Sterilization and Disinfection

Lecture 14

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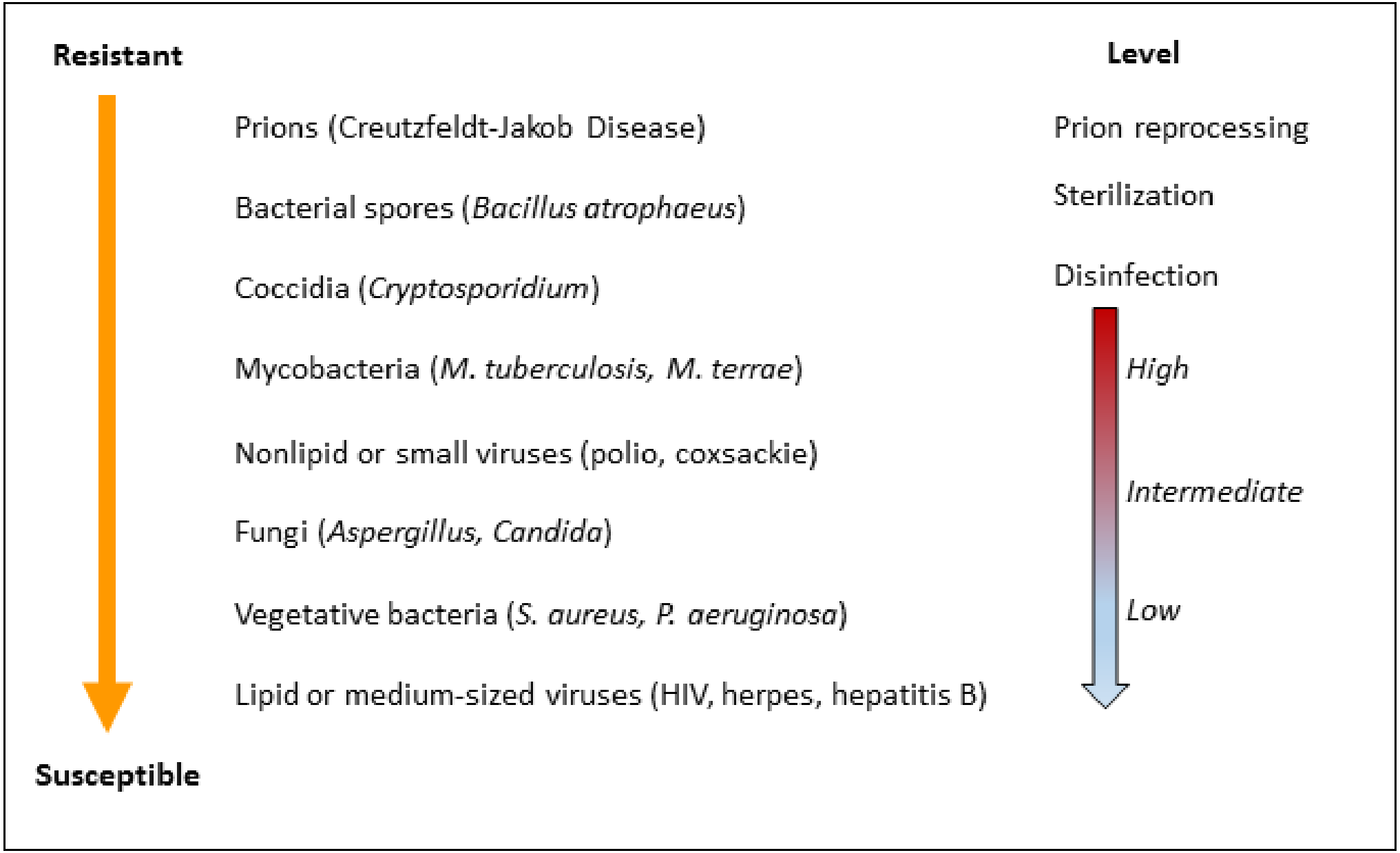
2025-2026

Learning Outcomes

- Student will be able to define sterilization, disinfection, antiseptic, bactericidal, and bacteriostatic.
- Classified the different methods of sterilization.
- Categorized disinfectant reagent.
- Mechanism of sterilization and disinfection.

Definitions

Sterilization:	**The complete destruction or removal of all forms of microbial life, including spores.**
Disinfection:	The process of eliminating or reducing harmful microorganisms (except bacterial spores) from inanimate objects and surfaces.
Disinfectant	chemical substance used to achieve disinfection.
Bacteriostatic:	Refers to agents that inhibit bacterial growth without necessarily killing them.
Bactericidal	Refers to agents that kill bacteria.
Antiseptic:	disinfectant that can be safely used on living tissues.


