Sterilization and Disinfection

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Historical Background

- The Birth of Sterilization
  - 1867: Joseph Lister introduced carbolic acid spray in surgery
    - Reduced surgical mortality from 45% to 15%
  - 1876: Robert Koch developed steam sterilization
  - 1880s: Introduction of surgical autoclaves
  - Modern era: Advanced methods like plasma sterilization
- Why This Matters Today:
  - 1 in 31 hospitalized patients develops healthcare-associated infections
  - Proper sterilization prevents surgical site infections
  - Essential for medical device safety

Key Terminology - I

- **Sterilization التعقيم:**
  - Removal or destruction of ALL forms of microbial life, including endospores→ 100% killing
  - Includes bacteria, spores, fungi, viruses, and prions
  - Used for: surgical instruments, implants, parenteral fluids
- **Commercial Sterilization:**
  - limited heat treatment is termed commercial sterilization
  - Less rigorous than complete sterilization
  - Destroys specific pathogens of concern
  - Example: Canned food processing
    - Targets C. botulinum spores
    - Some thermophilic spores may survive

Key Terminology - II

- **Disinfection:تطهير**
  - Destruction of vegetative pathogens on **inanimate objects**
  - Elimination of most pathogenic organisms
  - Does not necessarily kill bacterial spores
  - Three levels:
    - High-level: All microbes (including bacterial spores)
    - Intermediate-level: Mycobacteria, viruses, fungi (but not sporocidal)
    - Low-level: A wide range of activity against microorganisms but no sporocidal or tuberculocidal activity
- **Antisepsis:**
  - Application of antimicrobial substances to **living tissue**
  - Examples:
    - Surgical hand scrub
    - Preoperative skin preparation
    - Wound cleansing

Medical materials

- Medical materials into three device classifications
  - Critical materials → materials that invade sterile tissues → are most likely to produce infection if contaminated, and they require sterilization.
  - Semicritical materials → materials that come into contact with mucous membranes → require high-level disinfection agents
  - Noncritical materials → require intermediate-level to low-level disinfection before contact with intact skin.
- High-level disinfectants have activity against bacterial endospores,
- Intermediate-level disinfectants have tuberculocidal activity but not sporicidal activity.
- Low-level disinfectants have a wide range of activity against microorganisms but do not demonstrate sporicidal or tuberculocidal activity.

TABLE 4.1 Device Classification and Methods of Effective Disinfection

Device Classification	Disinfection Method	Killing Action Against				
		Spores	Mycobacteria	Nonlipid Viruses	Fungi	Bacteria
Critical	Sterilization					
	Steam	+	+	+	+	+
	Dry heat	+	+	+	+	+
	Gas	+	+	+	+	+
	Chemical	+	+	+	+	+
	Ionizing radiation	+	+	+	+	+
Semicritical	High-level disinfection					
	2% glutaraldehyde	±	+	+	+	+
	Chlorine dioxide	±	+	+	+	+
	Wet pasteurization	–	+	+	+	+
	Low-level disinfection					
	Sodium hypochlorite	–	+	±	+	+
	Quaternary ammonium compounds	–	–	±	+	+
	Ethyl alcohol, isopropyl alcohol (70%–90%)	–	–	+	+	+
	Phenolics	–	±	+	+	+
	Iodophors	–	–	+	+	+

+, Positive kill; –, no kill; ±, variable.

Factors That Influence the Degree of Killing

1. Types of organisms
2. Number of organisms
3. Concentration of disinfecting agent
4. Presence of organic material (e.g., serum, blood)
5. Nature of surface to be disinfected
6. Contact time
7. Temperature
8. pH
9. Biofilms
10. Compatibility of disinfectants and sterilants

