

# Sterilization and Disinfection

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Dr. Hala Mahmoud Altarawneh



# Learning Objectives

- Define sterilization, disinfection, antisepsis, and related terms
- Understand factors affecting microbial death
- Describe physical and chemical methods of sterilization/disinfection
- Apply appropriate methods in clinical settings

# Terminology - Key Definitions

**Sterilization:** complete destruction or elimination of all forms of microbial life, including bacterial spores

- **Result:** Absolute term - no degree of sterilization (either sterile or not)
- **Example:** Autoclaving surgical instruments

**Disinfection:** elimination of most or all pathogenic microorganisms, EXCEPT bacterial spores, on inanimate objects

- **Result:** Relative term - has levels (high, intermediate, low)
- **Example:** Using glutaraldehyde on endoscopes

# Terminology - Key Definitions

**Antisepsis:** application of antimicrobial agents to **living tissue** to reduce microbial load

- **Result:** Cannot sterilize living tissue (would damage cells)
- **Example:** Applying alcohol to skin before injection

**Decontamination:** process of removing pathogenic microorganisms to make items safe to handle

- **Result:** First step before disinfection/sterilization
- **Example:** Cleaning blood from surgical instruments

# Quick Check Question

**A nurse applies povidone-iodine to a patient's arm before inserting an IV catheter. Is this:**

- A) Sterilization
- B) Disinfection
- C) Antisepsis
- D) Sanitization

**Answer: C - Antisepsis (applied to living tissue)**

# Levels Of Disinfection

Disinfection is NOT an absolute term - it has **THREE levels** based on microbial kill spectrum:

## **1. High-level disinfection (HLD)**

- Kills ALL microorganisms EXCEPT high numbers of bacterial spores
- Effective against: Vegetative bacteria, mycobacteria, fungi, viruses (enveloped & non-enveloped)
- May kill some spores with prolonged exposure
- Clinical applications: semi-critical items as Endoscopes

# Levels Of Disinfection

## **2. Intermediate-level disinfection (Ild)**

- Kills vegetative bacteria, most fungi, mycobacteria, and most viruses
- Does NOT kill bacterial spores
- May not kill some non-enveloped viruses
- Clinical applications: items contaminated with blood

## **3. Low-level disinfection (Lld)**

- Kills most vegetative bacteria, some fungi, and enveloped viruses
- Does NOT kill mycobacteria, bacterial spores, or non-enveloped viruses
- Clinical applications: non-critical items: stethoscopes and blood pressure cuffs, furniture

# Factors Affecting Efficacy of Sterilization & Disinfection

## 1. **Number of microorganisms:**

- The more microorganisms present, the longer it takes to achieve sterilization/disinfection

## 2. **Type of microorganism** (intrinsic resistance):

- Different microorganisms have different resistance levels

# Factors Affecting Efficacy of Sterilization & Disinfection: Resistance Hierarchy

**Resistant**

Prions (extremely resistant)

Bacterial spores (Bacillus, Clostridium)

Mycobacteria (waxy cell wall)

Non-enveloped viruses (Hepatitis A)

Fungi

Vegetative bacteria (most species)

Enveloped viruses (Influenza, Coronavirus)

**Susceptible**

Method effective against spores = Effective against everything

Method that kills only vegetative bacteria = Inadequate for spore-forming organisms

# Factors Affecting Efficacy of Sterilization & Disinfection

## 3. Concentration of disinfecting agent & exposure time

- Both concentration of agent AND duration of contact are critical
- Too low → Ineffective kill, may only inhibit growth (bacteriostatic)
- Too high → May be toxic, corrosive, or wasteful

### Exposure Time:

- Minimum contact time MUST be maintained
- Premature removal = incomplete disinfection
- Different organisms require different exposure times