Resistant
Susceptible
Prions (Creutzfeldt-Jakob Disease)
Bacterial spores (Bacillus atrophaeus)
Coccidia (Cryptosporidium)
Mycobacteria (M. tuberculosis, M. terrae)
Nonlipid or small viruses (polio, coxsackie)
Fungi (Aspergillus, Candida)
Vegetative bacteria (S. aureus, P. aeruginosa)
Lipid or medium-sized viruses (HIV, herpes, hepatitis B)
Level
Prion reprocessing
Sterilization
Disinfection
High
Intermediate
Low

Methods of sterilization:

There are two methods of sterilization:

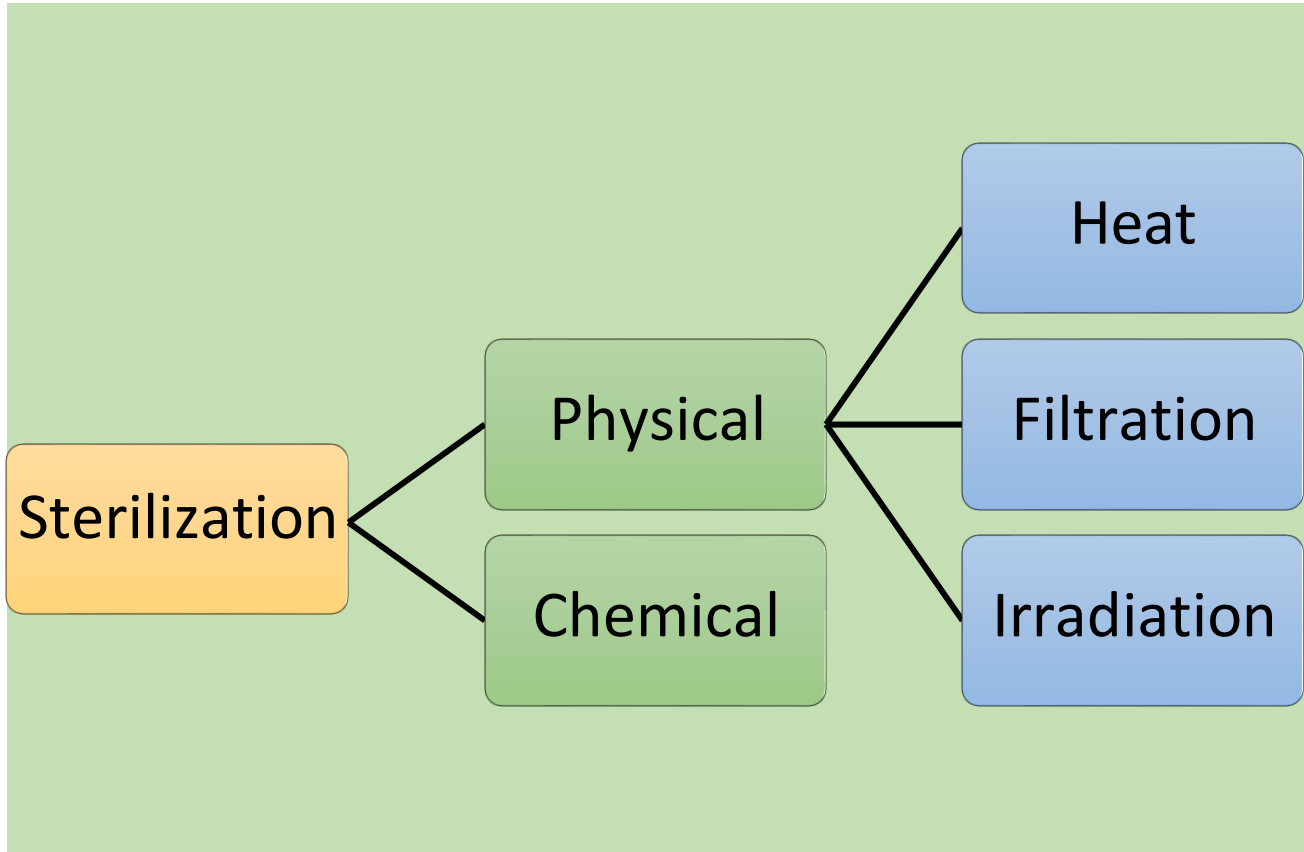
A- **Physical methods**:

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

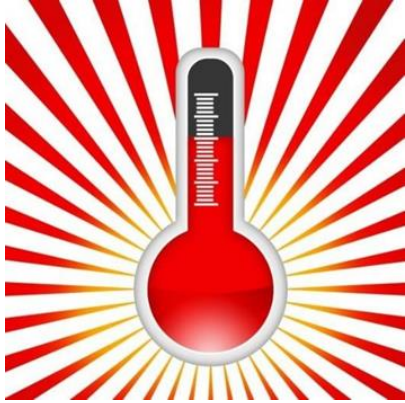
B- **Chemical methods**

(used for heat sensitive equipment's)

1. Ethylene Oxide
2. Gluteraldehyde



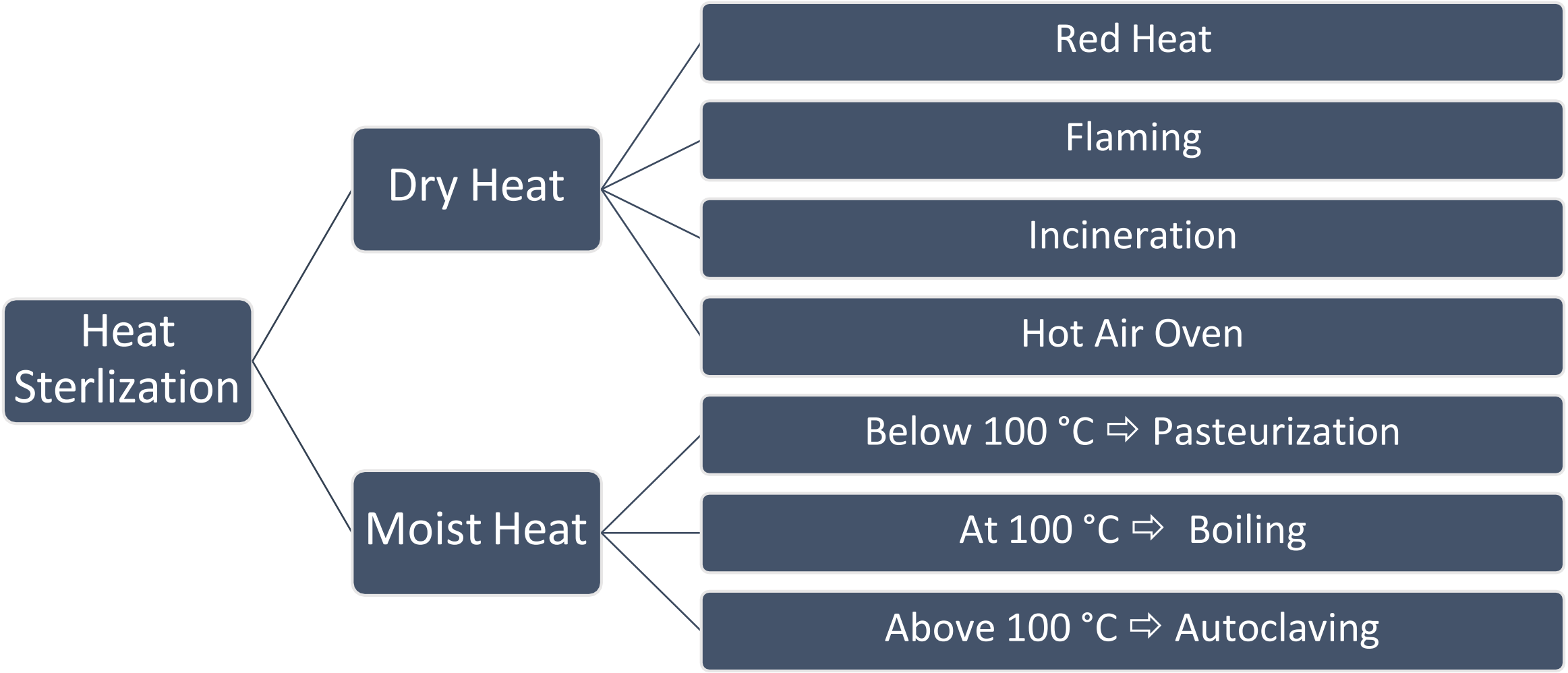
Simplest methods is to sterilize by naked flame



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
- For sterilizing bacteriological loops, knives, blades