Factors: **1-** Types of organisms

Microbial Resistance Hierarchy

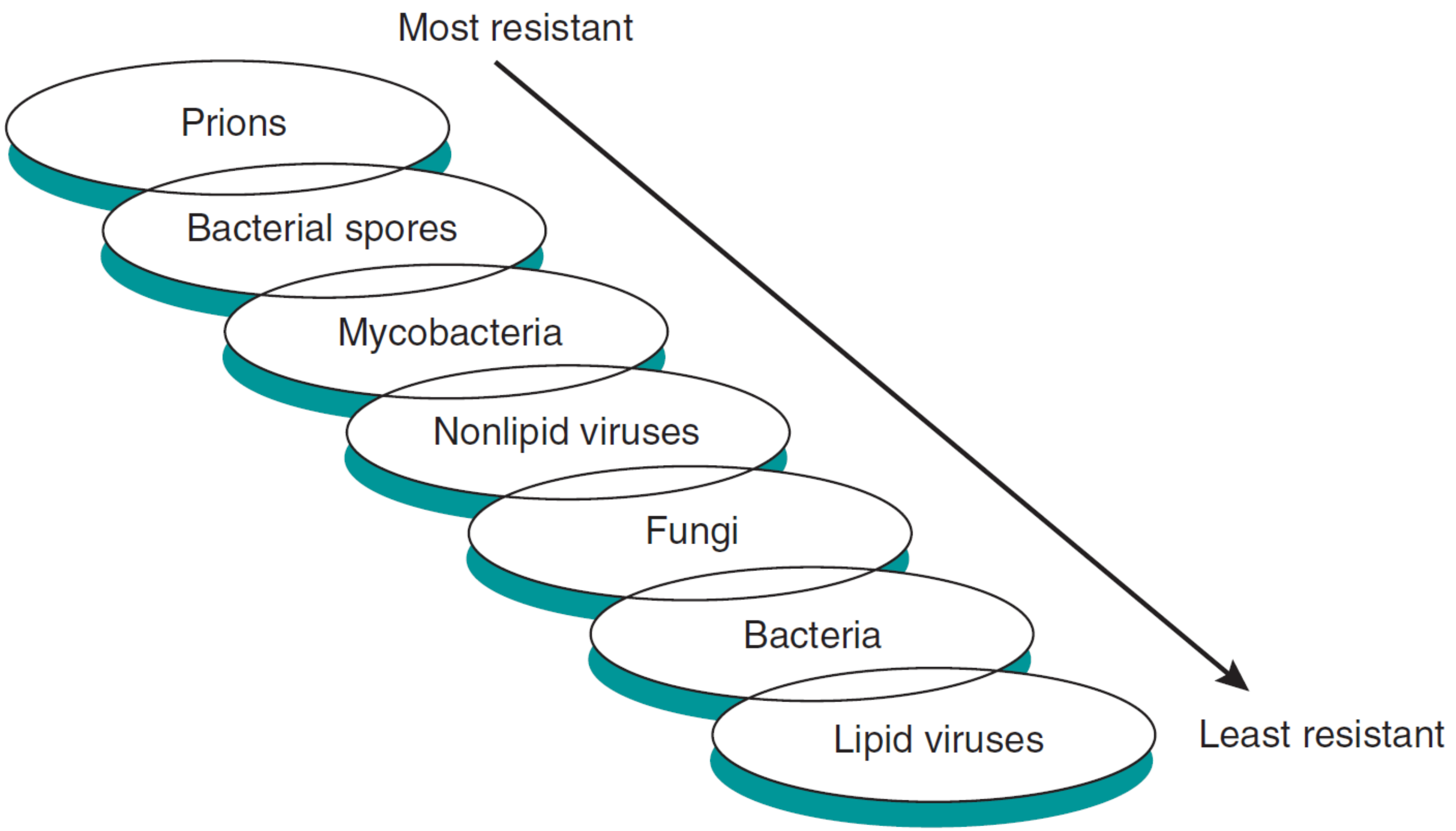


FIG. 4.1 Different types of organisms and their resistance to killing agents.

