Lab – 4

Sterilization and Disinfection

- **Sterilization: is the killing or removal of all microorganisms, including bacterial spores, which are highly resistant.**

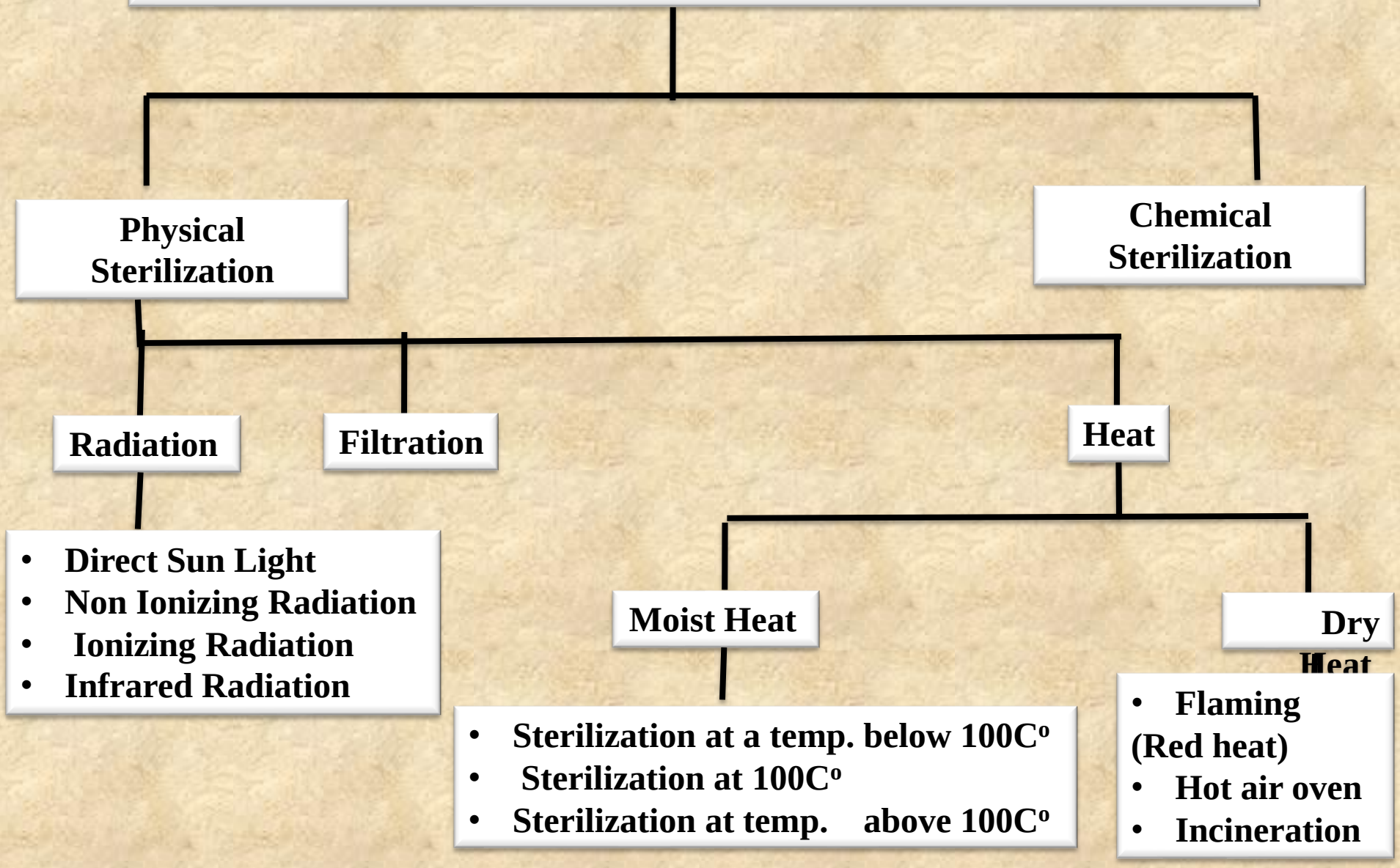
e.g.:
- Sterilization by autoclave (121^{o}C , 15Klb).
- Ethyl oxide gas for surgical instruments.
- Filtration for intravenous solution.

- **Disinfection: is the killing of most, but not all microorganisms, mainly the pathogenic ones.** For adequate disinfection, pathogens must be killed but some organisms and bacterial spores may survive.

- Disinfectants **vary in their tissue-damaging** from the **phenol**, which should be used only on inanimate objects, to less toxic materials such as **ethanol** and **iodine** which can be used on skin surface.

- Chemicals used to kill microorganisms on the surface of skin and mucous membrane are called **antiseptics**.

Classification of Sterilization Methods



Classification of sterilizing agents:

1-physical agents:

A. heat

B. Filtration

C. Radiation.

2- Chemical agents.

