

Advanced laboratory technique

Lecture 1



Sterilization and
Disinfection

Objectives



Define	Define [bactericidal, bacteriostatic, sterilization, disinfection]
Classify and enumerate	Classify and enumerate different methods of sterilization
Explain	Explain the principle and applications of each type
Explain	Explain the principle and operating conditions of autoclave and hot air oven.
Enumerate	Enumerate categories of disinfectants
Enumerate	Enumerate some commonly used disinfectants.

Definitions

Bactericidal	kills bacteria
Bacteriostatic	inhibits growth of bacteria

Definitions

Sterilization	removal or killing of all living microorganisms including bacteria and their spores
Disinfection	removal or killing of disease-causing microorganisms (not necessarily all microorganisms)

Decreasing
order of
resistance

Most Resistant

Prions

Endospores of bacteria

Mycobacteria

Cysts of protozoa

Vegetative protozoa

Gram-negative bacteria

Fungi, including most fungal spore forms

Viruses without envelopes

Gram-positive bacteria

Viruses with lipid envelopes

Least Resistant

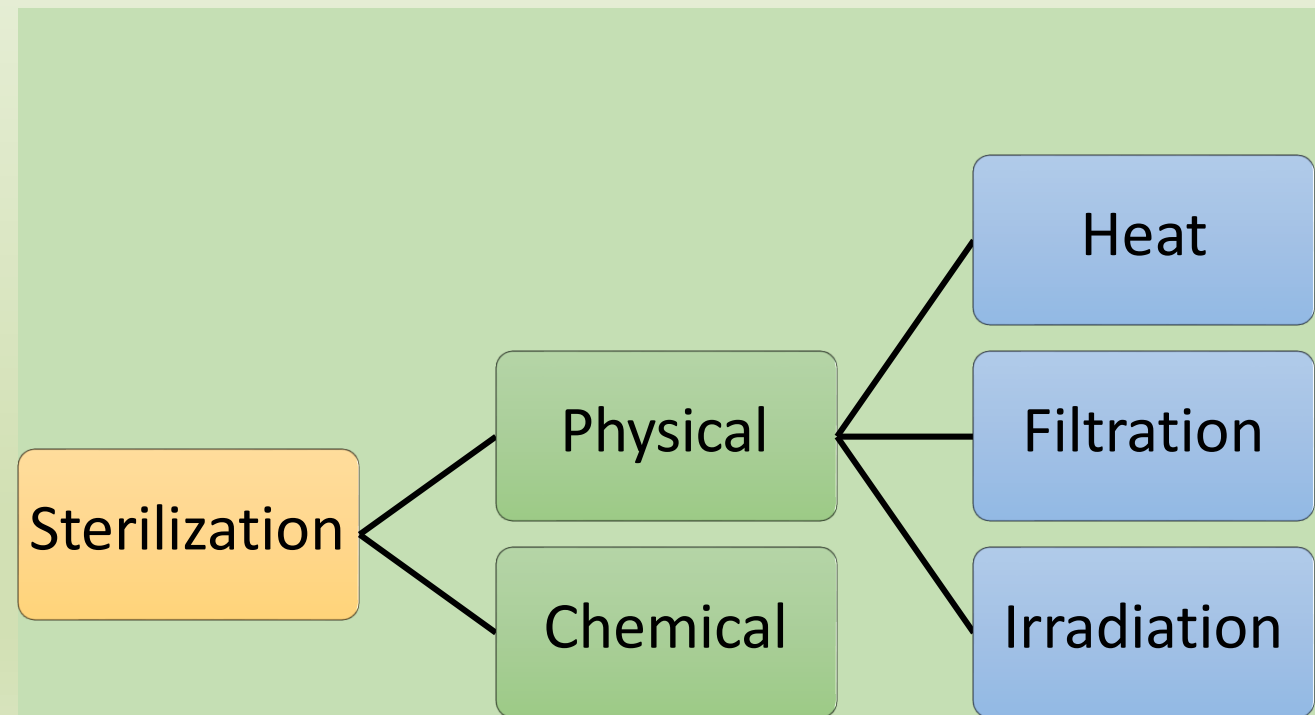
Methods of sterilization:

There are two methods of sterilization:

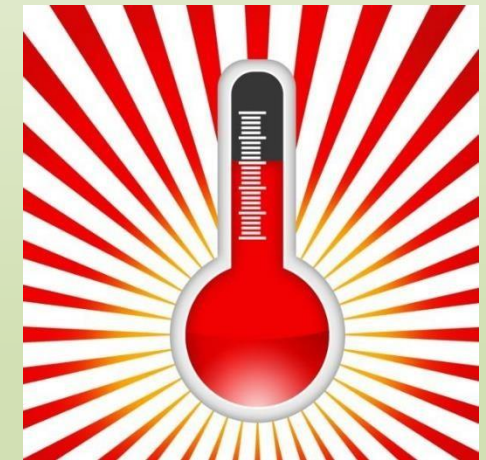
A- **Physical methods:**

1. Sterilization by Heat
2. Sterilization by Filtration
3. Sterilization by Irradiation

B- **Chemical methods**



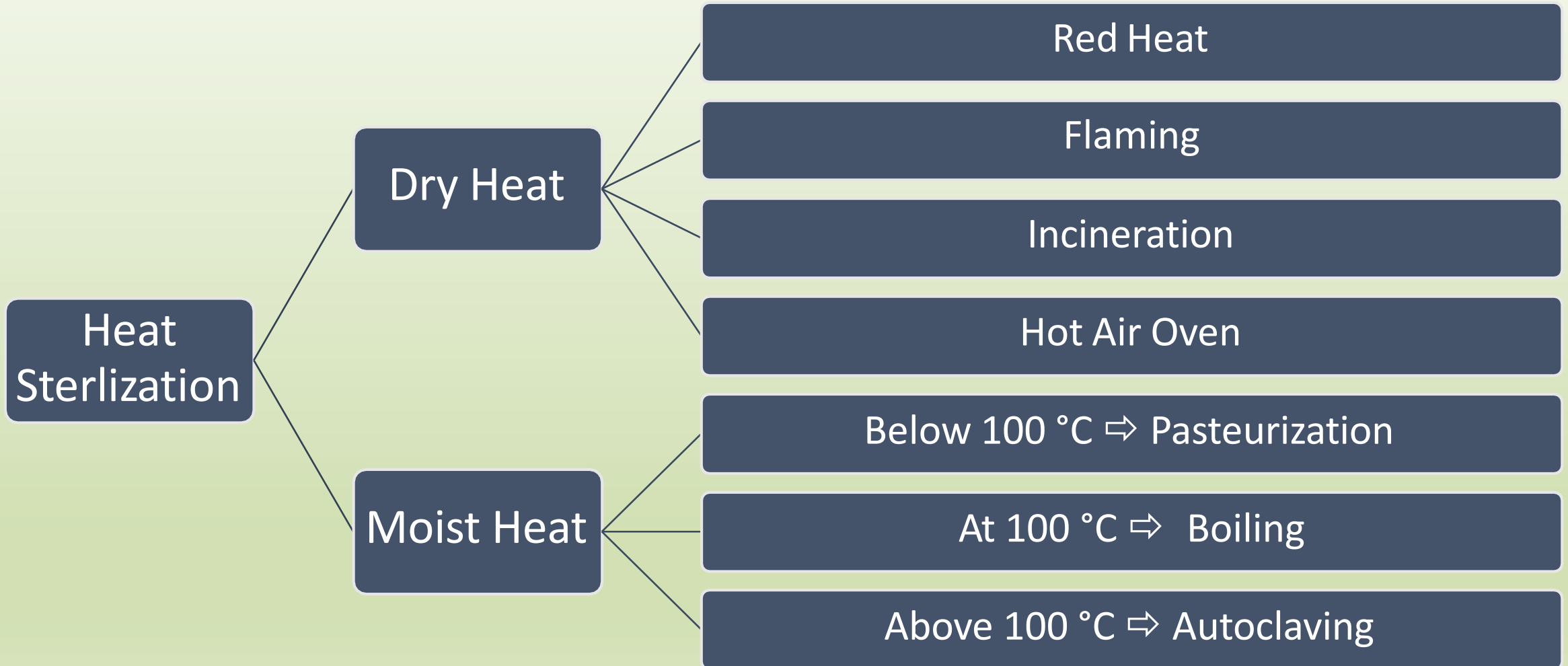
A. Sterilization by heat



Sterilization by heat

- Heat is the **most practical, reliable,** and **inexpensive** method of sterilization.
- It is used for sterilization of objects and materials that can **withstand** high temperatures.
- It can be either:
 - Dry heat
 - Moist Heat

Methods of Sterilization



1. Red Heat

Principal:

Holding object in Bunsen flame till they become red hot.