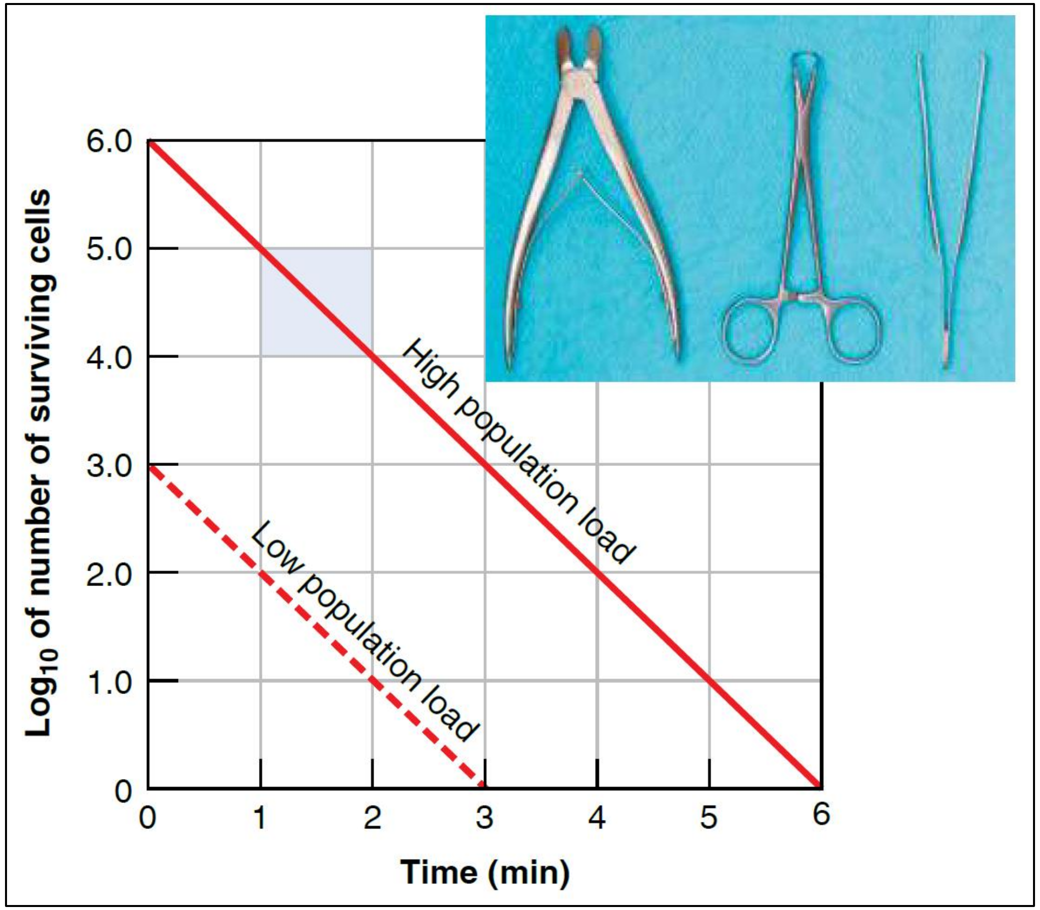
Factors: **2-** Number of Organisms

- Another factor to consider is the total number of organisms present, referred to as the microbial load (bioburden).
- If the number of organisms is plotted against the time they are exposed to the killing agent (exposure time) logarithmically, the result is a straight line (Next Slide)
- In general, higher numbers of organisms require longer exposure times.

Number of Organisms
The Rate of Microbial Death

- Microbial Death Follows Predictable Patterns:
  - Logarithmic rate of death
  - 90% reduction in each time unit

- Example:
  - Starting population: 1,000,000 organisms
  - After 1 minute: 100,000
  - After 2 minutes: 10,000
  - After 3 minutes: 1,000



Factors: **3-** Concentration of Disinfecting Agent

- The amount of disinfectant needed to destroy microorganisms varies with the different agents
- Concentrated disinfectants, such as povidone-iodine, may actually allow microorganisms to survive because there is not enough free iodine to kill microorganisms.
- Proper concentrations of disinfecting agents ensure the inactivation of target organisms and promote safe and cost-effective practices.

Factors: **4-** Presence of Organic Material

- Organic material, such as blood, mucus, and pus, affects killing activity by inactivating the disinfecting agent.
- In addition, by coating the surface to be treated, organic material prevents full contact between object and agent
- Bleach (sodium hypochlorite) is easily inactivated by organic material. For optimal killing activity, instruments and surfaces should be cleansed of excess organic material before disinfection.

Factors: **5-** Nature of Surface to Be Disinfected

- Certain medical instruments are manufactured of biomaterials that exclude the use of certain disinfection or sterilization methods because of possible damage to the instruments.
- For example, endoscopic instruments are readily damaged by the heat generated in an autoclave.
  - Alternative methods must be used for this class of instruments.

