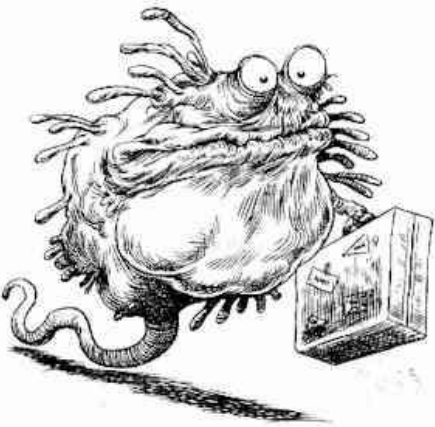




Inhibiting Microbial Growth *in vitro*

CLS 212: Medical Microbiology
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Microbicidal or Microbistatic?

Microbicidal

Microbicidal is the process or an agent that **kills** the microorganism.

- The suffix **-cidal** or **–cide** means "killing".

Microbistatic

Microbistatic is the process or an agent that **inhibits** the growth and reproduction of the microorganism.

- The suffix **-static** or **–state** means "inhibiting or stopping".

Sterilization or Disinfection?

Sterilization

Sterilization is the **complete destruction** of all forms of microbial life including bacteria, viruses, fungi, parasites, and spores.

Disinfection

Disinfection is **reducing or eliminating the number** of pathogenic microorganisms to the point where they no longer cause disease. But this method does not affect spores.

Pasteurization and Sanitization

Pasteurization

Is a **disinfecting** method used to eliminate pathogens from **liquids e.g.** milk, juice,..

Sanitization

Sanitization is the use of chemical agents on **food-handling equipment** to meet public health standards and minimize chances of disease transmission **e.g.** use of hot soap & water in cleaning restaurants.

STERILIZATION AND DISINFECTION METHODS

Methods used to destroy or inhibit microorganisms are either **physical or chemical**, and sometimes both types are used.

Physical Methods

Physical Methods

- They are commonly used in hospitals, clinics, and laboratories. Physical methods include:
 1. Heat.
 2. Cold.
 3. Desiccation.
 4. Radiation.
 5. Ultrasonic waves.
 6. Filtration.
 7. Gaseous atmosphere.

I- HEAT

- Heat is considered the most common method for sterilization because it is practical, efficient, and inexpensive.
- Heat kills microorganisms by denaturing their enzymes and other proteins.
- There are **three methods** of sterilization or disinfection by heat: **dry heat, moist heat, and using autoclave.**

1. Dry Heat

- An effective way to sterilize metals, glassware, some powders, oils, and waxes.

1. Hot Air Oven:

It is done in a 160-165°C oven for 2 hours or in 170-180°C oven for 1 hour.

2. Burning (incineration):

Is used to destroy contaminated disposable materials.

3. Direct Flame:

Bunsen burner or electrical heating device is used to sterilize wire loops and forceps used in the laboratory.



