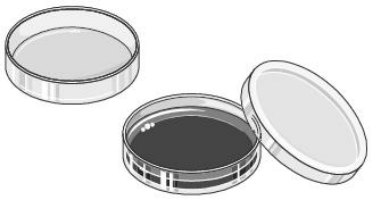
1. Basic manipulation methods (General manipulation techniques) :

1.1. Equipment

Glassware and equipment

Glassware : Petri dishes, Pasteur pipettes, test tubes, hemolysis tubes, beakers, pipettes, measuring cylinders/graduated cylinders, flasks, bottles/jars, microscope slides ……



Petri dishes

Pasteur pipettes

Test tubes

Hemolysis tube

Pipette

Microscope slides

Measuring cylinders

Flask

Bottles

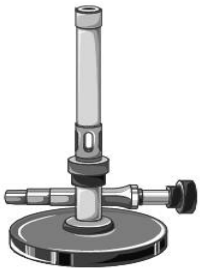
Beaker

Equipment: water bath, bacteriological incubator, spatula/scraper, Bunsen burner, platinum loop/wire, autoclave or moist heat generator, pasteur oven or dry heat generator, refrigerator, freezer, shaker/stirrer, grinder/crusher, balance, optical microscope.

Bain marie

Bacteriological incubator

Scraper



Bunsen burner

Platinum loop

Autoclave

Pasteur oven

Magnetic stirrer

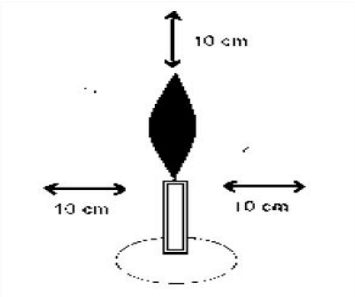
Balance

Optical microscope

Bunsen burner

A Bunsen burner, named after Robert Bunsen, is a kind of ambient air gas burner used as laboratory equipment; it produces a single open gas flame, and is used for heating, sterilization, and combustion

It should be noted that the blue flame, the hottest, creates a sterile zone approximately 20 cm in diameter.



Sterile zone of a Bunsen burner.

Pasteur oven :

This is a hot-air drying oven. It operates at 180°C for 90 minutes and is used exclusively for sterilizing pre-cleaned and dried glassware or metal instruments that can tolerate very high temperatures. It should be noted that the objects to be sterilized must be clean and perfectly dry, possibly stoppered with cotton plugs and wrapped in strong paper or aluminum foil.

Autoclave :

This is an essential piece of equipment in a microbiology laboratory (The autoclaving). It is a device that exposes the material to be sterilized to the action of pressurized steam, at temperatures ranging from 115 to 140°C for a determined period of time. Bacteria and spores are destroyed in 15 minutes at 120°C under a pressure of 2 bars.

Bain marie

In microbiology, Bain marie can be used as a sterilization tool. Indeed, heating for 10 minutes at 80°C is sufficient to destroy all vegetative forms of bacteria. However, spores are not destroyed under these conditions. The effectiveness of boiling can be improved by adding concentrated saline solutions to the water (which raises the boiling point).

This method can be used for sterilizing delicate liquid products: albuminous media, milk, gelatin, etc.

Stérilisation

Sterilization is a technique intended to destroy all living cells, viable spores, and acellular entities (viruses) in any material (glassware, plastic, food, etc.).

Pasteurization :

As the name suggests, this technique was invented in 1865 by the French scientist, Louis Pasteur.

It is used for natural food preservation for a limited time, destroying the vegetative forms , but not the

spores. It consists of heating the product to 65–75°C for 30 minutes (slow pasteurization) or to 85–90°C for 20 to 30 seconds (high-temperature pasteurization) and then cooling it abruptly to 10°C.

Ultra-High Temperature (UHT). This involves heating a product to a very high temperature (between 140 and 150°C) for a very short time (2 to 5 seconds).

This preserves its quality while destroying all microorganisms, thus providing a long shelf life (a long preservation time) (approximately 3 months).

Tyndallization :

This technique was invented in 1871 by the Irish physicist John Tyndall (1820-1893). Tyndallization is a series of 3 brief heatings at temperatures of 70°C at regular intervals. This is done to allow resistant forms (such as bacterial spores) to germinate so they can be killed in the subsequent heating cycle. It should be noted that this method can be used for the stabilization of certain media containing non-filterable thermosensitive substances of a certain viscosity, such as serum, vaccine, and egg.

1.2.Culture media and general culture techniques.

1.2.1. Culture media

A bacterial culture medium is a preparation in which bacteria can multiply. It must therefore satisfy the nutritional and physicochemical needs of the microorganism under study, which implies:

- Meeting the requirements for mineral ions and growth factors, and providing the carbon, nitrogen, and energy source.
- Having a pH close to the optimum pH (buffer system).
- Having an optimal ionic strength.

Before use, a culture medium must be sterile.