Factors: **6-** Contact Time

- The amount of time a disinfectant or sterilant is in contact with the object is critical.
- When disinfecting or sterilizing a contaminated object, it is critical to know what organisms may be present and the contact time to use, which is based on the microorganism that is most resistant.
- The amount of time that an agent is in contact with an object can also determine whether it is disinfecting or sterilizing the object.
- For example, glutaraldehyde can be used as a disinfectant or a sterilant, with the difference being the amount of time the glutaraldehyde is in contact with the contaminated object. When glutaraldehyde is used as a sterilant, the contact time is much longer than when it is used as a disinfectant

Factors: **7-** Temperature

- Disinfectants are generally used at room temperature (20° to 22° C).
- Their activity is generally increased to some degree by an increase in temperature and decreased by a decrease in temperature.
- Disinfection of blood spills in a refrigerator can take longer than disinfection of blood spills on a room temperature countertop.
- Disinfectants and sterilants can be rendered inactive by too high or too low a temperature.

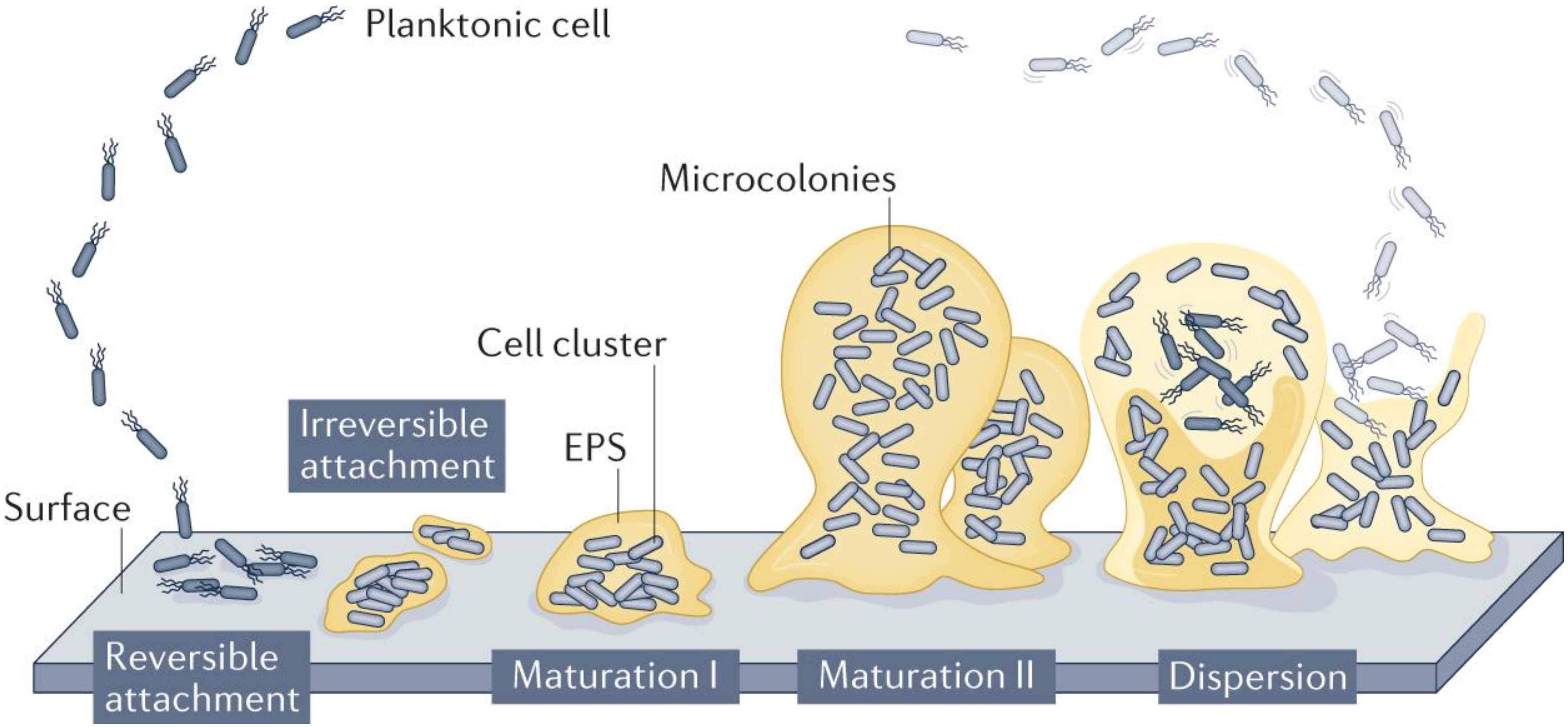
Factors: **8-** pH

- The pH of the material to be disinfected or sterilized can affect the activity of the disinfecting or sterilizing agent.
- It is critical to make sure at what pH the agent is active and what the pH of the material to be exposed to the agent is at the time the process will be done.