Used for:

Sterilization of:

- Bacteriological loops
- Tips of forceps



2. Flaming

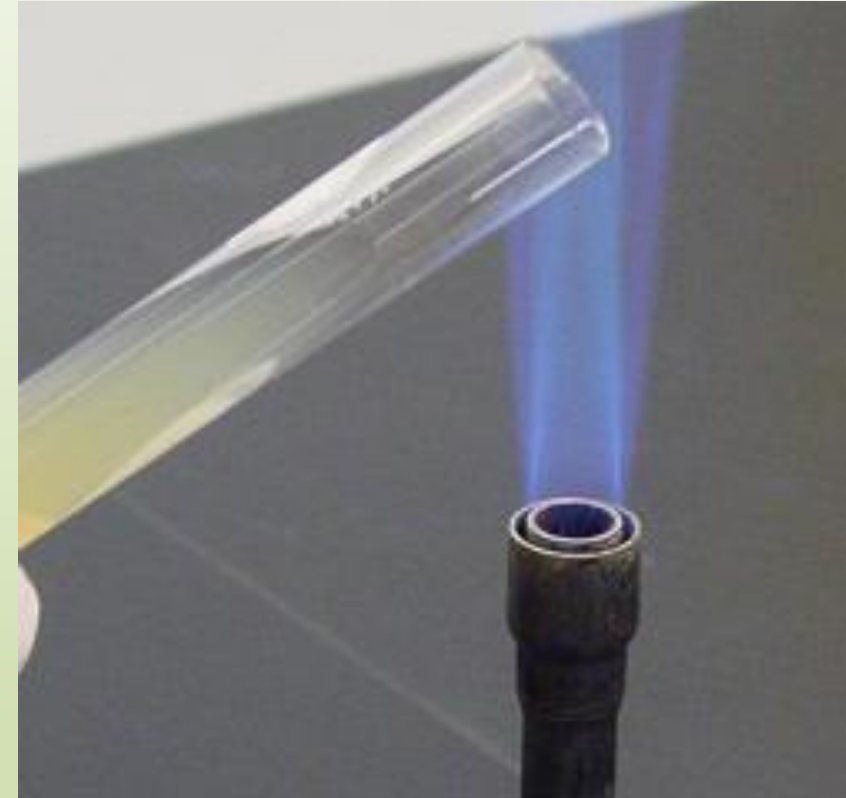
Principal:

Passing the object through the flame of Bunsen burner without heating to redness.

Used for:

Sterilization of:

- glass slides
- mouth of culture tubes.



3. Incineration

Principal:

Infective materials is converted to sterile ash by burning in incinerator

Used for:

Destruction of contaminated disposable materials (**waste**)



4. Hot air oven

Principal

- Articles to be sterilized are exposed to high temperature in an electrically heated oven.
- Even distribution of heat throughout the chamber is achieved by a fan.