**Example:** Alcohol (concentration 70%) → exposure time 30-60 sec → intermediate level disinfection (ILD)

# Factors Affecting Efficacy of Sterilization & Disinfection

## 4. Temperature & pH

**Temperature:** Higher temperature = Faster microbial death

### **pH Effects:**

- Many disinfectants are pH-dependent
- **Hypochlorite (bleach):** More effective at acidic pH
- **Glutaraldehyde:** Alkaline pH increases activity

# Factors Affecting Efficacy of Sterilization & Disinfection

## 5. Presence of organic matter

- The most common cause of disinfection failure!
- Organic material includes blood, pus and exudates, faeces

### How Organic Matter Interferes:

- **Physical barrier:** Shields microorganisms from disinfectant
- **Chemical inactivation:** Proteins react with disinfectant, depleting it
- **Dilution effect:** Reduces effective concentration

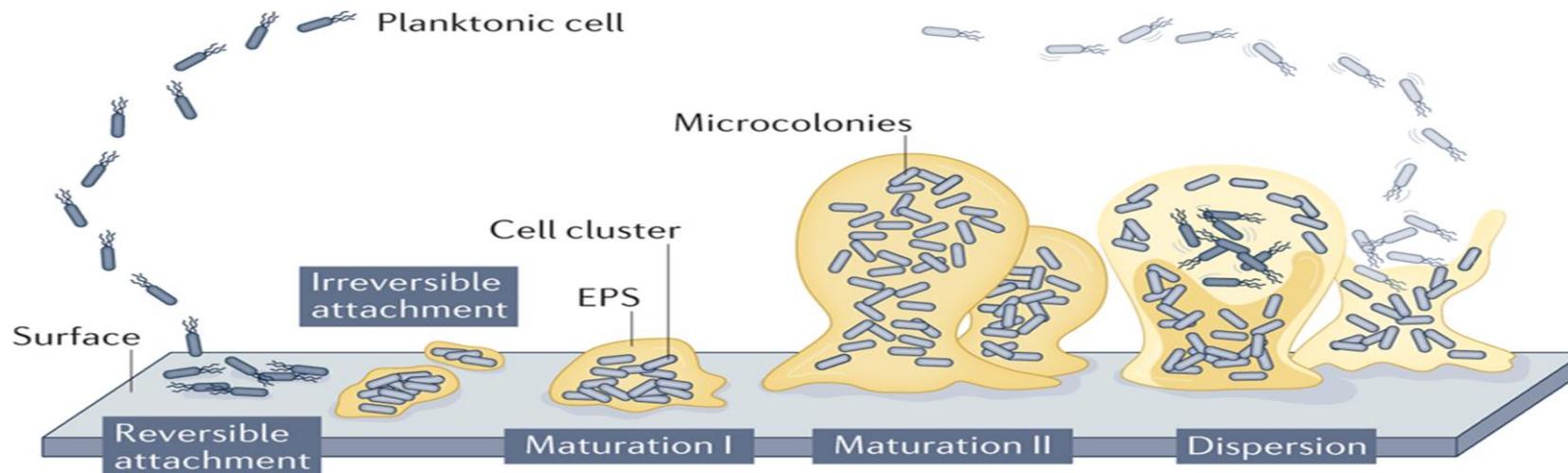
# Factors Affecting Efficacy of Sterilization & Disinfection

## 6. Biofilms

**Definition:** Communities of microorganisms embedded in protective matrix (extracellular polymeric substances)

### Why Biofilms Are Problematic:

- 10-1000× more resistant than planktonic (free-floating) bacteria
- Protective matrix prevents penetration of disinfectants



# Factors Affecting Efficacy of Sterilization & Disinfection

## **7. Nature of surface to be disinfected**

- Instruments with lumens, crevices, or joints are harder to sterilize
- Larger surface area = more time needed