2. Flaming

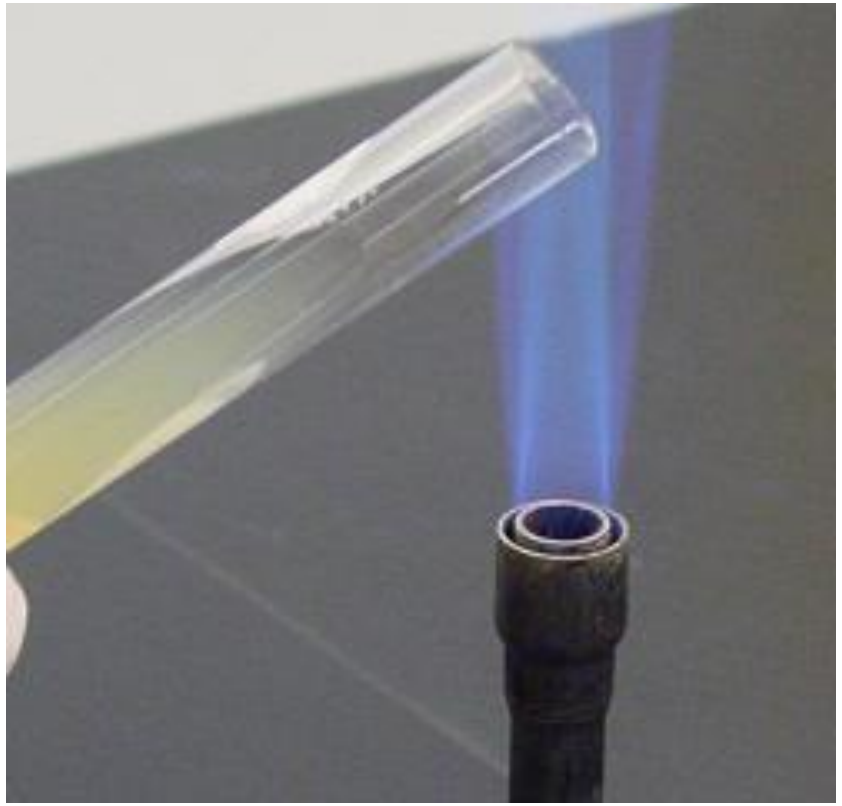
Principal:

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

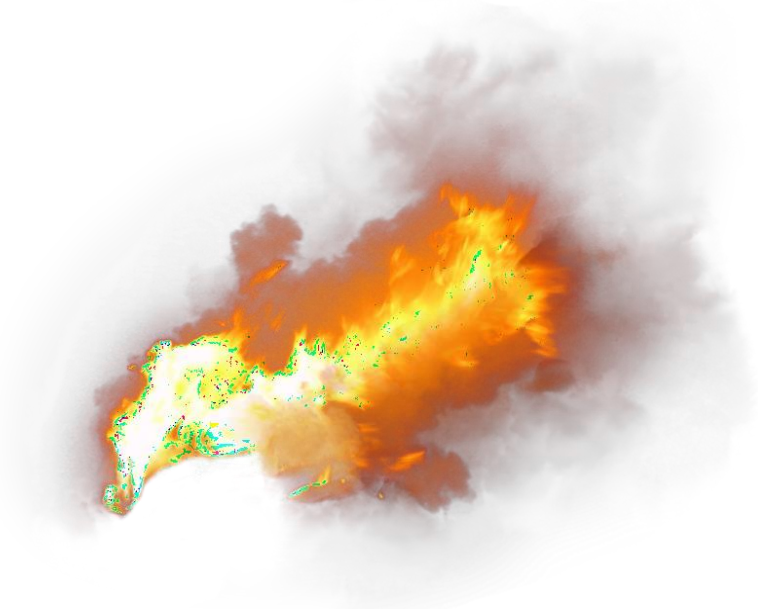
Used for:

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

- Has electric element in chamber as source of heat plus a fan to circulate air for even distribution of heat in chamber.
- Oven without fan is dangerous.
- Used for items that are lacking water such as:

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

Holding time:

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

Moist Heat

Can be used at different temperatures:

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

Uses hot water. Moist heat kills microorganisms by denaturing proteins.

1. Pasteurization (Below 100)

- **Pasteurization**

✓ Used heat at temperatures sufficient to inactivate harmful organism.

✓ The temperatures of **sterilization** is not achieved .

✓ Temperature may be

➢ 74° C, for 3-5 seconds. (Flash methods)

or

➢ 62° C for 30 minutes. (Conventional method).

✓ Pasteurization of the milk to prevent diseases like :

➢ Typhoid fever
➢ Brucellosis
➢ Tuberculosis
➢ Q fever
➢ Salmonella

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:

✓ Common especially in domestic circumstances.