Holding time:

- 160°C for two hour
- 180°C for one hour

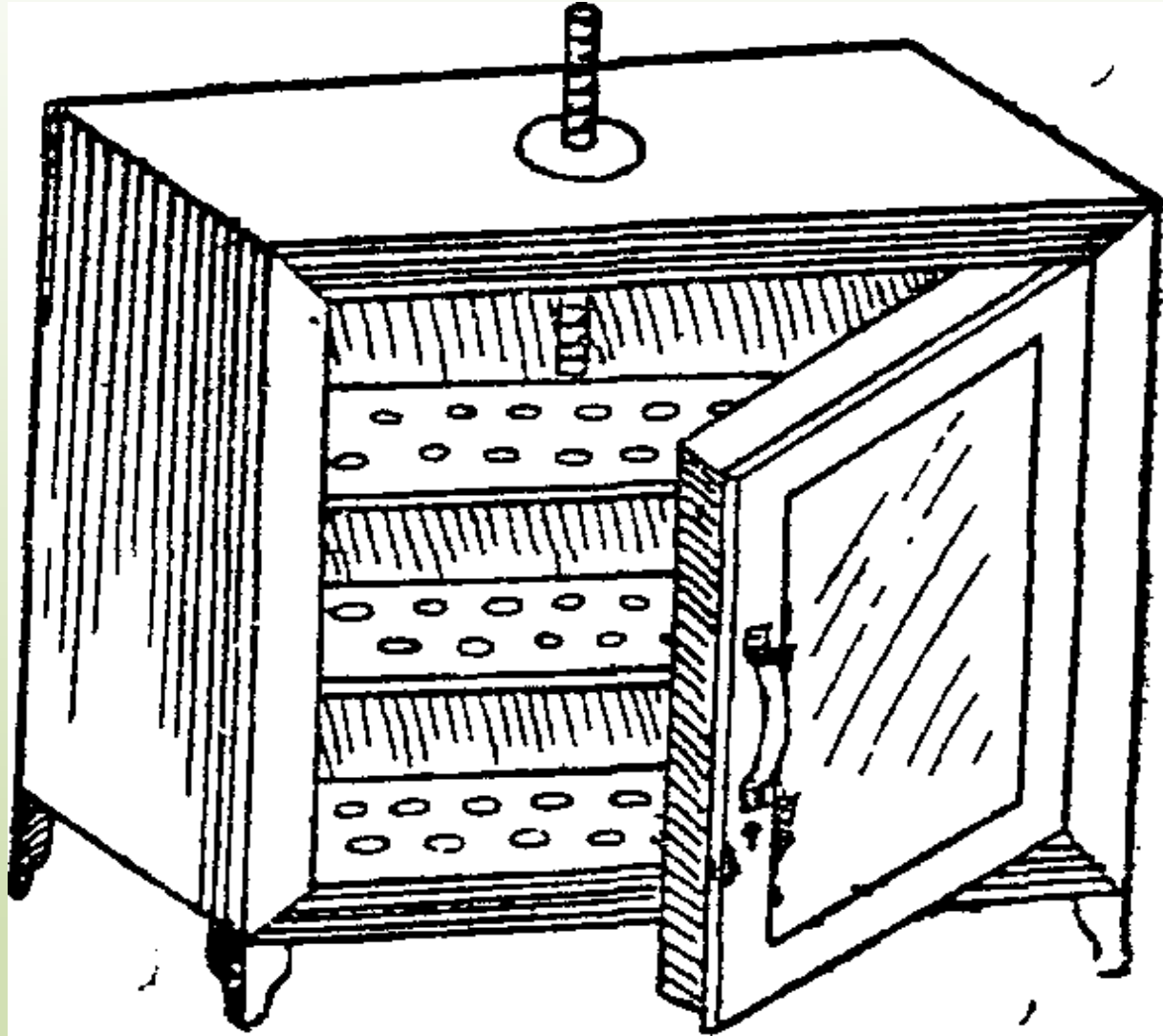


4. Hot air oven

Used for Sterilization of:

- All **glasses**: test tubes, Petri dishes, flasks, pipettes.
- **Instruments**: as forceps, scalpels, scissors
- **Dry material** in sealed containers as fat, oils, powder.





