Inhibiting of Microbial Growth *in vitro*

CLS 212

Microbicidal

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

+ The suffix **-cidal** or **–cide** means ??(**See chapter 8 page 131**)

Microbistatic

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

+ The suffix **-static** or **–state** means ? (**See chapter 8 page 131**)

Sterilization & Disinfection

+ **Sterilization :**

*(See page 131 , chapter 8)

*all forms of microbial life including bacteria, viruses, fungi, parasites, and spores.

- **Can be accomplished by physical or chemical method**

+ **Disinfection (elimination or reducing) :**

*(See page 131 , chapter 8)

***Can be accomplished by pasteurization or liquid chemical**

Disinfection

Pasteurization

- (See page 131 , chapter 8)
- Is a **disinfecting** method
- **For 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 water & soap in cleaning restaurants.

Antiseptic :

(See page 131 , chapter 8)

STERILIZATION AND DISINFECTION METHODS

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

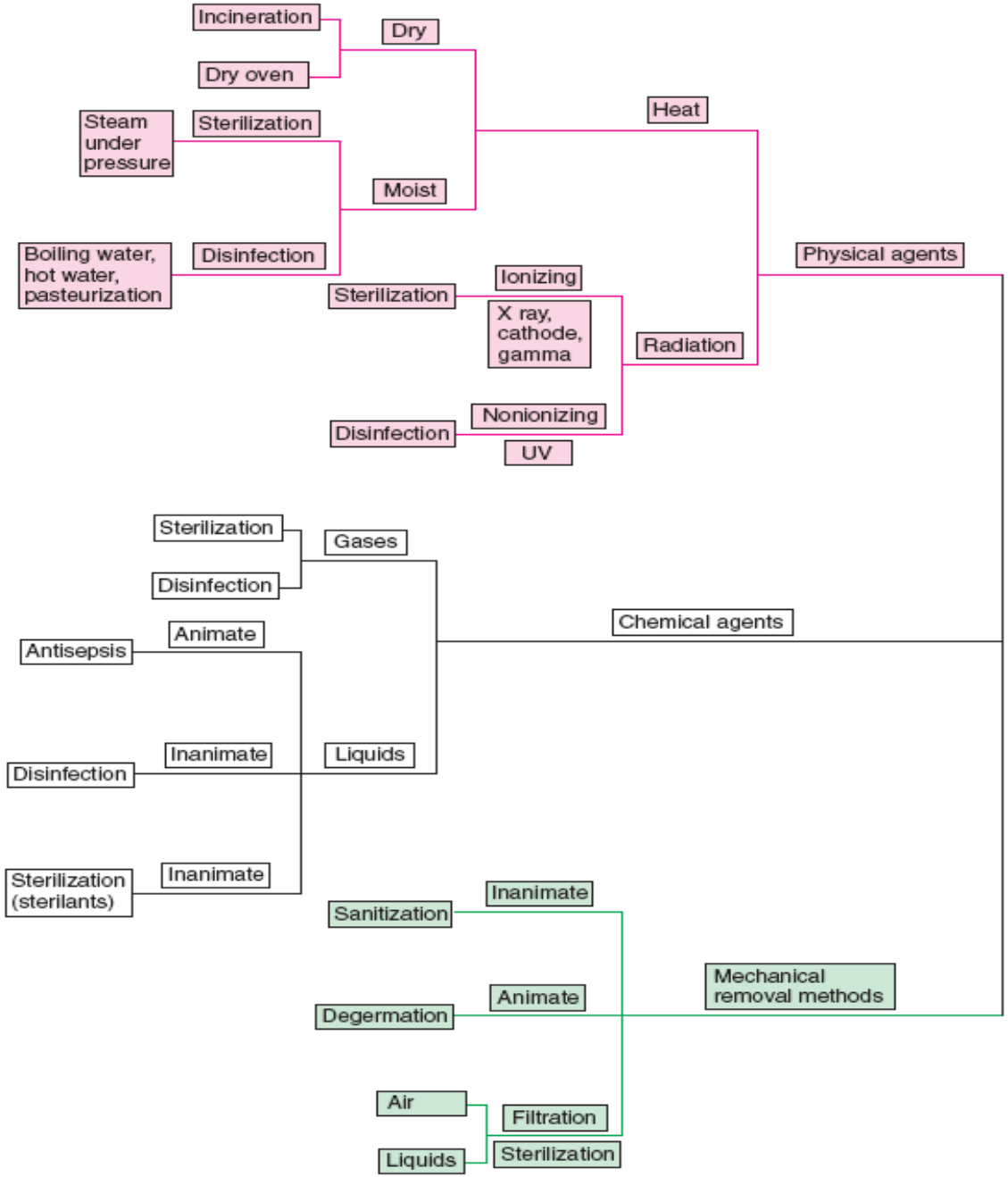


FIGURE 11.1
Flowchart of microbial control measures.

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 **two methods** of sterilization or disinfection by heat:

1. **dry heat**
2. **moist heat**

I- HEAT
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 2hours or in 170-180°C oven for 1hour.

2. **Burning (incineration)**:

   Is used to destroy contaminated disposable materials.

3. **Direct Flame:**

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

I- HEAT

Dry heat Oven

Bunsen Burner

Electric Bunsen

I- HEAT
2. Moist Heat

- Use in sterilization or disinfection method
- There are **three methods** of sterilization or disinfection by moist heat:

1. Boiling water >>>>disinfection
2. Pasteurization >>>>>disinfection
3. Autoclave >>>>>>>>> sterilization

- Faster and more effective than dry heat

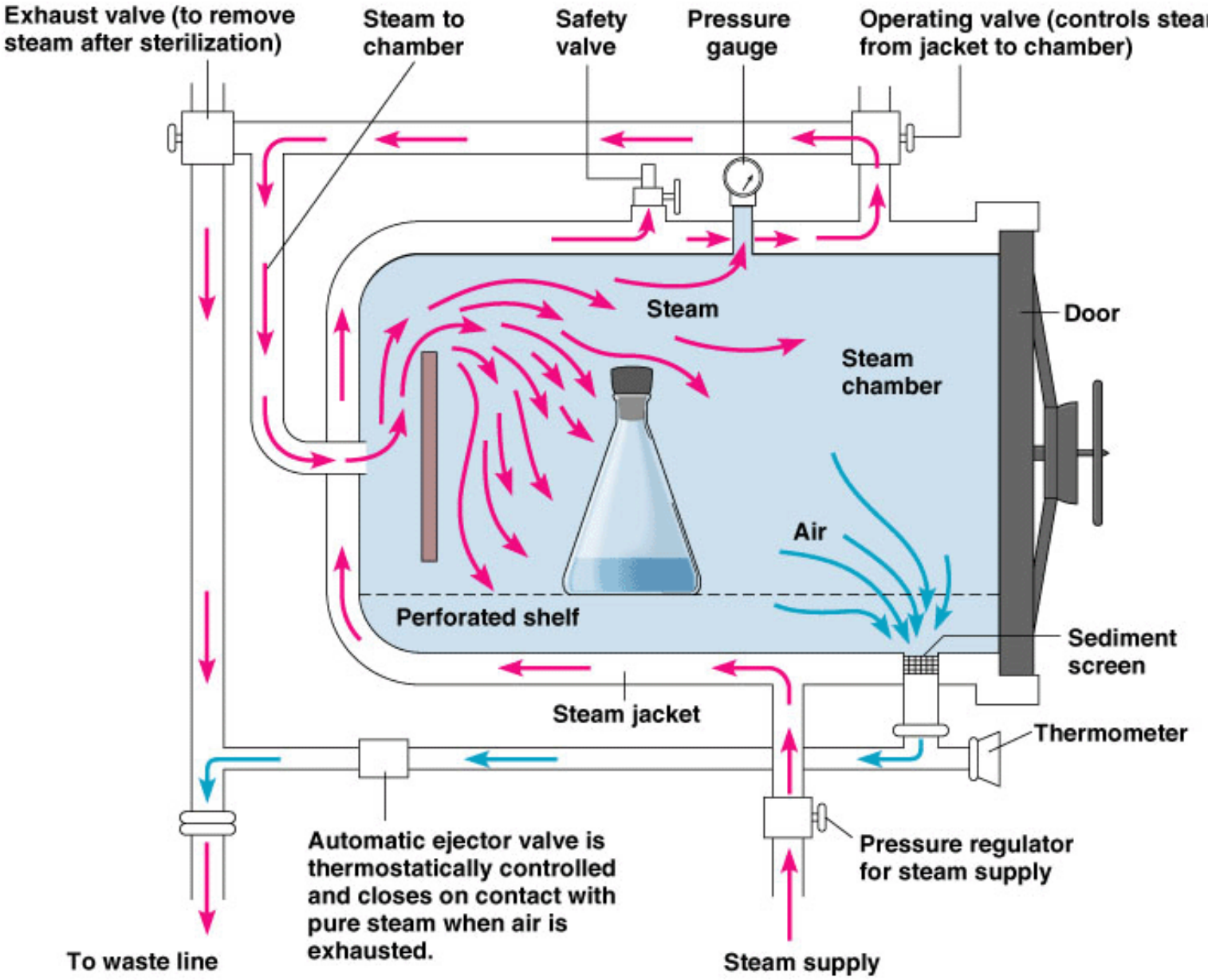
I- HEAT
2. Moist Heat

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

I- HEAT
3. Autoclave

- **Autoclave is :**

- Definition : See page 133 , chapter 8
- For sterilization
- Autoclaving is done at : See page 133 , chapter 8


Exhaust valve (to remove steam after sterilization)
Steam to chamber
Safety valve
Pressure gauge
Operating valve (controls steam from jacket to chamber)
Steam
Steam chamber
Door
Air
Perforated shelf
Sediment screen
Steam jacket
Thermometer
Automatic ejector valve is thermostatically controlled and closes on contact with pure steam when air is exhausted.
Pressure regulator for steam supply
To waste line
Steam supply

3. Autoclave

- An autoclave tape or strip (commercially available) is used to ensure proper functioning.

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.**

II- cold

+ **CAUSION:**

Thawing and refreezing of

food ???? See page 134 , chapter 8

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,antisera, antitoxin, antibiotics, and pure culture of microorganisms.

IV- Radiation

- There are **two types** of radiation that can be used to control microorganisms:

1. **Ionizing radiation.**
2. **(non- Ionizing radiation) :**

- **Ultraviolet radiation (non- Ionizing radiation)**
- **Microwave radiation (non- Ionizing radiation)**

IV- 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** heat-sensitive materials including medical equipment, disposable surgical supplies and drugs. Radiation can be carried out after packaging.
- Food industry is interested in using ionizing radiation **e.g.** chicken.
- **Disadvantages**: Penetrates human tissues and may cause genetic mutations in humans and cancer.

2. Ultraviolet light (Non-ionizing Radiation)

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

IV- Radiation

3. Microwave Radiation

- Wavelength ranges from 1mm to 1m.
- Very little effect on microbes
- 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, were short sound waves pass