Factors: **9-** Biofilms

- Biofilms are considered a community of bacteria or other microorganisms. These communities are generally layers of microorganisms that often have a protective material over them that shields them from outside environmental factors.
- A critical place where biofilms are seen in the hospital is on catheters.
- When dealing with the disinfection of objects that may have a biofilm, it is critical to realize that the presence of the biofilm makes disinfection more difficult.
- Microorganisms in a biofilm can be characteristically different than when they are singular and free floating.
  - This means disinfectants that the microbes were susceptible to singularly can be resistant within a biofilm

Biofilms


Planktonic cell
Microcolonies
Cell cluster
Irreversible attachment
EPS
Surface
Reversible attachment
Maturation I
Maturation II
Dispersion

Factors: **10-** Compatibility of Disinfectants

- A common mistake is to believe that two disinfectants are better than one.
- This is not necessarily incorrect, but when more than one disinfectant is used, the compatibility of the disinfectants must be taken into consideration.
- Some disinfectants may inactivate other disinfectants.
- For example, the use of bleach and a quaternary ammonium compound together may negate the activity of both disinfectants.

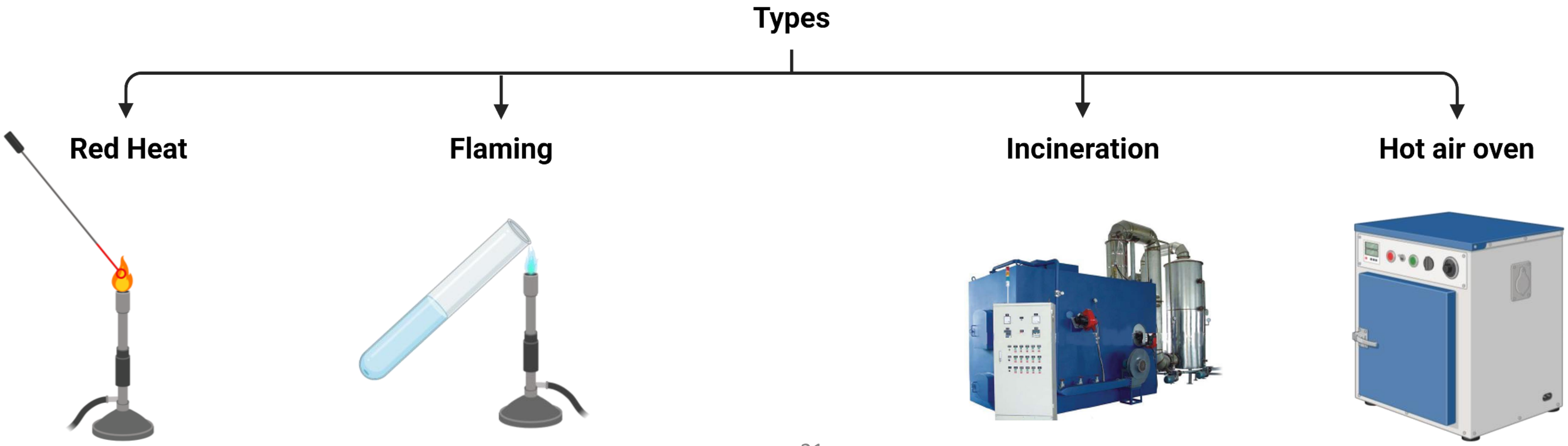
Methods of Sterilization

Methods of sterilization and disinfection

- **Physical**
  - Sunlight
  - Heat
    - Dry heat
      - Red heat
      - Flaming
      - Incineration
      - Hot air oven
      - Infra red
    - Moist heat
      - Below $100^{o}C$
      - At $100^{o}C$
      - Above$100^{o}C$
  - Radiation
    - Non-ionizing
    - Ionizing
      - Electromagnetic
      - Particulate
  - Filtration
- **Chemical**
  - Liquid
    - Alcohols
    - Aldehydes
    - Halogens
    - Phenolics
    - Dyes
    - Heavy metals
  - Gaseous
    - Ethylene oxide
    - Formaldehyde

Physical methods
Dry Heat Sterilization

- Death of micro-organism is by destructive oxidation effects of essential cell constituents especially protein denaturation.

