop of culture must be covered with a coverslip, avoiding air bubbles.

   ……

3. Gram-positive bacteria appear violet after Gram staining.

   ……
   ……

4. All bacteria have the same oxygen requirements.

   ……
   ……

5. Bacterial growth corresponds only to an increase in cell size.

   ……