through, removing organic

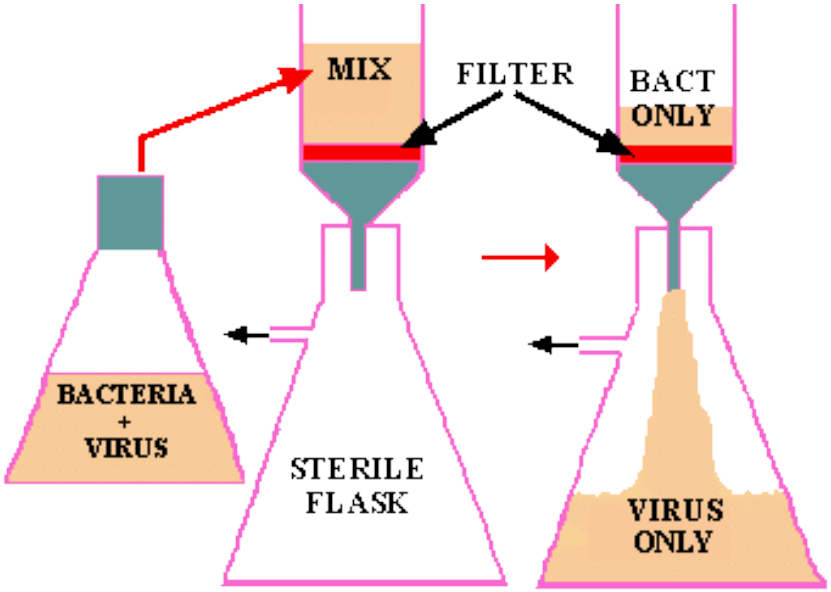
debris from equipments.

VI- Filtration

- **Filtration** is : (see page 135 , chapter 8)
- Filters of various pore sizes can be used.
- Used to **sterilize** heat sensitive materials like vaccines, enzymes, antibiotics, and some culture media.

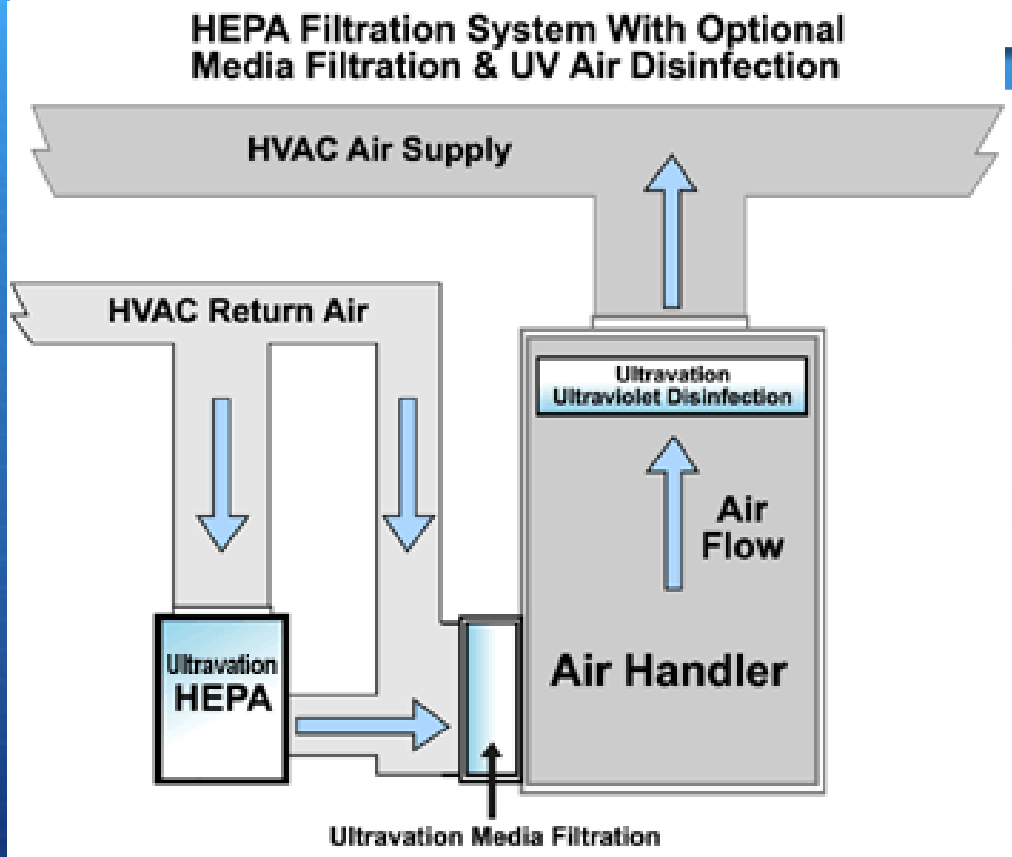
- **High Efficiency Particulate Air Filters (HEPA):**
- (see page 136 , chapter 8)