Dry heat Oven



Bunsen Burner



Electric Bunsen

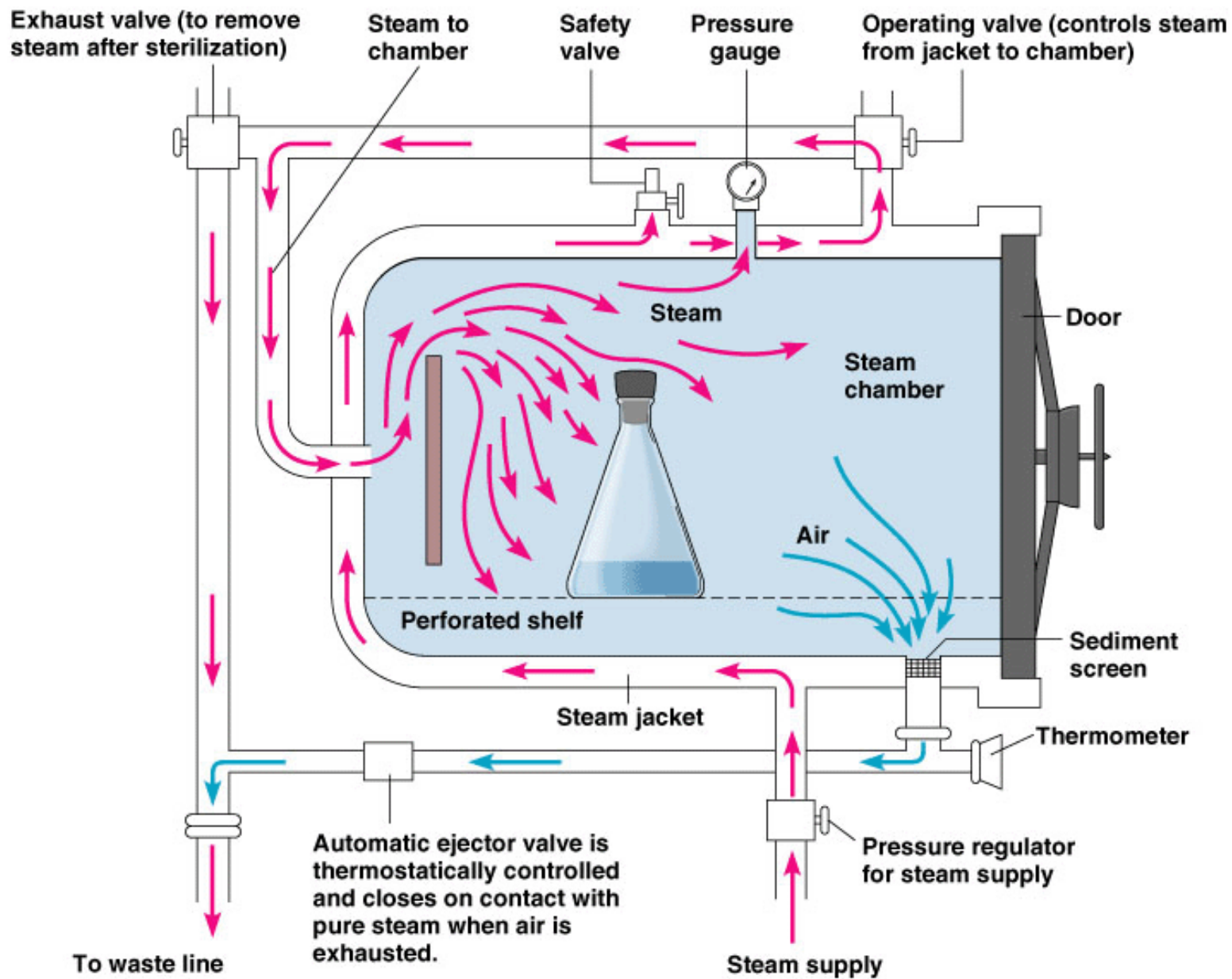
2. Moist Heat

- Is used to disinfect syringes, needles, and simple instruments by boiling (100°C) for 30 minutes.
- Boiling is not always effective as spores, like *Mycobacteria*, and some viruses are not affected.



3. Autoclave

- An autoclave is a large metal chamber that uses **steam under pressure for sterilization** (like the pressure cooker).
- Autoclaving is done at 121.5°C and 15psi pressure for 20 minutes.
- An autoclave tape or strip (commercially available) is used to ensure proper functioning.



Autoclave Tapes



II- Cold

1. Freezing (below zero)

Freezing will greatly slow the metabolic activities of microorganisms leading to inhibition of their growth.

2. Refrigeration (4°C)

Refrigeration will slightly affect the metabolic activities of most microorganisms but it would not completely inhibit growth.

- Slow Freezing is the most effective way as ice crystals that forms may rupture the cell membrane and cell wall of microbes.
- **CAUTION:**
Thawing and refreezing of food will allow the bacteria and its spores to resume growing.