Physical sterilization

1. Heat: Most effective, **cause stope cellular activates by coagulate proteins or oxidize cell components.**

A. Dry Heat

1. Direct flame: the simplest method is direct flaming. Used for rapid and repeated sterilization. This Type of sterilization is used for metal instruments such as platinum wire loop, forceps and scissors etc.

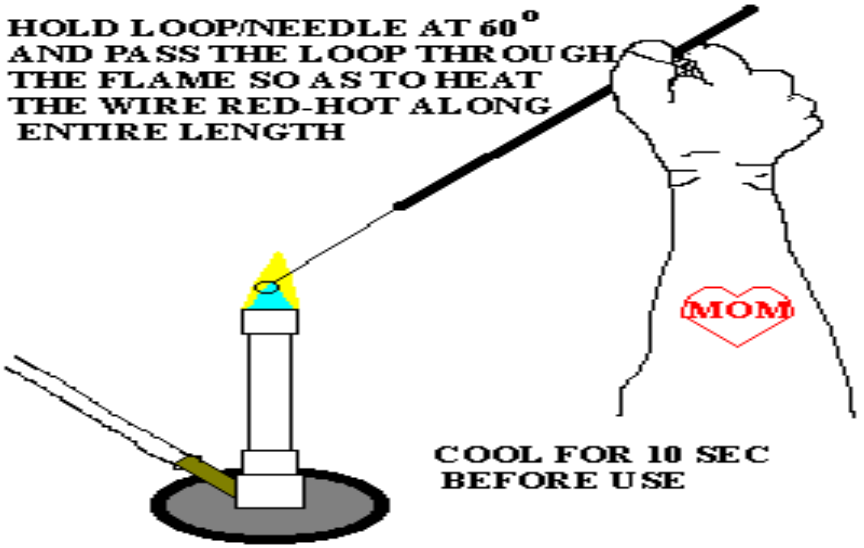
Bunsen burner is commonly used for sterilization by dry heat (direct flame).

2. Hot air oven: All glassware and metal instrument are usually sterilized by this method. Generally, the instruments are left in a **Hot Air Oven** on a **temperature of 160-180ºC** for **one hour.**

3. Incineration: provide an excellent means of rapidly destroying articles such as solid dressings, pathological material, animal caresses and bedding.

FLAMING

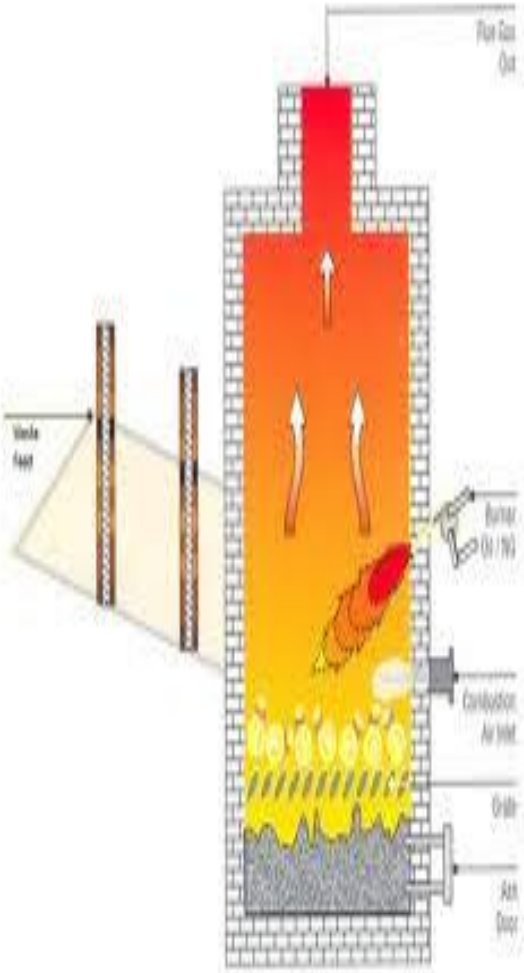
- A simple & effective method.
- Loops or wires, needle.
- Instruments held on a Bunsen flame till red-hot.