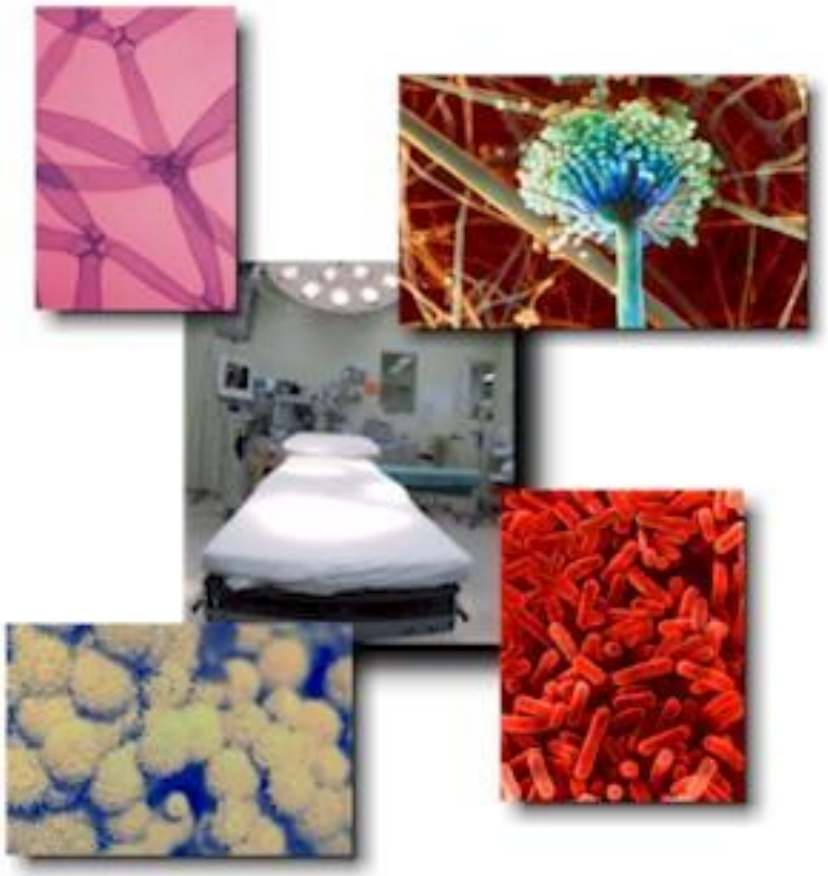

TWILLED
TWILLED DUTCH WEAVE


MIX
FILTER
BACT
ONLY
BACTERIA
+
VIRUS
STERILE
FLASK
VIRUS
ONLY

VI- Filtration

HEPA





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.**

Medical devices, equipment, and surgical materials are divided into three general categories based on the potential risk of infection involved in their use:

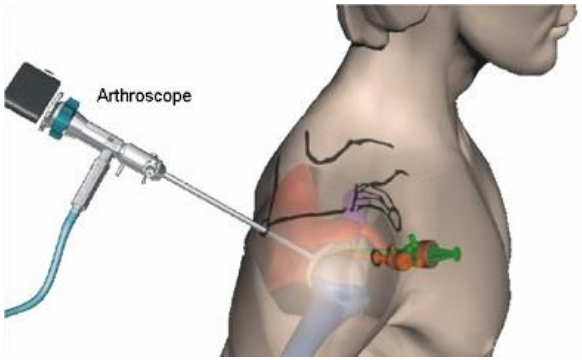
- critical items
- semicritical items
- noncritical items

+ High risk (critical items):

Contacting tissue or blood, includes

(surgical instruments and arthroscopes)

must be sterilized



+ Intermediate risk (semicritical items):

Contact mucous membrane or non intact skin, includes (G-I endoscopes and respiratory tubes) need high level disinfection.