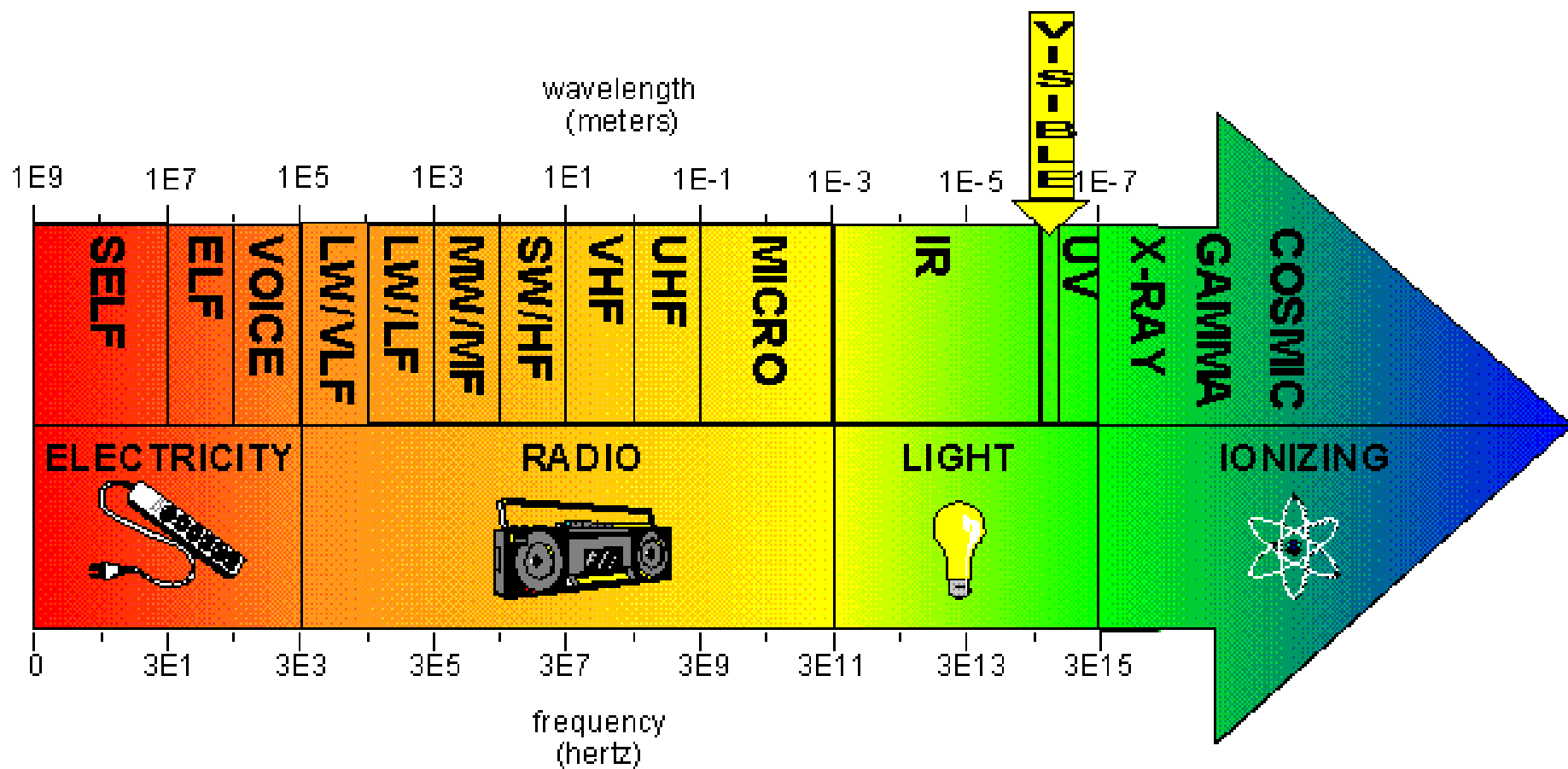


III- Desiccation (Drying)

- Many microorganisms stay viable even after drying but they cannot reproduce i.e. desiccation will inhibit the growth of microorganisms.
- When suitable moist and nutrient rich environment is available, the microorganism will grow rapidly.
- It is a method mainly used for food preservation.

IV- Radiation

- There are **three different types** of radiation that can be used to control microorganisms:
 1. **Ionizing radiation.**
 2. **Ultraviolet radiation.**
 3. **Microwave radiation.**



1. Ionizing Radiation

- Gamma rays, X-rays, and Beta rays from radioactive materials have short wavelengths (less than 1 nanometer).
- Cause death or mutations in microorganisms as they damage the DNA and proteins.
- Used to sterilize pharmaceuticals, disposable medical supplies, heat-sensitive surgical equipment.
- Food industry is interested in using ionizing radiation **e.g.** chicken.
- **Disadvantages:** Penetrates human tissues and may cause genetic mutations in humans and cancer.