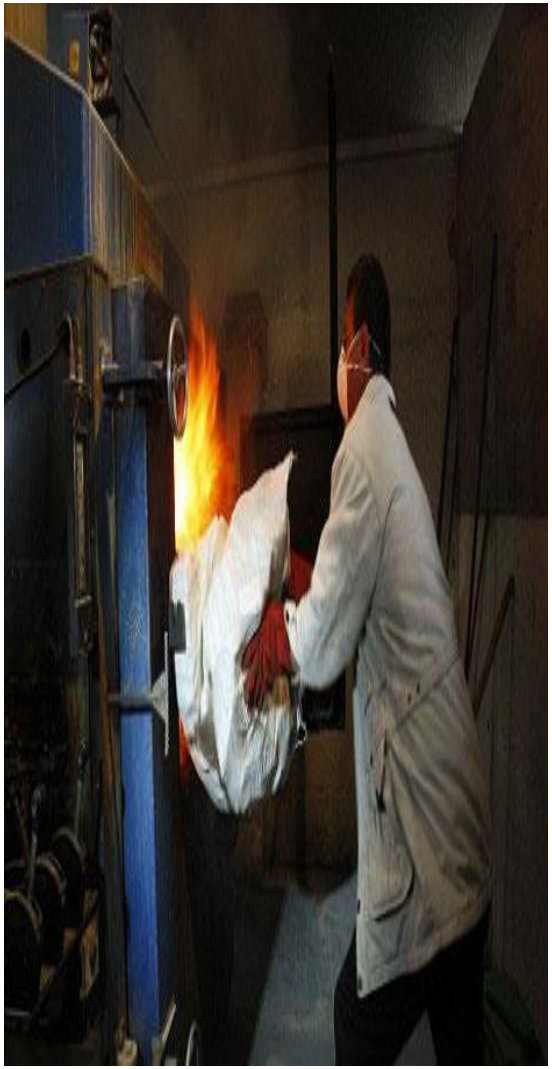


Hot air oven

Glassware

Incinerator





B-moist heat

1.Pasteurization (Below 100ºC): uses heat 63ºC at 30ºC minutes.

The organisms such as Brucella or Salmonella and tubercle bacilli which contribute to milk born-disease are readily killed by this process.

N.T: bacterial spores are not killed by this method.

2.Boiling water (At 100ºC): A temperature at 100ºC will kill all non-sporing and vegetative organisms within 10 minutes. Most spores will be killed in 30 minutes at this temperature, but some spores will resist boiling for several hours.

- The addition of **2% sodium carbonate** increases the disinfecting power of the water, and spores resistant to boiling water for 10 hours have been killed in 30 minutes by this addition.

This method is suitable for infected instruments or small pieces of infected glassware.

3. Tyndallization (At 100ºC): the process of sterilization by **intermittent steaming**. It is a form of sterilization that involves boiling at 100 ºC for about 30 minutes a day, for three successive days. After heating the medium is incubated to allow spores to germinate and vegetative organisms to grow which will be killed by the second steaming, the third heating is a safety precaution. Used for culture media containing sugar or gelatin and for sterilization of canned food.

4.Steam under pressure (High pressure steam, above 100ºC):

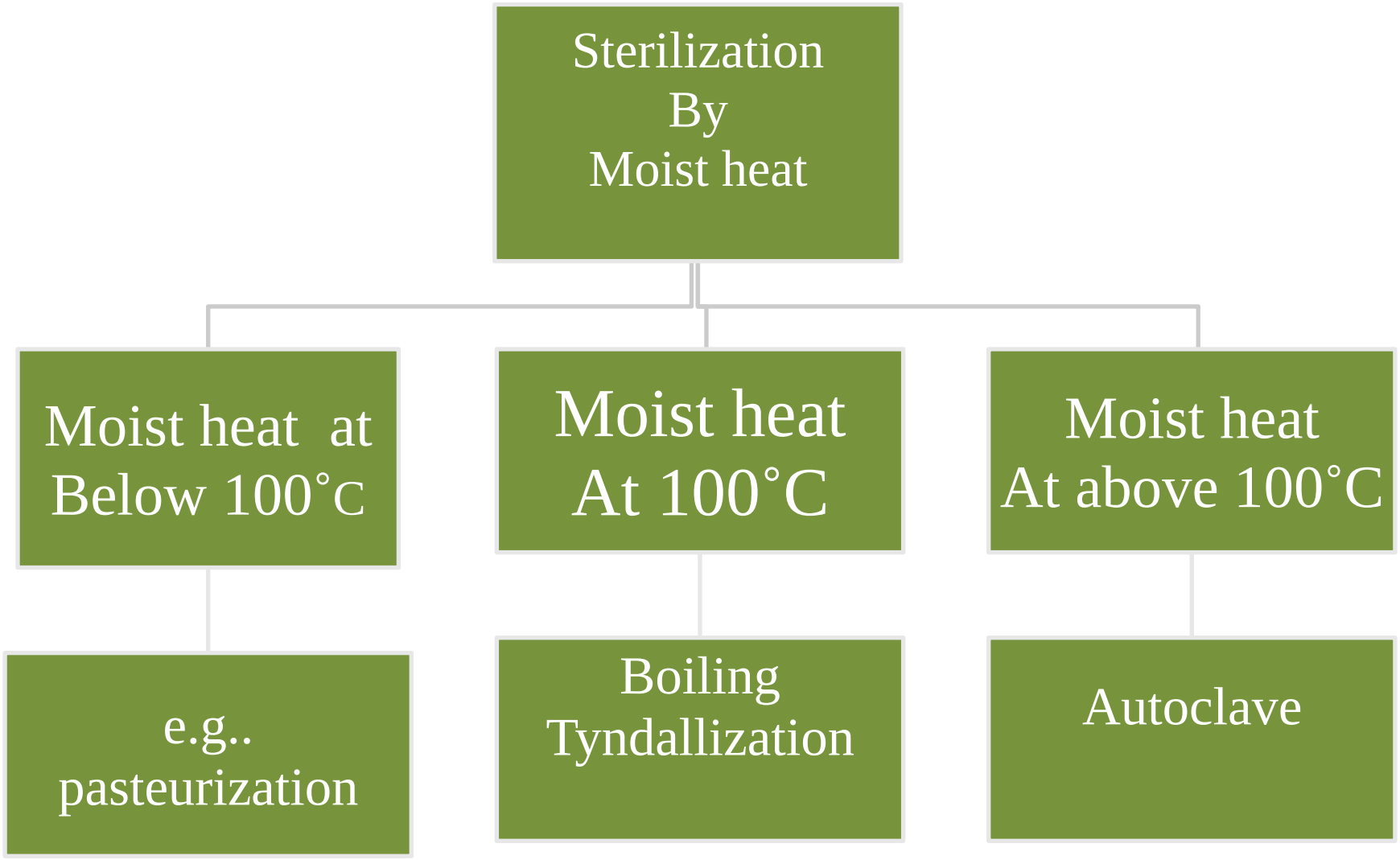
The autoclave is the instruments used for high pressure steam sterilization. the minimum values required for sterilization are **121ºC, 1atm (15 pound / inch) for 15 minutes.**