# Memory Aid

## The "3 C's and 3 B's" Rule

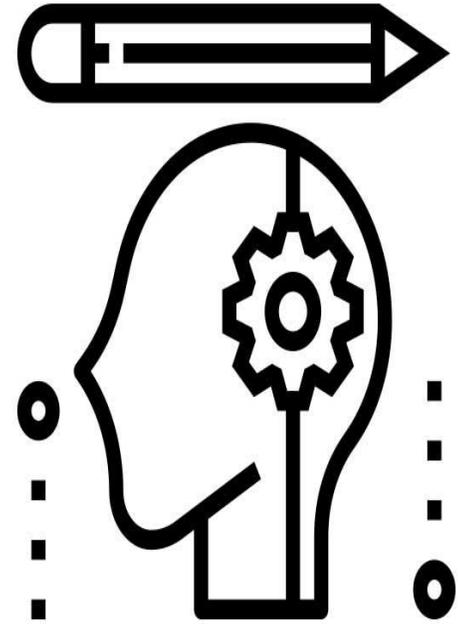
### The 3 C's (What You Control):

- Clean first (remove organic matter)
- Concentration of disinfectant
- Contact time (keep wet!)

### The 3 B's (What You're Fighting):

- Bioburden (number of organisms)
- Bugs (type/resistance of organisms)
- Biofilms (protective communities)

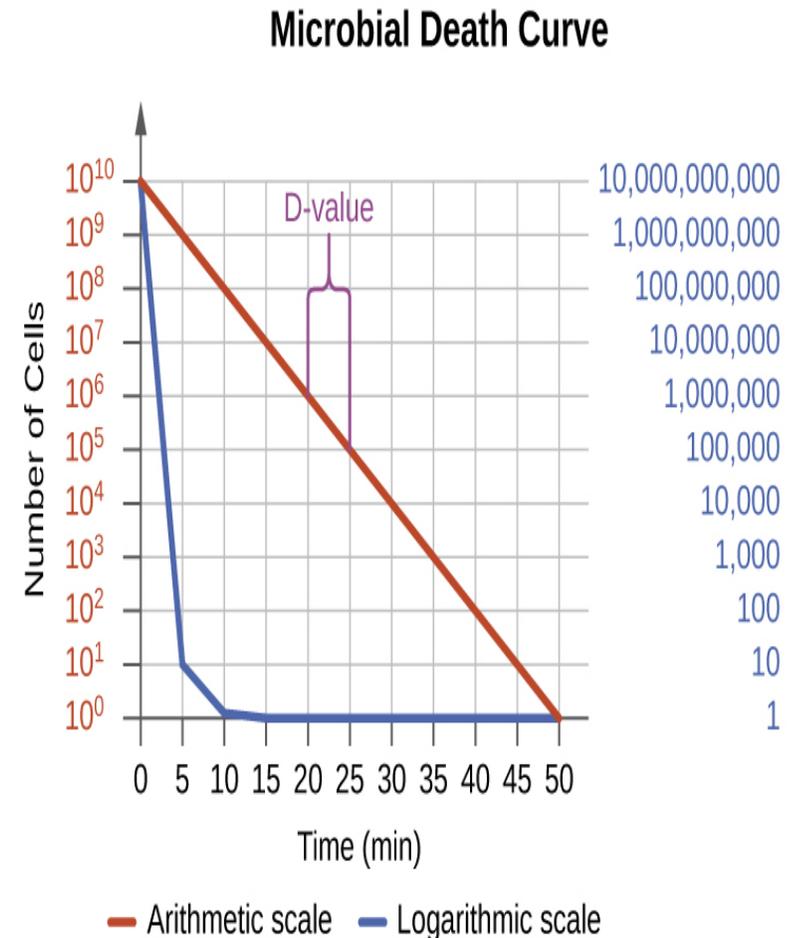
+ T<sup>2</sup> (Temperature & Time)