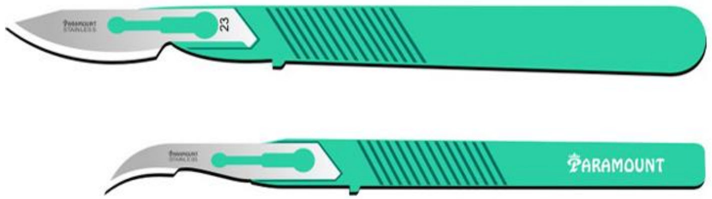


Physical methods
Dry Heat Sterilization - Red heat

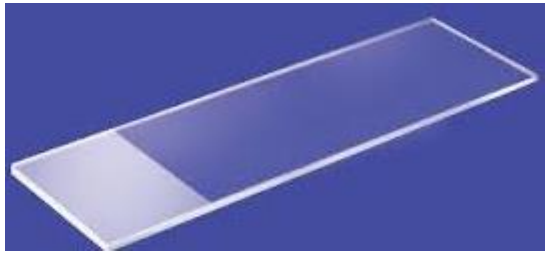
- Articles such as bacteriological loops, tips of forceps, and spatulas are sterilized by holding them in Bunsen flame till they become red hot. This method is limited to those articles that can be heated to redness in flame.

Physical methods
Dry Heat Sterilization - Flaming

- This is direct exposure of materials to naked flame. Inoculation loop or Wire, the tip of Forceps and spatulas are held in a Bunsen flame till they are red hot.



scalpels



glass slides

mouth of test tubes

Flasks

Physical methods
Dry Heat Sterilization – Incineration

- Excellent method of destroying materials such as contaminated cloth, animal carcasses and pathological materials, surgical dressings, sharp needles and other clinical waste at high temperatures (1500°C).

Physical methods
Moist heat

- It is a more effective method compared to dry heat.
- Mechanism of killing: proteins coagulation.
- Forms:
  - below 100°C: Pasteurization.
  - at 100°C: Boiling.
  - above 100°C: Autoclave.

Physical methods
Moist heat - Below 100^{o}C

A. Pasteurization

- Do not kill spores
- Employed in the food and dairy industry.
- Types:
  - Holder method at 63°C for 30 minutes.
  - Flash method at 72°C for 15 minutes rapid cooling to 13°C.

B. Vaccine bath:

- The contaminating bacteria in a vaccine preparation can be inactivated by heating **in a water bath at 60^{o}C for one hour**.
- Only vegetative bacteria are killed and spores survive.

Physical methods
Moist heat - at 100^{o}C (Boiling)

- kills most microorganisms in 10 min. at 100C except spores and certain bacterial toxin.
- Tyndallization: an exposure of 100°C for 20 minutes on 3 successive days; sporicidal

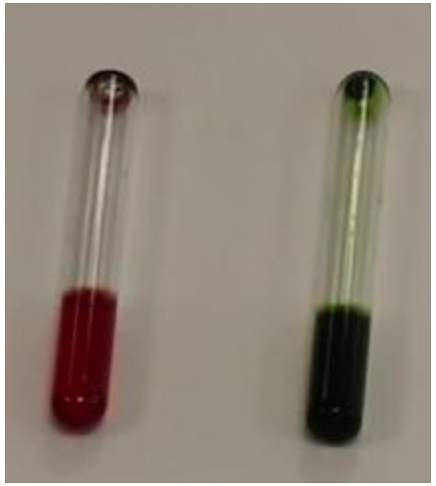
Physical methods
Moist heat - Above 100^{o}C

- By using autoclave
- Destroy **ALL** microorganisms and their spores
  - Steam under 1 atm of pressure, at 121^{o}C, 15 minutes of exposure in autoclaves
  - Destroy ALL microorganisms **(including prions)** and their spores
    - Using longer times: 135^{o}C for at least 1 hour under 2 atm
- **Application:** the sterilization method of choice for heat-stable objects

Controls used in sterilization using Autoclaves

The efficiency of an autoclave should be tested by

1. Temperature recorder(thermometer) .
2. Pressure recorder(barometer)
3. Browne's tubes: **are glass tubes that contain heat-sensitive dyes. These change color after sufficient time at the desired temperature.**
    - Red to green means complete sterilization.
    - Red to brown means incomplete sterilization.