This is the usual method of sterilizing Bacteriological media,

surgical instruments, towels, dressings, gloves …

Autoclave

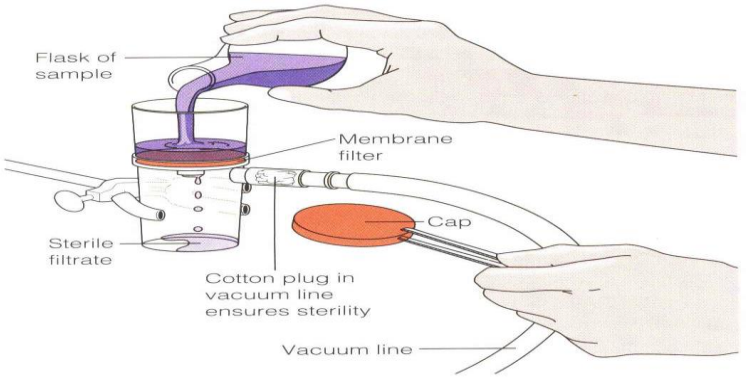
MOIST HEAT


Sterilization
By
Moist heat
Moist heat at
Below 100˚C
Moist heat
At 100˚C
Moist heat
At above 100˚C
e.g..
pasteurization
Boiling
Tyndallization
Autoclave

2. Filtration: The principle of this method is to pass the material to be sterilized through special bacterial filters which hold back any bacteria present.

- This method is used for the sterilization of **fluids** that do not withstand heating, **e.g. Sera, Plasma, Vitamins and Antibiotic solutions.**

- **Seitz filters is** a kind of bacterial filters that used in filtration.



3.Radiation: It is employed commercially for the sterilization of large amount of pre-packed disposable items such as **plastic syringes** and **Catheters** that are unable to withstand heat.

- It is done by applying **ultra-violet rays or ionizing radiation (X ray)**

sterilized by ionizing radiation

Chemical sterilization

It act on lipid content of cell membrane, denaturation of protein and on nucleic acid, it either kill or stop the growing of m.o. we had 2 types:

A. potent: called **disinfectant**

1-Phenols: sterilization of **surgical instruments**, **bathroom**, **hospital floor**.

2- Chlorine: treatment of swimming pools and water supplies.

3- Strong alkaline and acids: e.g NaOH used for treating sputum for detection TB (*Mycobacterium tuberculosis*) and to decrease viscosity

B. Mild: **called Antiseptics**, it's less toxic and can be applied to living tissue like skin.

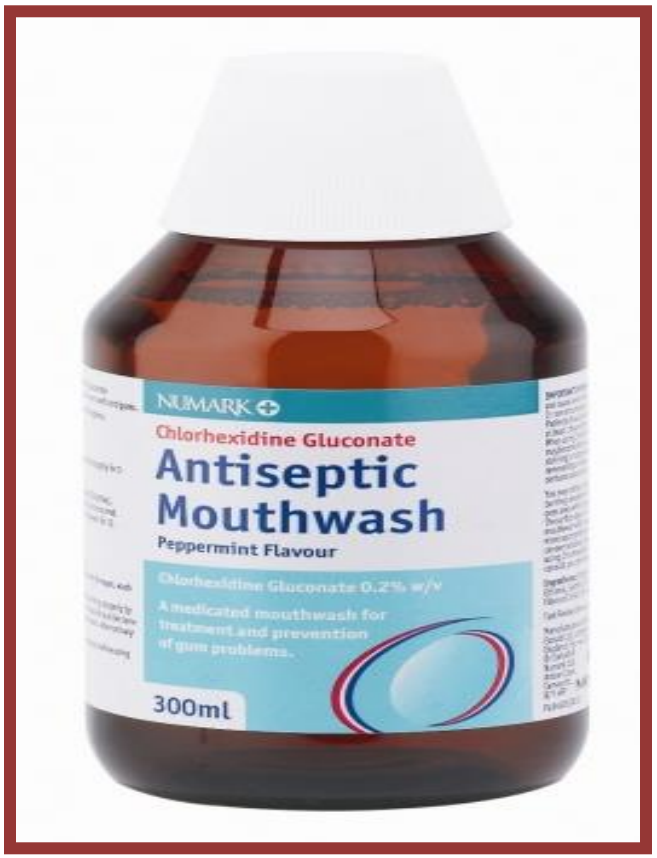
1- Dettol: Chlorohexidin ; as skin Antiseptics used to treat surgical wounds because it work on both G +ve and G –ve.