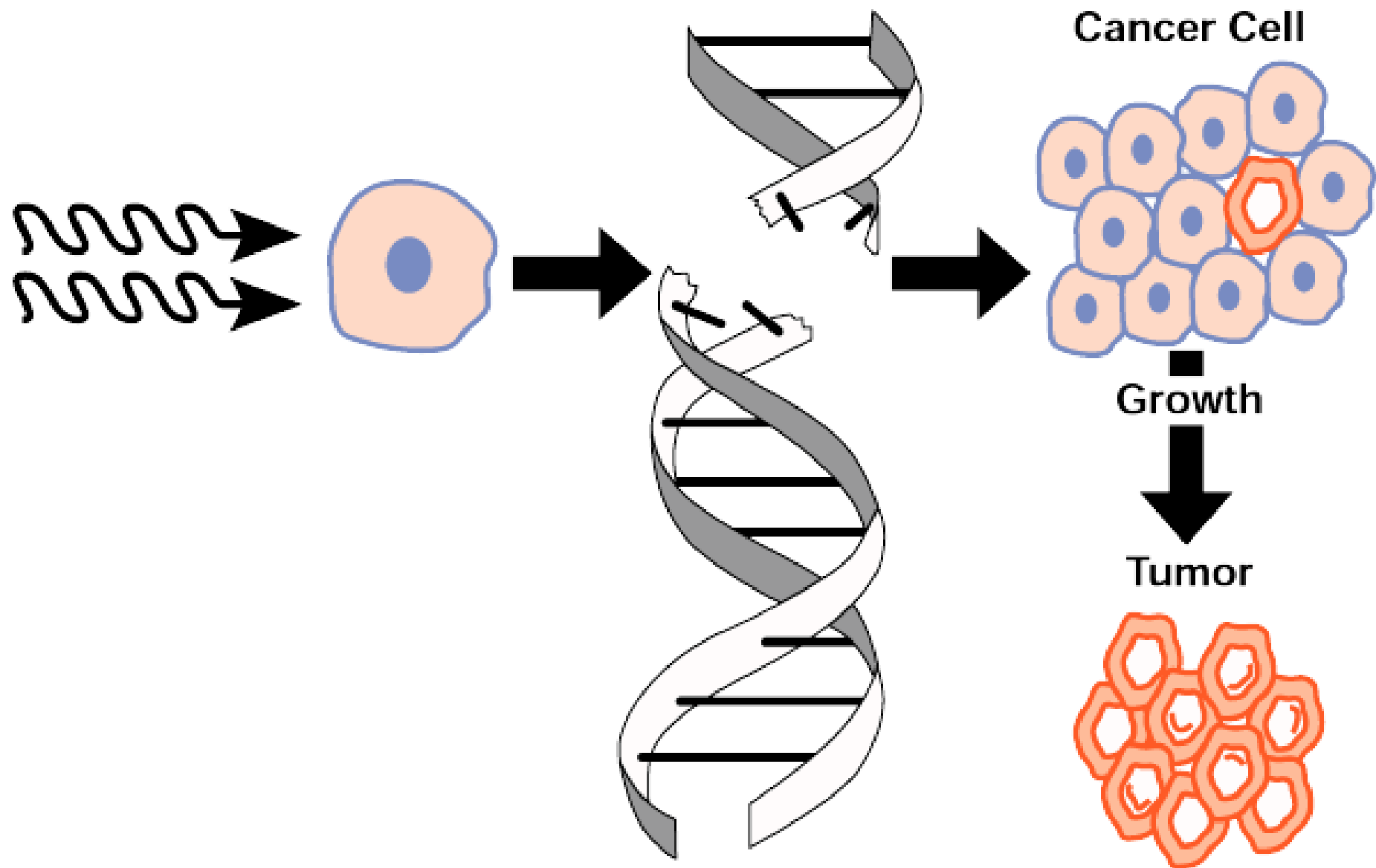


Figure 1. Development of cancer from mutation produced by ionizing radiation.