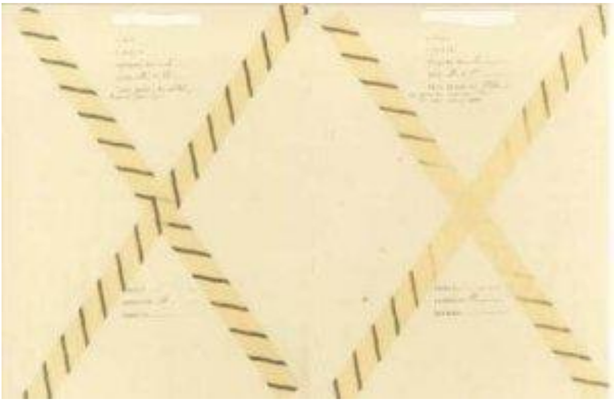


Red to green

Controls used in sterilization using Autoclaves

4. Spore indicator (biological control): They are biological indicators including spores of Bacillus stearothermophilus. If grows after sterilization, this means incomplete sterilization.

5. Bowie Dick tape: before heat exposure, the tape is uniformly buff in color. After adequate heating, the tape develops dark brown stripes.



The color-changing indicator of tape is usually lead carbonate based, which decomposes to lead(II) oxide

Physical methods
Liquid filtration

- Filtration methods may be used with both liquid and air.
- The membrane filters are composed of plastic polymers or cellulose esters containing pores of a certain size
- The liquid is pulled (vacuum) or pushed (pressure) through the filter matrix.
    - Organisms larger than the size of the pores are retained.
- **Pore size of:**
    - 0.45 and 0.80 µm: most bacteria, yeasts, and molds
    - 0.22 µm: for critical sterilizing, e.g. parenteral solutions
    - 0.01 µm: for retaining small viruses
- **Application:** parenteral solutions (serum), vitamins, vaccines and antibiotic solutions