3. Autoclaving

Principal:

- When the pressure is increased inside a closed container, the temperature at which water boiling point 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.
- The air in the chamber is evacuated and filled with saturated steam
- The items to be sterilized get completely surrounded by saturated steam (moist heat) which on contact with the surface of material to be sterilized condenses to release its latent heat of condensation which adds to already raised temperature

3. Autoclaving

Temperature of sterilization:

- 121°C for 15minutes (or 134 C for 10 minutes)

Used for sterilization of:

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

Advantages of Autoclave

1. Temp. > 100 C therefore spores killed.
2. Condensation of steam generates extra heat.
3. The condensation also allows the steam to penetrate rapidly into porous materials.

Note: for all invasive procedures at operating room or clinics, autoclavable equipment's should be used.

Monitoring of Autoclaves

1. Physical method:
use of thermocouple to measure accurately the temperature.

2. Chemical method:
it consists of heat sensitive chemical that changes color at the right temperature and exposure time.
e.g. **Autoclave tape**

3. Biological method:

Geobacillus stearothermophilus
where a spore-bearing organism is added during the sterilization or autoclaving process and then cultured later to ensure that it has been killed.

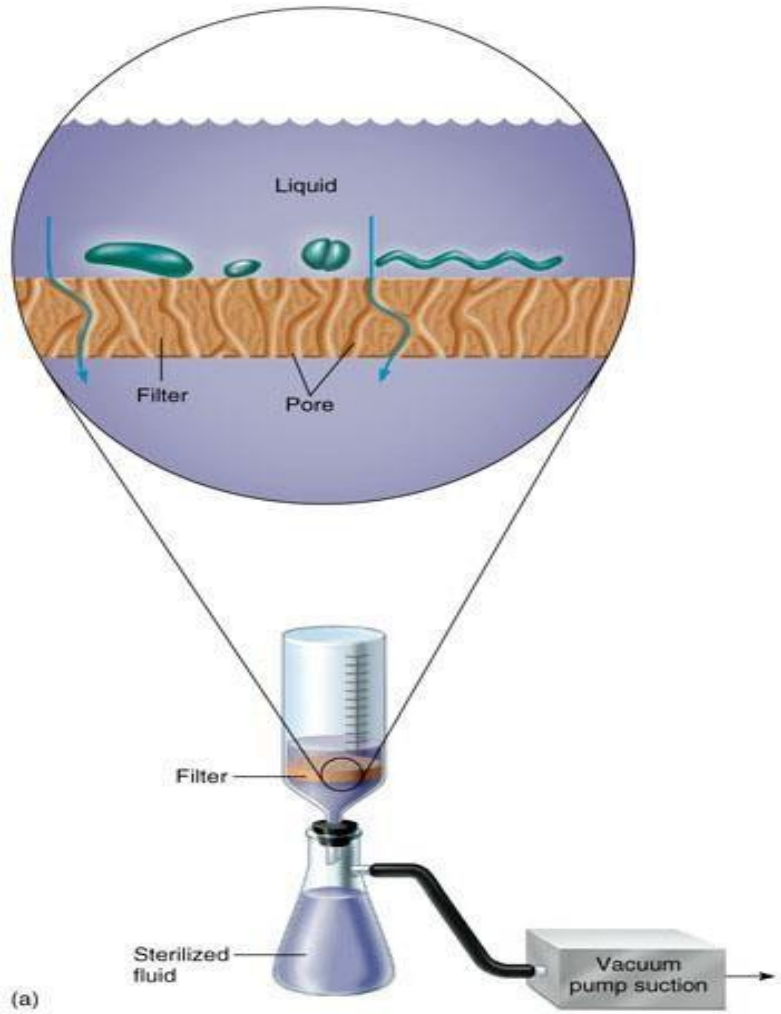
Filtration

- Use of membrane filter

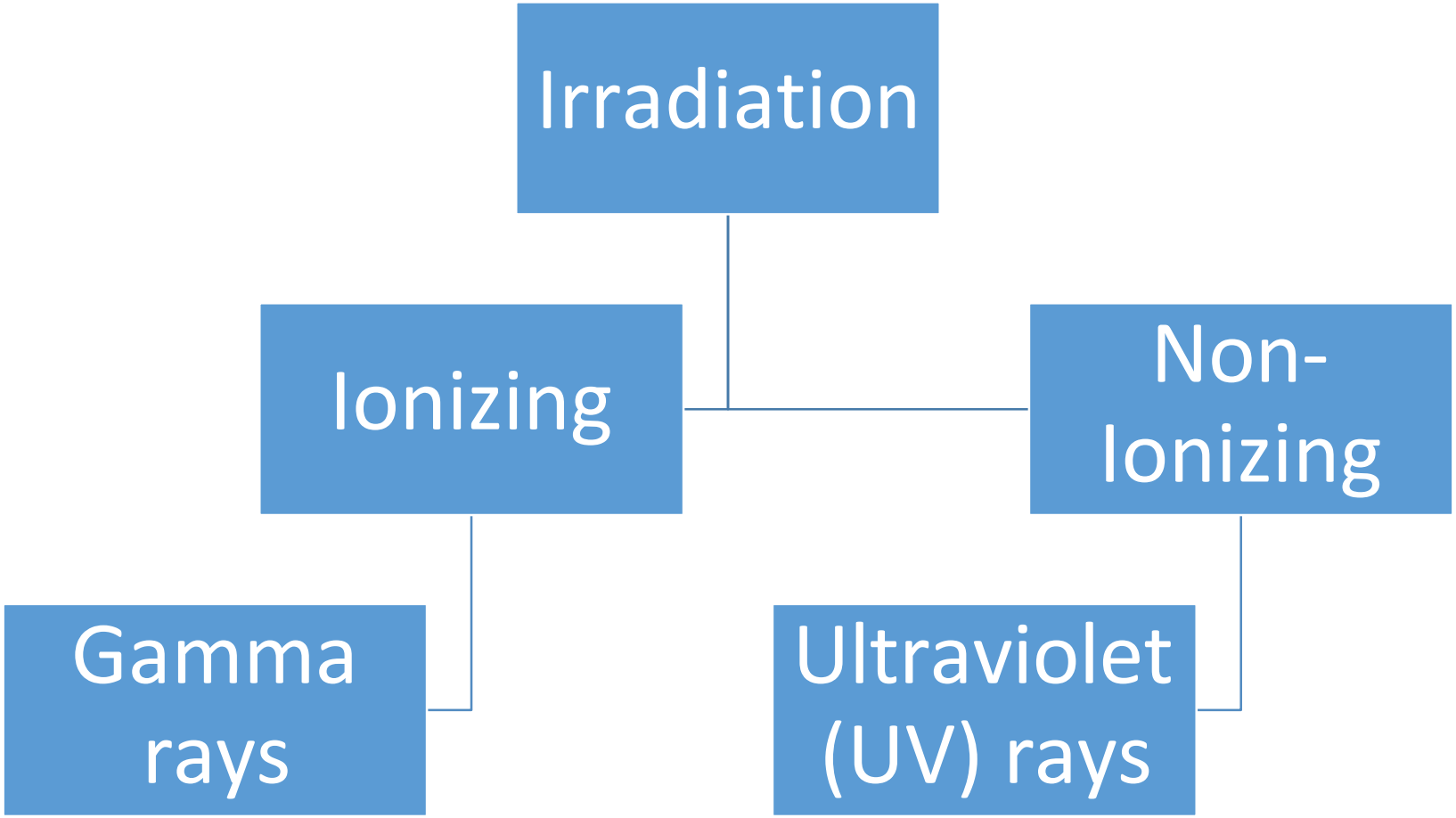
Example ; membrane filter made of **cellulose acetate**.

- Generally removes most bacteria but viruses and some small bacteria e.g. *Chlamydia* & *Mycoplasma* may pass through.

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



Sterilization by irradiation



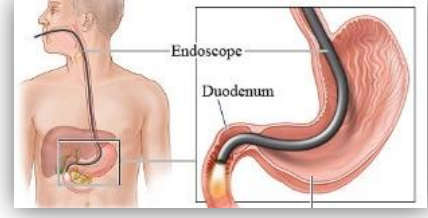
Non- Ionizing irradiation(Ultraviolet radiation)

- Has limited sterilizing power because of poor penetration into most materials.
- Generally used in irradiation of air in certain areas such as operating rooms and tuberculosis labs.
- Sterilization of the interiors of biological safety cabinets.

Ionizing irradiation (gamma rays)

- Has greater energy than U.V. light, therefore more effective.
- Used mainly in industrial facilities e.g. sterilization of disposable plastic syringes, gloves, specimens containers and Petri dishes.

Chemical Methods:



Used for **heat sensitive** equipments. e.g. plastics and lensed endoscopes.