2- Iodine: used in surgery to sterile skin pre-operation.

3- H_2O_2: for disinfection of contaminated wounds or by anaerobic bacteria.

4- Soap and Detergents: Soaps to clean the skin.

5– **Alcohol** at concentration 70% because it can penetrate tissue easily at that concentration better than at 99% (absolute). Used to sterilize the **thermometer** and **skin** before injection.

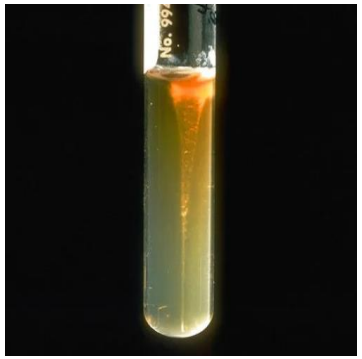


Chlorhexidine agents

Iodine (Betadine): used in surgery to sterilize skin pre-operation

Bacterial Motility

(Hanging Drop Technique)



Motility testing

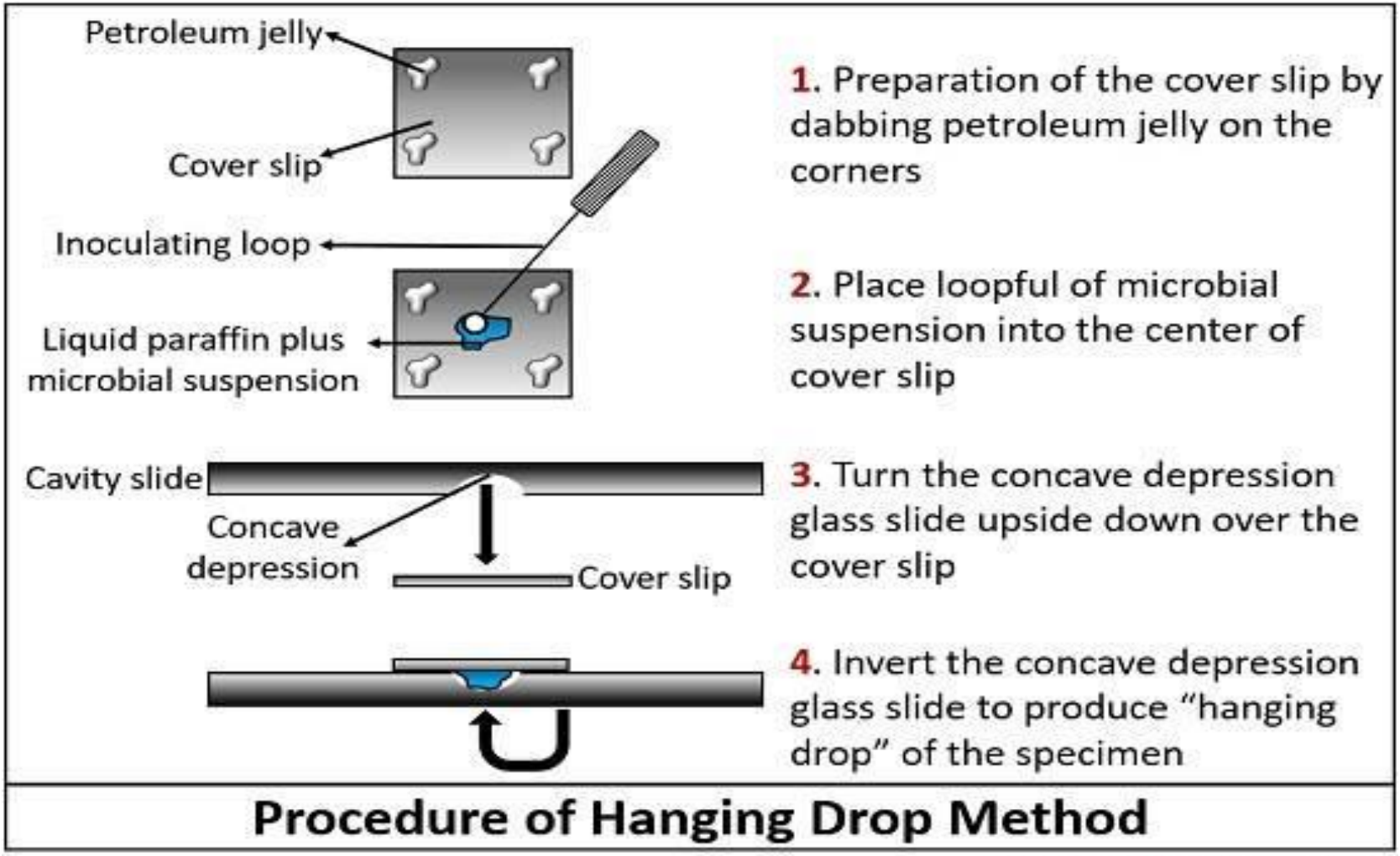
Motility could be detected by:

1) Hanging Drop technique.
2) Semi-Solid media Inoculation.
3) Flagella stain.

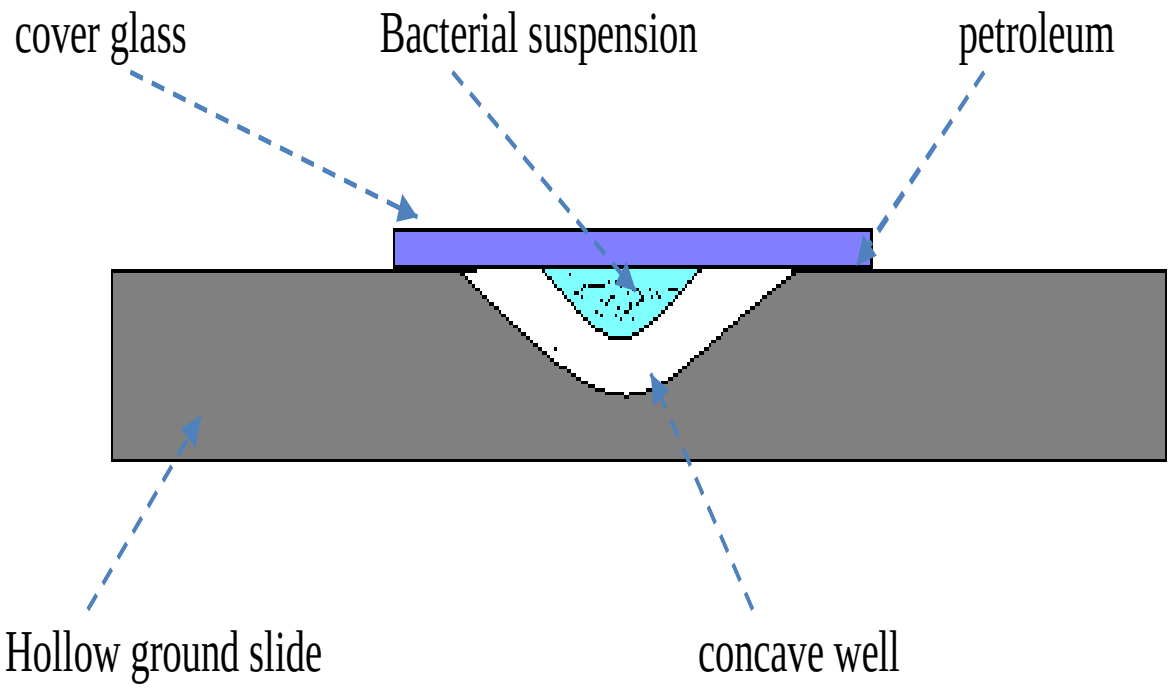
1. The Hanging-Drop Preparation

- The simplest method for examining living microorganisms is to suspend them in a fluid (water, saline, or broth) and prepare a "hanging drop," using a cover glass and a **hollow ground slide**.
- The slide is ground with a concave well in the center
- This slide is used to determine whether an organism is motile or not.
- Such a slide can be observed for a fairly long time period, because the drop does not dry up quickly.