2. Ultraviolet light (Non-ionizing Radiation)

- Wavelength is longer than 1 nanometer.
- Damages DNA which cause mutations or death.
- Most commonly used as **UV-lamps that disinfect** operating rooms, nurseries, cafeterias,...
- **Disadvantages:** Damages skin, eyes and does not penetrate paper, glass, and cloth.

UV-Lamps



3. Microwave Radiation

- Wavelength ranges from 1mm to 1m.
- May **kill** microbial cells in moist foods but not spores.



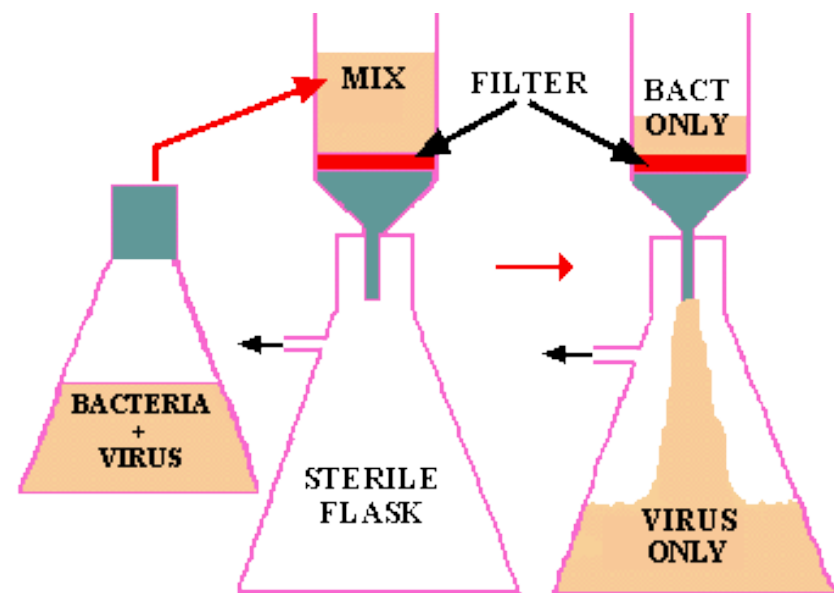
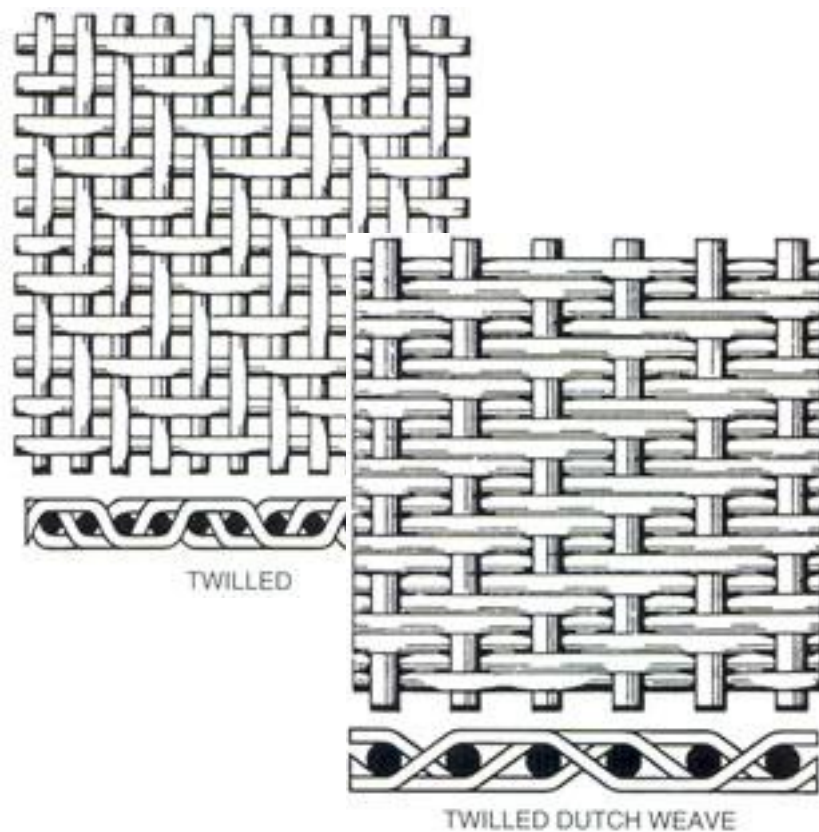
V- Ultrasonic Waves