Hot air oven

Moist Heat

Can be used at different temperatures:

- below 100 °C → Pasteurization
- At 100 °C → Boiling
- Above 100 °C → Autoclave

1. Pasteurization (Below 100)

Principal:

Pasteurization is a process of **heating** a liquid to a specific temperature for a definite length of time and then **cooling** it **immediately**.

Used for:

Pasteurization is commonly used in milk processing.



2. Boiling (At 100 °C)

Principal:

Boiling in water for fifteen minutes will kill most vegetative bacteria and inactivate viruses.

However boiling is ineffective against many bacterial and fungal **spores**.

Used for:

Kill most vegetative bacteria and inactivate viruses.

3. Autoclaving

Principal:

- When the pressure is increased inside a closed container, the temperature at which water boils exceeds 100°C.
- At **double** atmospheric pressure the temperature of the steam reaches **121°C**.
- Autoclaving is the most **reliable** method of sterilization that kills all kinds of bacteria and spores.

3. Autoclaving

Temperature of sterilization:

- 121°C for 20 – 30 minutes.

Used for sterilization of:

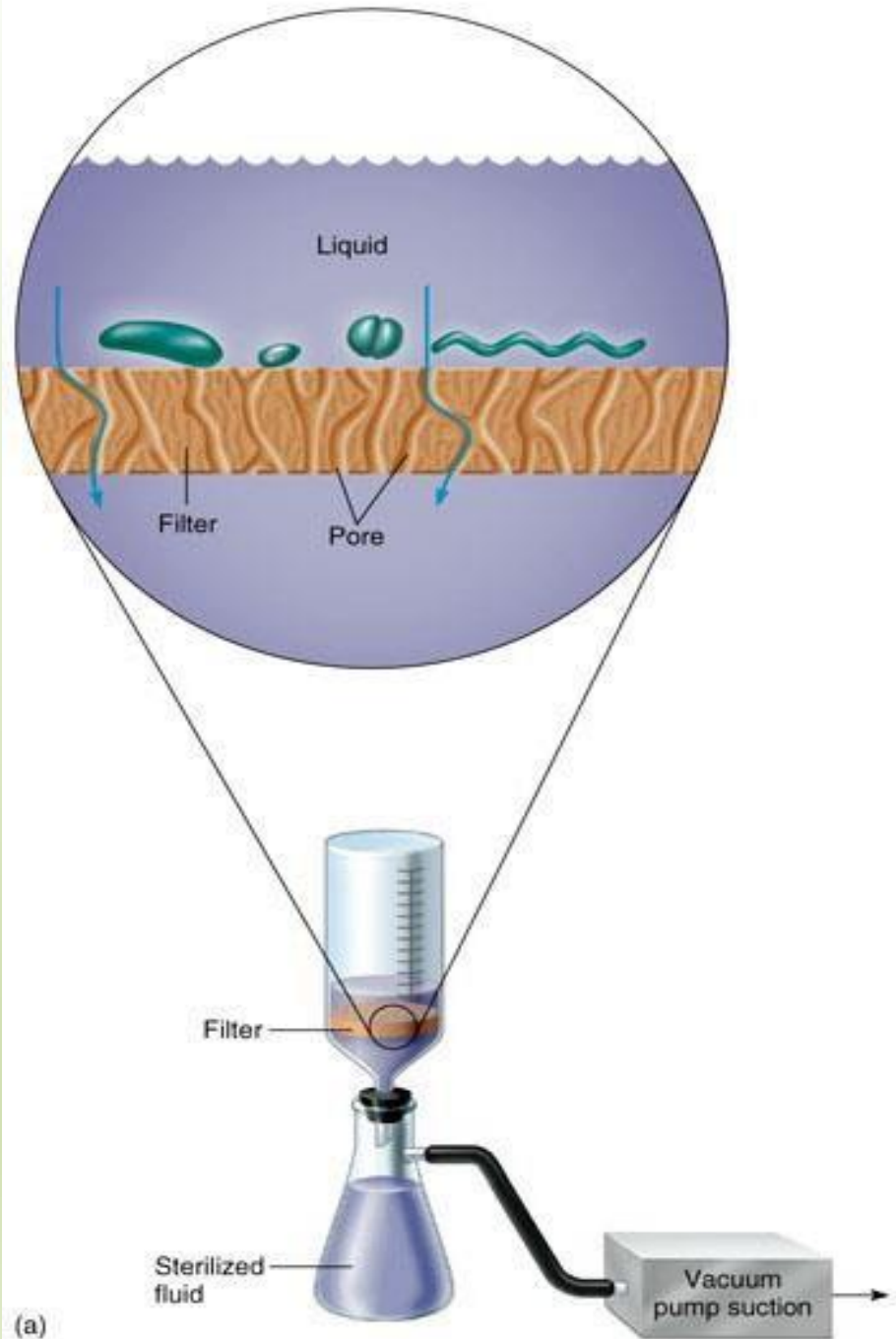
- Culture media.
- Surgical supply e.g. dressing, and surgical instruments.