1.2.2. Types of Culture Media

Several types of culture media are distinguished:

- **According to their consistency**

  - **Liquid medium:** Does not contain agar-agar. Example: nutrient broth. Growth is indicated by turbidity, deposits, or surface pellicles.
  - **Solid medium:** Contains 1.5-2% agar-agar. They can be used in several ways: as slanted agar in test tubes, as a deep in upright tubes, or as a thin layer in Petri dishes. Microbial growth is indicated by the formation of colonies.
  - **Semi-solid medium:** Contains 0.5 - 0.75% agar-agar.

- **According to their composition :**
  - **Complex or undefined media:** These have a complex and poorly defined composition. They can be:
    - Of animal origin: milk, serum, nutrient broth and agar, gelatin, etc.
    - Of vegetable origin: soy peptone, potato, etc.
  - **Synthetic or defined media:** In which all components are known.

- **According to their use :**

  - **Isolation media:** Depending on the techniques used and the bacteria involved, they can be:
    - Basic media (e.g., Columbia agar, Trypticase soy agar...)
    - Media enriched with biological products (e.g., Blood agar, Milk agar...)
    - Selective media
  - **Enriched solid media**: allow for the growth of fastidious bacteria. Example: Blood agar medium, supplemented with blood (5 to 10%). Streptococci grow well on this medium since it contains growth factors. Furthermore, it allows for the determination of the hemolytic character of these microorganisms by the presence of a hemolysis zone surrounding the colonies.
  - **Selective solid media** allow for the isolation of a specific bacterial species while inhibiting other species that may be present in a sample.
    Example: Chapman's medium selects for halophilic bacteria like ***Staphylococcus*** because it contains up to 7.5% NaCl.
  - **Liquid enrichment media**: are used to promote the growth of a species present in small quantities in a sample.
    For example: Sometimes, the proportion of pathogenic bacteria may be minimal compared to that of the commensal flora, and there may be an absence of suspicious colonies on selective isolation. This is not sufficient to confirm the absence of these bacteria in the sample. Therefore, an enrichment culture is initiated at the same time as the (selective) isolation.
    Enrichment means increasing the representation (proportion) of a subgroup of microorganisms within a larger population.
    An enrichment medium combines two characteristics:
    1. It contains selective molecules that completely or partially inhibit the culture of non-target microorganisms, or it uses a specific incubation temperature, or a specific atmosphere.
    2. It is liquid (broth) so that its selective action can operate on a large and homogeneous population.

  - **Solid or liquid identification or differential media** : are used to reveal one or more metabolic characteristics of a previously isolated and purified bacterial strain. (The study of bacterial

metabolism can and should only be performed on a pure bacterial culture).

Example: Hugh and Leifson medium ("liquid and green"), which allows for the distinction between the two processes: respiratory and fermentative.

- **During respiration:** The carbohydrate is oxidized to CO_2 by oxygen (or another inorganic oxidant); there is **no change** in the color of the medium.
- **During fermentation:** The carbohydrate is oxidized into acids, alcohols, which are released into the medium, acidifying it. There **is a change** in the color of the medium due to the presence of a pH indicator, bromothymol blue, which turns yellow in an acidic medium.

- **Transport media:** They are used for shipping or temporarily storing material (such as a sample) that will be examined later for the presence of certain organisms. The primary function of the medium is to keep these organisms alive, in case they are present. It is not necessary for a transport medium to support growth; in fact, growth can be a disadvantage, as the waste products formed are likely to have an unfavorable effect on the survival of the organisms.
  Example:*Stuart's transport medium (salts, 0.5% agar, sodium thioglycollate, methylene blue) is suitable for a wide range of anaerobic bacteria and for "fragile" organisms, such as *Neisseria gonorrhoeae*.
- **Preservation media:** These are nutrient-poor media that maintain bacteria in a state of slowed or dormant life.

1.2.3. General culture techniques

Inoculation (Seeding), which is the transfer of bacteria to a new culture medium, must be carried out under completely aseptic conditions. Cultures are grown in liquid or solid media.

3.1.Cultures in liquid media

- **Culture from a liquid sample**

The sterilized (flamed) and cooled loop is introduced aseptically into the sample and then inserted sterilely into the medium to be inoculated.

- **Culture from a solid sample**

After loading the inoculating loop with the sample, it is introduced into the tube to be inoculated and brought just into contact with the liquid. The loop is then scraped against the wall of the tube to obtain a thick, well-mixed suspension, which is subsequently mixed into the entirety of the culture medium.

After inoculation, the broth is incubated at 37°C. Following 24 to 48 hours of incubation, observe the presence or absence of, its appearance, its color, as well as the presence of a pellicle (film).

3.2.Cultures on Solid Media

- **Culture in Tubes**

A. Slanted Solid Media (agar slant):

- **Cross-streak inoculation**

  Starting 0.5–1 cm from the bottom of the slope, use the inoculated loop to make tightly spaced, parallel streaks on the agar surface without scratching it. Incubate at 37°C for 24–48 hours.