Hanging drop technique



Hanging Drop slide



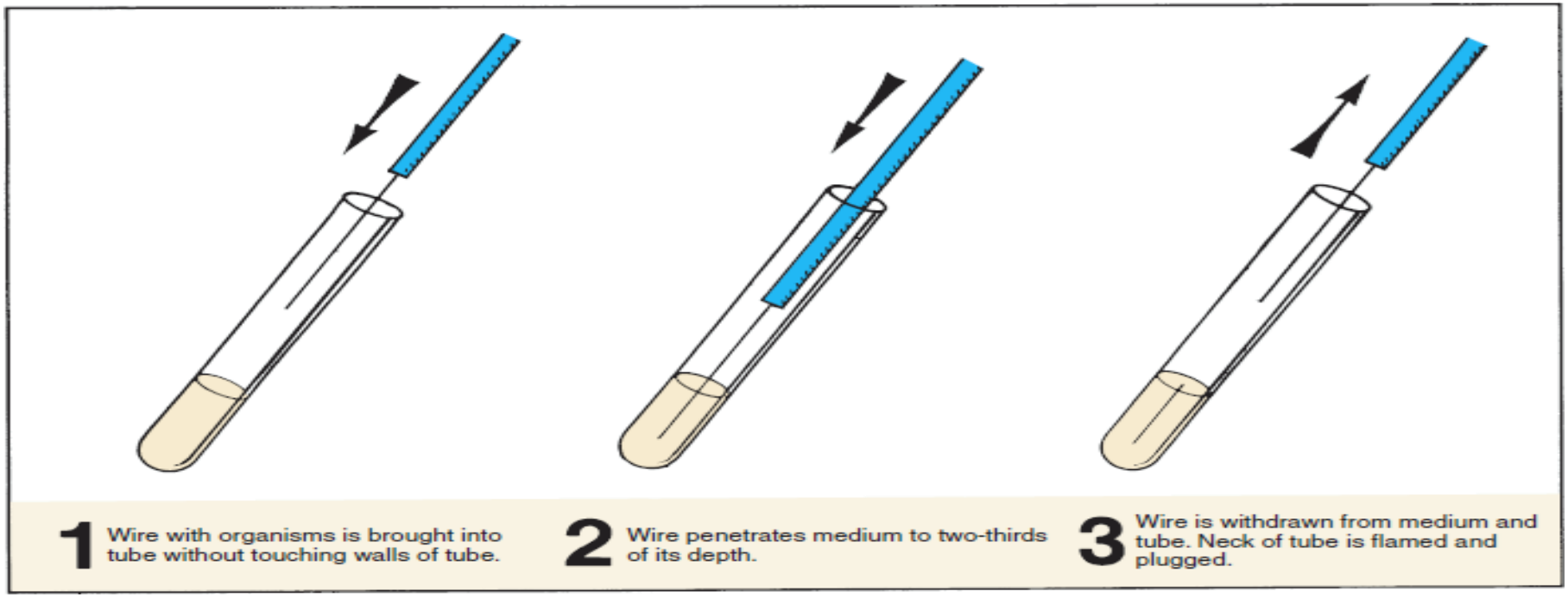
2. Semi-Solid media Inoculation

- The most commonly used test for motility in microbiology lab.
- It depends on the ability of motile bacteria to move through semi-solid media.
- Ordinary solid media contain 2 % Agar
- Semi solid media contain about 1% Agar