Simple
disinfectants / antiseptic

- alcohol,
- phenolic
- chlorhexidine

Most disinfectant doesn't achieve full sterilize EXEPT glutaraldehyde 2% can achieve full sterilize

Strong
chemical substances may be used to achieve sterilization (kill spores)

Ethylene oxide
(inactivates microorganisms by alkylates DNA molecules)

Ethylene oxide may cause explosion if used pure, so it is:
1- mixed with an inert gas.
2- Requires high humidity (50- 60%).
3- Temperature : 55-60°C
4- exposure period 4-6 hours.

Gluteraldehyde 2%
Activated alkaline
Ethylene oxide

Immerse item (endoscopes) in solution (Gluteraldehyde) for:
1. **HIV or hepatitis B/C** > about 20 m.
2. **Mycobacterium tuberculosis** or spores > immersion period 2-3 h.

Chemical methods of Sterilization

Disinfectant:

- Are chemical materials used for sterilization but are toxic to the human tissues and cells.
- Examples of toxic disinfectants include hydrogen peroxide and sodium hypochlorite (bleach)
- cause chemical burns, irritation, and damage to human tissues

Antiseptics:

- They are generally **milder** than disinfectants but can still be toxic to human cells (e.g., skin.
- e.g. "mouth gargles".
- Ethyl alcohol (60-95%), hand sanitizer. Can be drying to skin; toxic if ingested or used on eyes.

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

Factors Affecting the Efficacy of Disinfection and Sterilization

Seven major factors influence disinfection and sterilization:

1. Number and Location of Microorganisms
2. Innate Resistance of Microorganisms
3. Concentration and Potency of Disinfectants
4. Physical and Chemical Factors
5. Organic and Inorganic Matter
6. Duration of Exposure
7. Biofilms

Factors Affecting the Efficacy of Disinfection and Sterilization

Number and Location of Microorganisms

- More microorganisms require more germicide.
- Clumped cells are harder to kill than dispersed ones.
- Disassemble medical tools for thorough cleaning.
- Crevices and joints are harder to disinfect than flat surfaces.

Innate Resistance of Microorganisms

- Some microbes naturally resist disinfectants and sterilization.
- Stronger or longer exposure needed for resistant species.
- Choose appropriate method and agent for target organisms.

Factors Affecting the Efficacy of Disinfection and Sterilization

Concentration and Potency of Disinfectants

- Higher concentration increases germicidal activity.
- Reduces required exposure time.
- Some agents (e.g., quaternary ammonium compounds, phenols) not strongly affected by concentration.

Physical and Chemical Factors

- Temperature: Generally increases activity but extreme heat may reduce efficacy.
- pH: Alters disinfectant or cell surface; effect depends on disinfectant.
- Humidity: Critical for gaseous agents (EtO, chlorine dioxide, formaldehyde).
- Water hardness: Decreases efficacy by forming insoluble precipitates.

Factors Affecting the Efficacy of Disinfection and Sterilization

Organic and Inorganic Matter

- Organic materials (blood, tissue) reduce disinfectant activity by reacting with germicides
- Inorganic salts can shield microbes from sterilization.
- Pre-cleaning is essential for effective disinfection.

Duration of Exposure

- Longer contact time = higher efficacy.
- Follow label exposure durations.
- Different microorganisms require different exposure times.

Factors Affecting the Efficacy of Disinfection and Sterilization

Biofilms

- Biofilms protect bacteria, increasing resistance up to 1,000×.
- Hard to remove; adhere strongly to surfaces.
- Chlorine and monochloramines effective against biofilm bacteria

Hospital disinfection methods

*Depends on Hospital's Policy

Article	Disinfectant
-Floors, walls:	Phenolics fluids 1-2%
-Surfaces tables:	Hypochlorite, Alcohol
-Endoscopes:	Gluteraldehyde 2% (Cidex)
	sub-atmospheric steam
-Thermometers:	70% Alcohol
Skin:	**Antiseptics**
-Surgeons' hands:	Chlorhexidine, Iodine alcohol
-Patient skin:	70% Alcohol, Iodine

❖ Disinfectants kill bacteria by disrupting their cellular structure by:

- **Oxidation**: Those containing chlorine or hydrogen peroxide, work by **oxidizing** the bacteria's cell walls and membranes.
- **Protein denaturation**: Alcohol-based ones, Dehydrates and break down the proteins that are essential for the bacteria's function.
- **Disruption of cell membranes**: Like certain **quaternary ammonium compounds** (Quats), bind to the negatively charged bacterial membranes and cause them to fall apart by creating holes.
- **DNA and RNA damage**: Like UV light, directly damage the bacteria's DNA and RNA, preventing it from replicating and causing the cell to die.
- **Protein cross-linking**: Such as glutaraldehyde, work by cross-linking proteins within the cell wall peptidoglycan, which makes them unable to function.