B. Stab culture media

- **Inoculation by stabbing (Stab Inoculation)**

  The perfectly straight metal wire (platinum or nichrome) (or a Pasteur pipette), loaded with the inoculum, is used to make a central stab. The tube is then incubated for 24–48 hours at 37 °C.

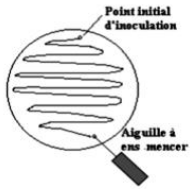
Stab Inoculation

Example: Tube containing Mannitol Motility Medium for the study of microbial motility.

- **Petri dish cultures using the streaking method**

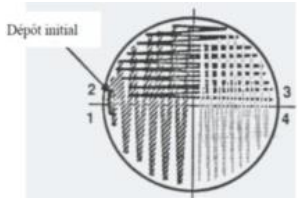
- **Parallel Streak Inoculation**

The inoculum, taken from a culture broth, is placed at a peripheral point on the agar plate. The small droplet of culture is spread across the entire agar surface by making parallel streaks. The plate is then incubated at 37°C for 24–48 hours.



- **Quadrant Streak Method**

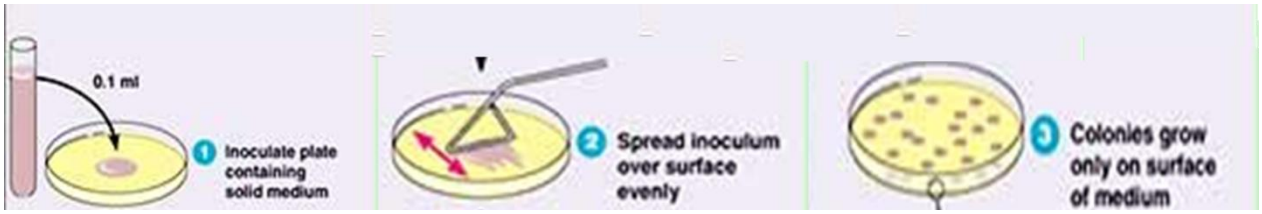
The inoculum, taken from a culture broth, is placed at the periphery of the agar. It is then spread by making parallel streaks across half of the plate. Rotate the plate a quarter turn and inoculate with streaks perpendicular to the initial ones. After another quarter turn of the plate, inoculate the final quadrant.



- **Spread Plate or Surface Inoculation**

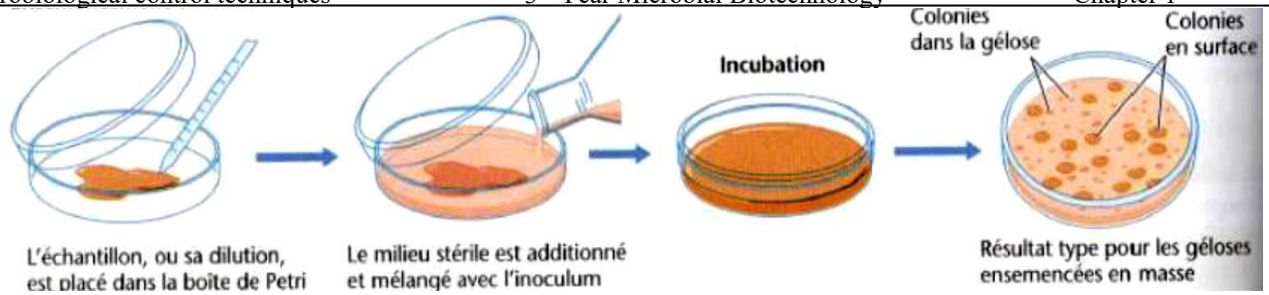
  This method is preferable when selective media are used for the enumeration of a specific group of aerobic microorganisms.

  It allows for the manifestation of colonial properties of these microorganisms, such as: morphology, pigmentation, hemolysis, precipitation halos, or changes in the color of the culture medium.



- **Inoculation by incorporation (pour plate method)**

Sterilely transfer 1 ml of inoculum into an empty, sterile Petri dish. Pour over it the contents of a tube of molten agar (at 45–50°C). Mix the medium and inoculum by swirling in opposite circular motions. Allow it to cool and solidify before incubating the inverted plate at 37°C for 24–48 hours.



- **ROLL TUBE COUNT**

As the name suggests, this technique involves the use of 25 mL screw-cap tubes. 2 to 4 mL of agar medium is poured into these tubes, which are then kept in a super-cooled state (45°C) in a water bath.

0.1 mL of each dilution is added to the tubes, which are then rolled horizontally under cold water until the medium forms a uniform layer on the walls.

It was proposed for the culture of strict aerobic bacteria and Koch's bacilli (tuberculosis bacteria), which are difficult to grow.

The medium used is transparent, allowing for easy colony counting.

- **Agar droplet technique**

This technique is proposed in a miniaturized and cost-effective format.

It involves mixing a diluted homogenate of any food product into a melted and cooled (45°C) agar medium, which is then distributed in 0.1 mL droplets onto the bottom of a Petri dish.

It is therefore possible to place 5 droplets from 4 successive dilutions in the same plate.

After incubation, a special projector enlarges the diameter of the droplets, allowing for the counting of pinpoint colonies.