- Used for cleaning and sterilizing delicate equipments.
- Ultrasonic cleaners consist of water tanks, where short sound waves pass through, removing organic debris from equipments.



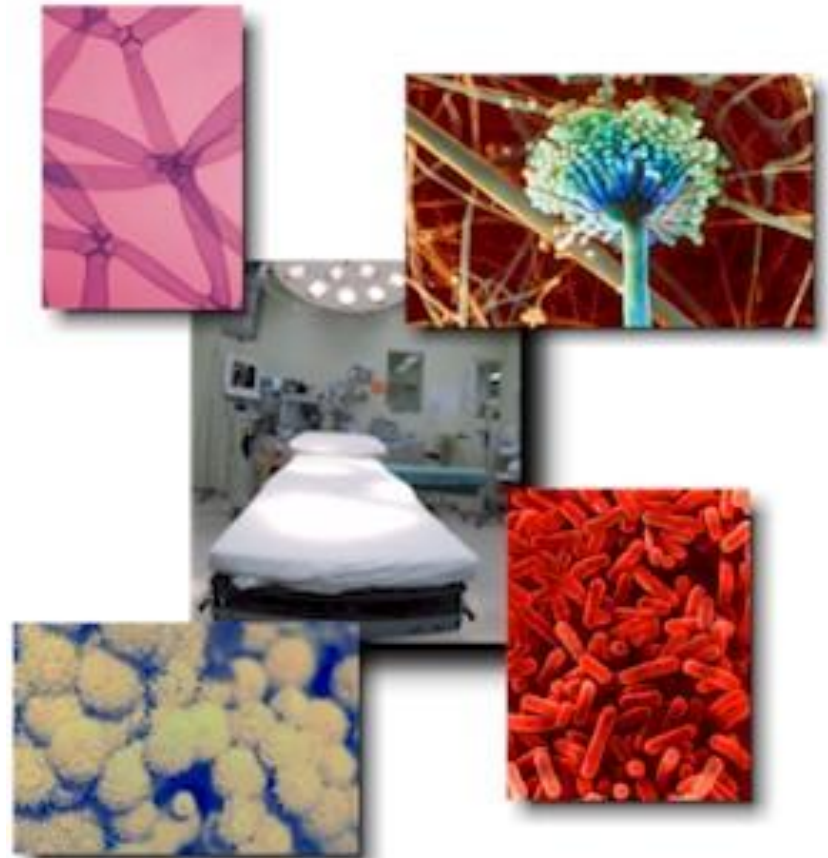
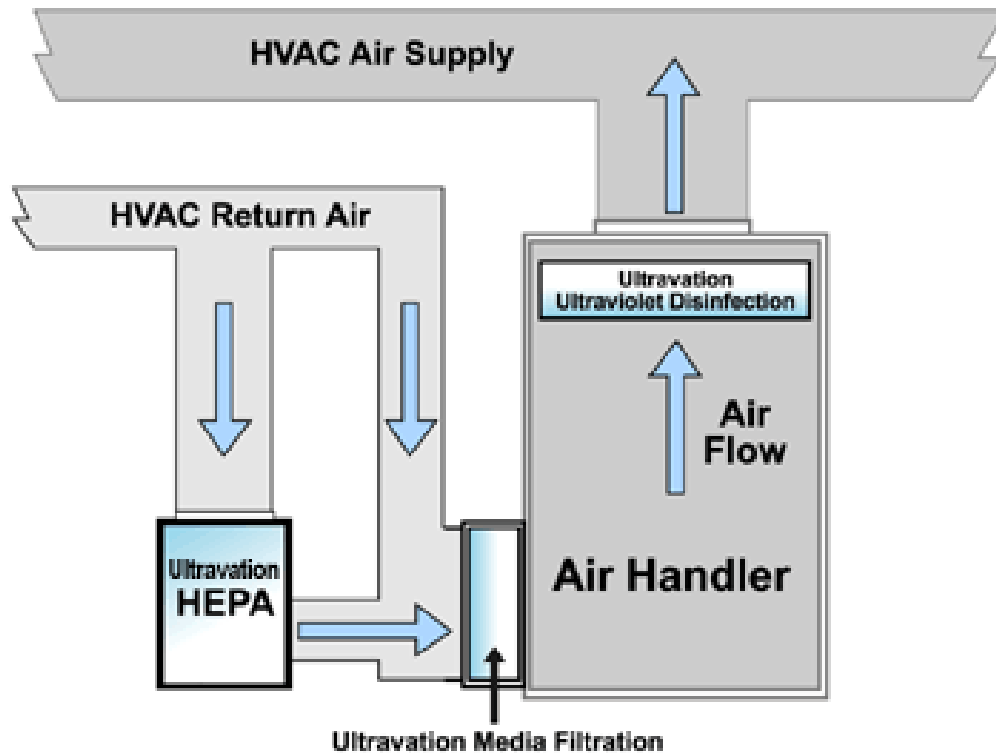
VI- Filtration