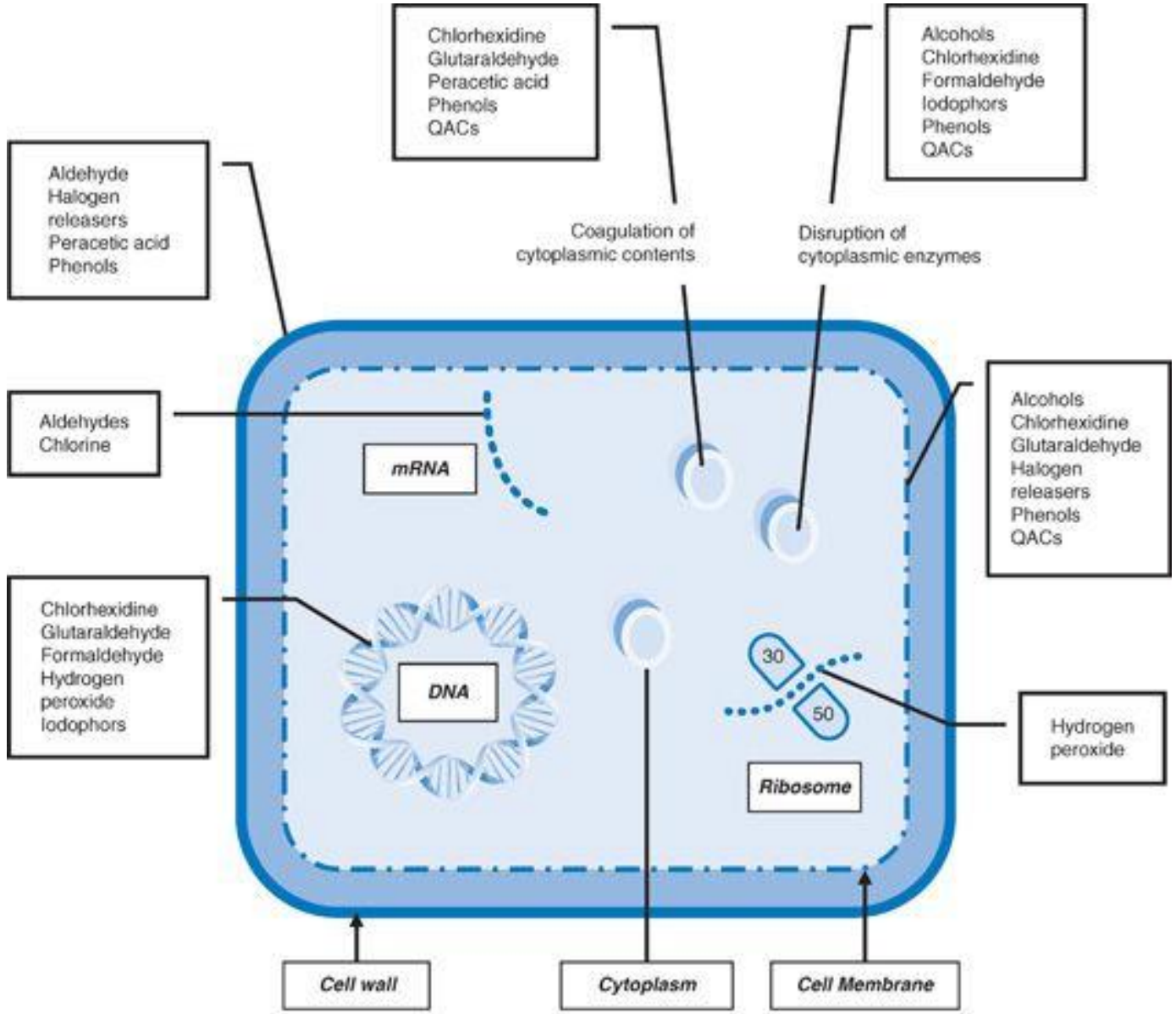


Figure 12.1 ■ Cellular targets of biocidal agents. (Adapted from Fanning S. Altered tolerance to biocides: links to antibiotic resistance? Paper presented at: International Association of Food Protection (IAFP), European Symposium on Food Safety; 2011; The Netherlands. http://www.foodprotection.org/events/european-symposia/11Ede/Fanning.pdf. Accessed November 15, 2012.)