+ Low risk (noncritical items):

Contact only contact skin, includes

(ward equipment and stethoscopes)

low level disinfection is sufficient

Most commonly used Disinfectants

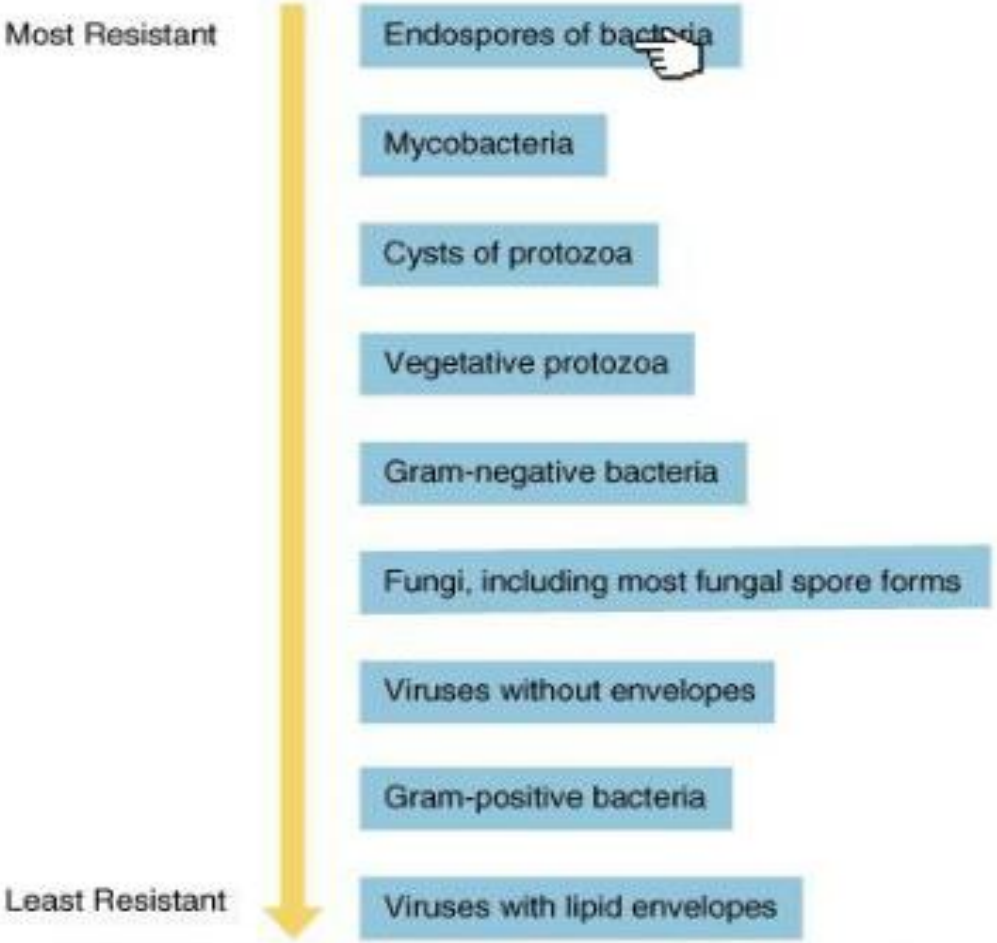
1. **Soaps and detergents, alcohols, and phenolic compounds:** destroy microbial cell membranes **e.g.** Dettol.

2. **Formaldehydes, hydrogen peroxide, halogens, and salts of heavy metals:** destroy enzymes and structural proteins **e.g.** Bleach, Clorox.

3. **Chlorine, ozone, iodine:** attach 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.



Microbial Sensitivity to Chemical Biocides

Infection Control