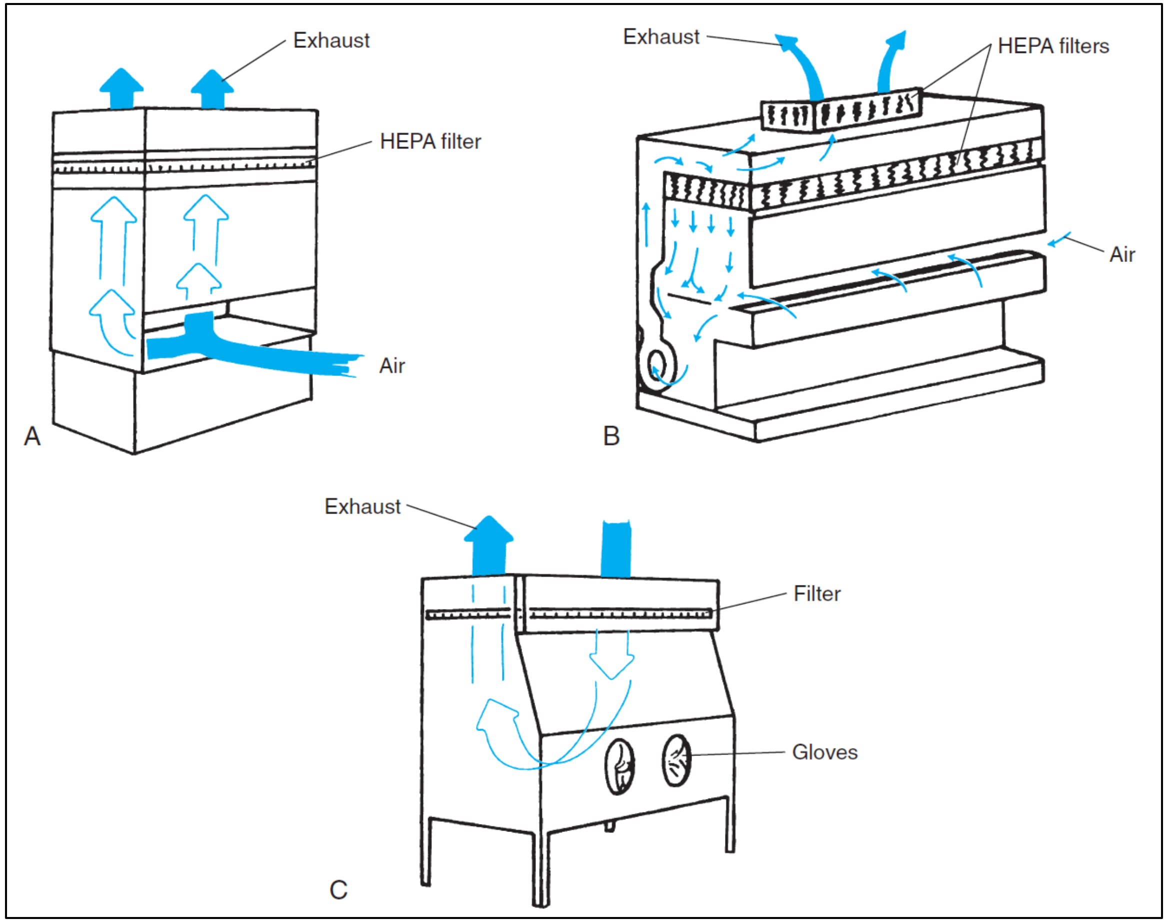
Physical methods
Air filtration

- Filtration of air is accomplished with the use of high-efficiency particulate air (HEPA) filters
  - HEPA filters are able to remove microorganisms larger than 0.3 μm and are used in laboratory hoods and in rooms of immunocompromised patients.

A. The class I biological safety cabinet (hood) uses an exhaust fan to move air inward through the open front. The air is circulated within the safety hood, passing through a high efficiency particulate air (HEPA) filter before reaching the environment outside the hood.

B. The class II biological safety hood **is the most common in microbiology laboratories**. Air is pulled inward and downward by a blower and passed up through the air flow plenum, where it passes through a HEPA filter before reaching the work surface. A percentage of the remaining air is HEPA filtered before reaching the environment.

C. The class III biological safety hood is a self contained ventilated system for highly infectious microorganisms or materials and provides the highest level of personal protection. The closed front contains attached gloves for manipulation on the work surface.



Physical methods
Radiation

- Radiation has various effects on cells, depending on its wavelength, intensity, and duration. Radiation that kills microorganisms (sterilizing radiation) is of two types:
    1. ionizing
    2. nonionizing.

Physical methods
Radiation

A. Ionizing radiation

- gamma rays or electron beams
- short wavelength and high energy

Application: for the medical industry: the sterilization of disposable supplies (syringes, bandages, catheters and gloves), and heat-sensitive pharmaceuticals,

B. Non-ionizing

- Rays of wavelength longer than the visible light are non-ionizing
- in the form of **ultraviolet rays (UV)(280-200 nm)**
- long wavelength and low energy
- Radiation is not very penetrating
- the use is limited

Application: disinfect smooth surfaces with ultraviolet lamps and to reduce airborne pathogens (hospital wards, operation theatres, virus laboratories)

Chemical methods
1- Alcohols

- **Ethanol 70%, isopropanol 70%, propanol 60%**
- Inactivate microorganisms by denaturing proteins
- Wide spectrum against **bacteria and fungi but not sporocidal!**
- **Tuberculocidal and virucidal for most viruses (15 min.)**
- Alcohols may be contaminated with spores –should be filtered through a 0.22 µm filter
- The most effective concentrations are between 60%-90% (water is needed in chemical reactions → because denaturation requires water)
- **Application:** surgical and hygienic disinfection of the skin and hands

Chemical methods
2- Aldehydes

- This is in form of water soluble gas that is lethal to all kinds of micro-organisms and spores.
- **Application:** disinfection of surfaces and objects (plastic and rubber items)
- The sterilizer of choice for heat-sensitive medical equipment

Chemical methods
3- Halogens: (iodine, and their derivatives)

Iodine (2 forms)

- **tincture (alcohol and iodine)**
- **Iodophors or called povidone-iodine (iodine and surfactants)**
- **bactericidal, not sporocidal**
- less irritant than pure iodine

- **Application: as antiseptics, disinfection of skin and small wounds**

Chemical methods
3- Halogens: (iodine, and their derivatives)

Phenols

- Denaturate proteins
- **Broad-spectrum, but not sporocidal, not virucidal**
- **Application: widely used, disinfection of hospital, institutional, and household environment (soaps)**