Filtration

Filtration

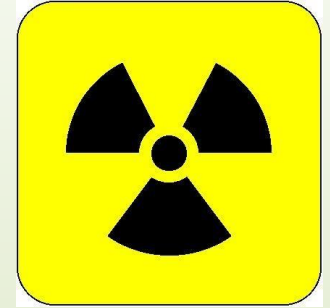
It is possible to remove bacteria from fluids by passing them through filters with pores so small that bacteria are arrested.



Filtration

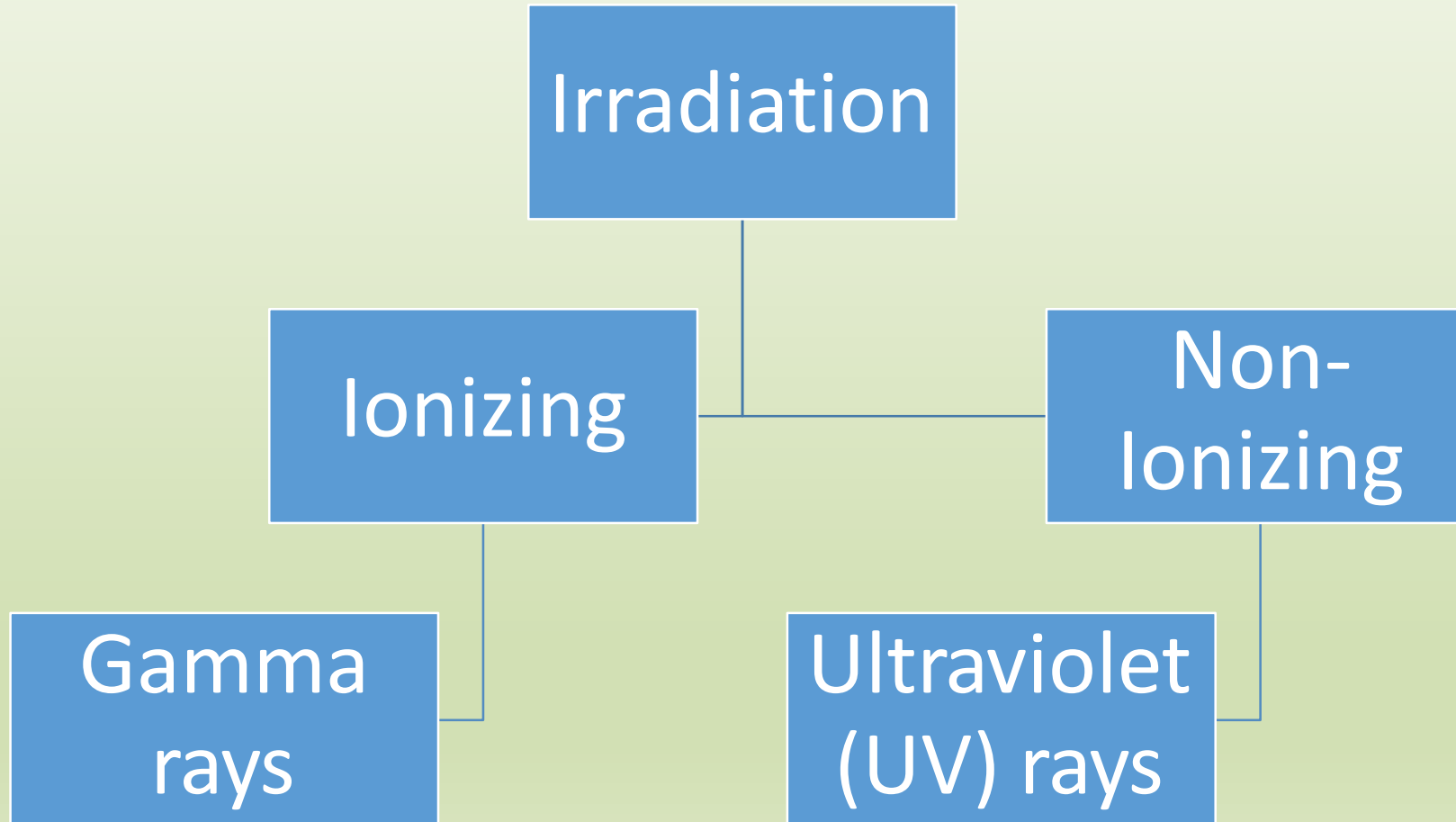
- Filtration is used to sterilize liquids that would be damaged by heat as **sera**, **antibiotic** solutions and **vaccines**.