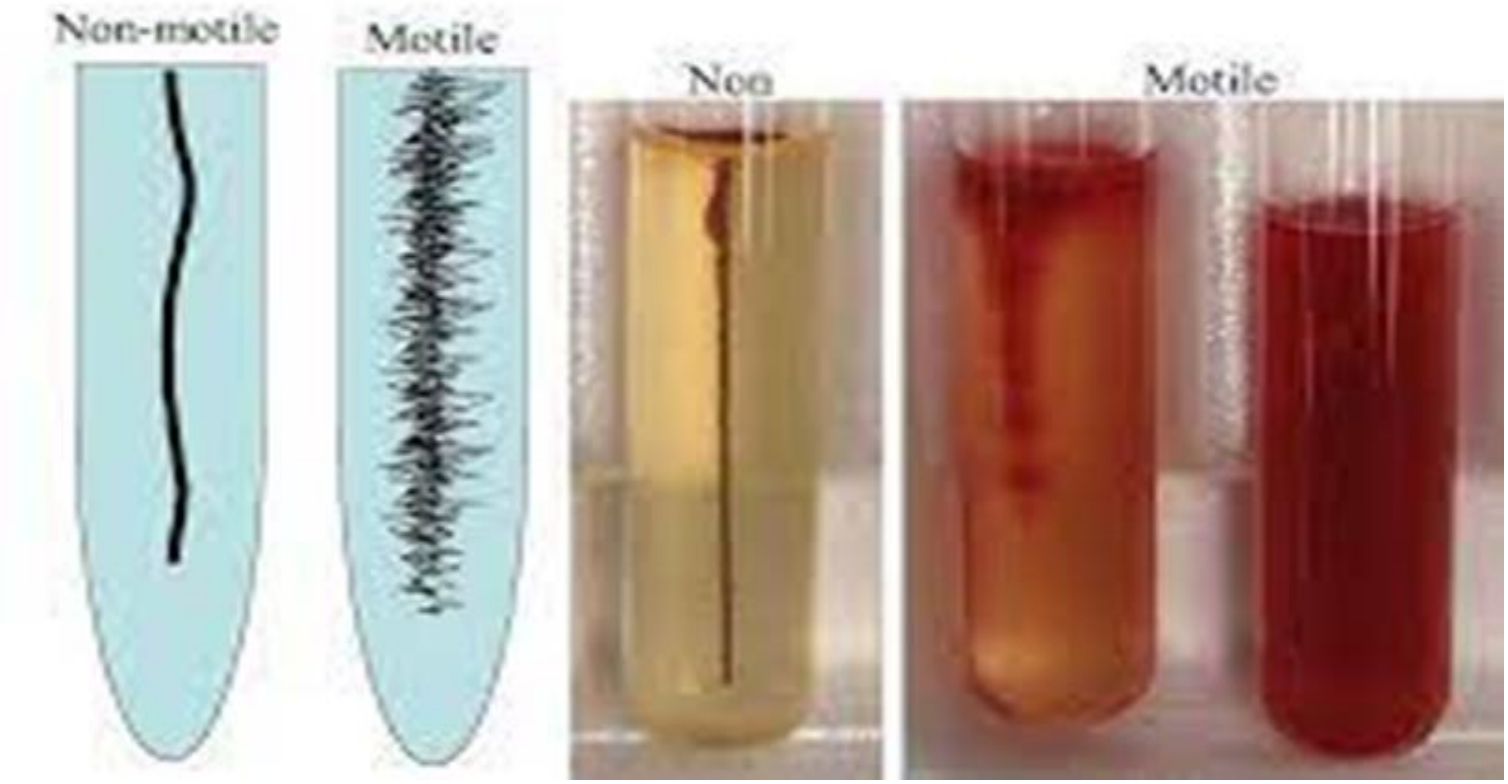
Procedure of Motility Test

How to Perform Test:

- Using a sterile bacteriological needle, pick a colony of the test organism
- Stab quickly a tube of semi solid media. (avoid using bent needles).
- Incubate the semi solid media for 24 hours

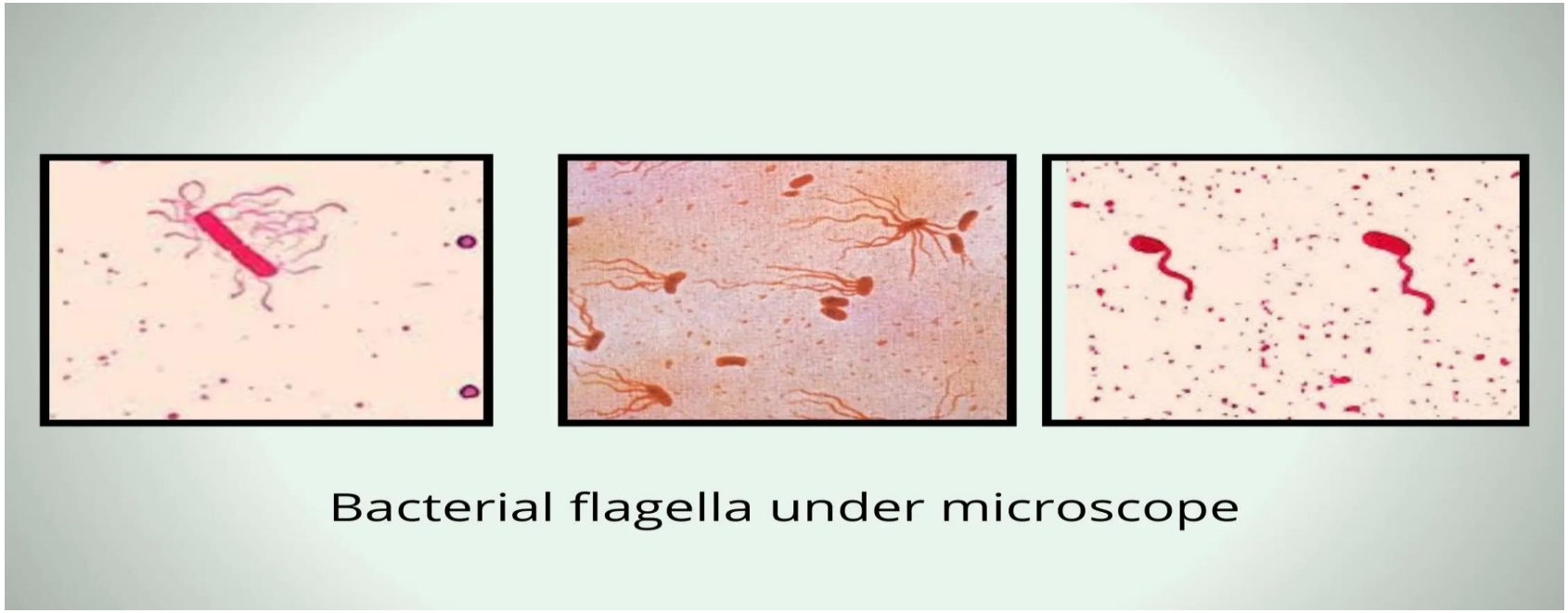


Motility Test Media Results



3. Flagella staining

1. Rosanalin dye
2. Silver nitrate + ferric tannate



Thank You