- Filtration is the removal of microbes by passage of a liquid or gas through a screen like material with small pores.
- Filters of various pore sizes can be used.
- Used to sterilize heat sensitive materials like vaccines, enzymes, antibiotics, and some culture media.
- **High Efficiency Particulate Air Filters (HEPA):**
Used in operating rooms and burn units to remove bacteria from air.



HEPA

HEPA Filtration System With Optional Media Filtration & UV Air Disinfection



VII- Gaseous Atmosphere

- In rare cases, changing the atmosphere can be used as a way to inhibit the growth of microorganisms.
- Aerobes and microaerophiles can be killed by placing them in oxygen-free atmosphere. Whereas, anaerobes can be killed by placing them in oxygen atmosphere.

Chemical Methods

Chemical Methods

Disinfectants

A disinfectant is a chemical agent that is used to **inhibit** microbial growth **on inanimate objects, surfaces, and floors.**

Antiseptics

An antiseptic is a chemical agent that is used to **inhibit** microbial growth **on human skin and mucous membranes.**

Most commonly used Disinfectants

1. **Soaps and detergents, alcohols, and phenolic compounds:** destroy microbial cell membranes **e.g.** Dettol.



2. **Formaldehydes, hydrogen peroxide, halogens, and salts of heavy metals:**

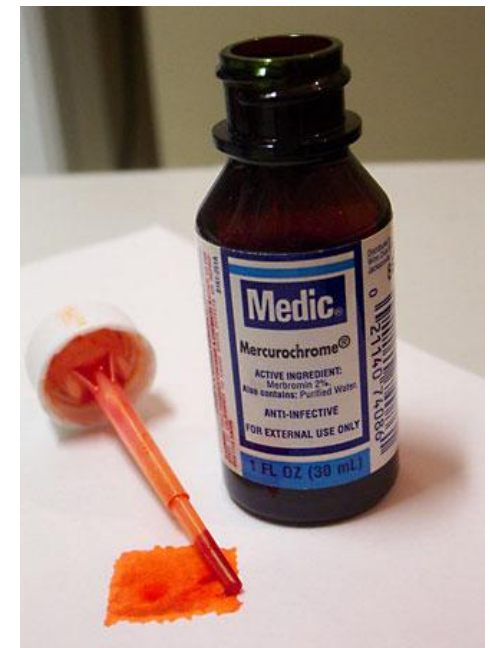
destroy enzymes and structural proteins **e.g.** Bleach, Clorox.

3. **Chlorine, ozone, iodine:** attack nucleic acids.



Most commonly used Antiseptics

1. **Iodophor:** used as skin antiseptic in surgery.
2. **Alcohol:** used on skin before needle pricking.
3. **Mercurochrome:** used to disinfect skin wounds.



INFECTION CONTROL



Germ Farm



Scrub'em!