C- Sterilization by irradiation

Sterilization by irradiation



Ultraviolet radiation

Used for.

1. Sterilization of operating theatre
2. Sterilization of the interiors of biological safety cabinets



Ionizing irradiation (gamma rays)

- Used for sterilization of an article not stand heat as rubber catheters, gloves, plastic syringes.



Chemical methods of Sterilization

- **Disinfectant:**

- Are chemical materials used for sterilization but are toxic to the human tissues and cells.

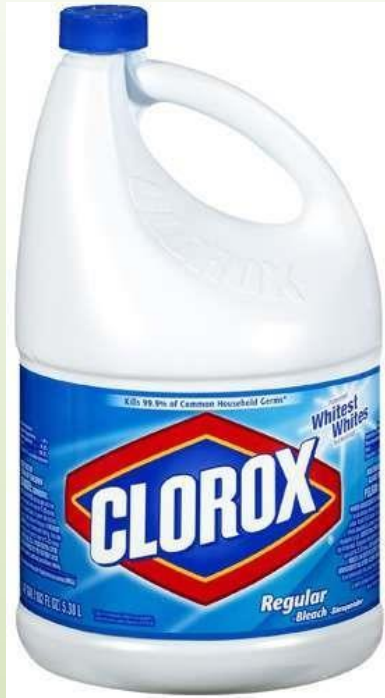
- **Antiseptics:**

- Are chemicals for sterilization but not toxic to the human body e.g. "mouth gargles".

Examples of disinfectant and antiseptics

There are a number of chemicals that can act as disinfectants or antiseptics. These include:

- **Phenol** and its derivatives e.g. Dettol.
- **Halogens** e.g. Chlorine, Tincture iodine.
- **Alcohols** e.g. ethyl alcohol.
- **Aldehydes** e.g. glutaraldehyde (Cidex), Formalin.
- **Quaternary Ammonium Compounds** (Cationic detergents).



Halogen



Halogen



**Phenol
derivative**