# Kinetics of Microbial Death

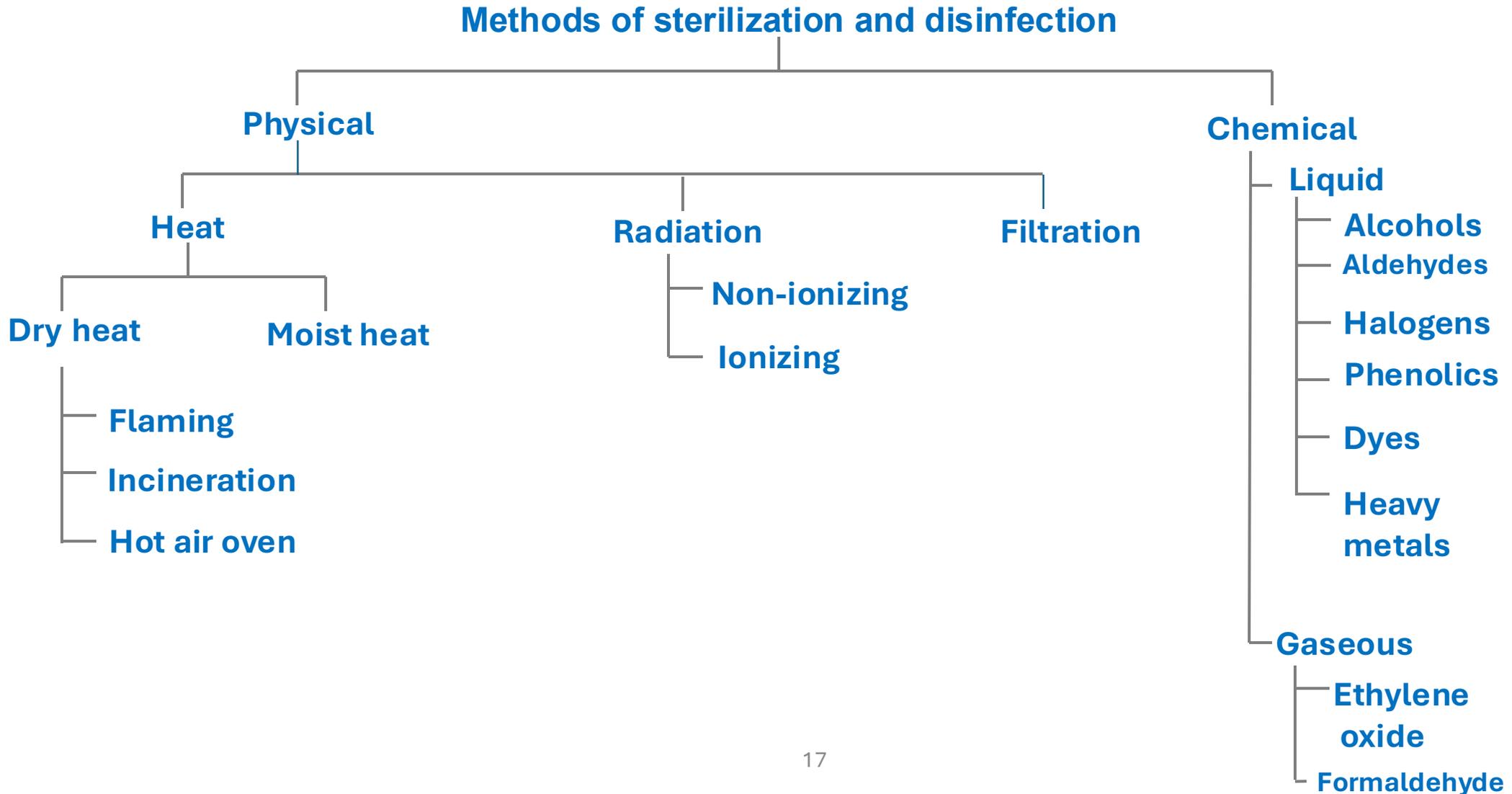
Microbial death follows predictable patterns:

- Microorganisms do NOT all die at once
- They die at a **constant rate (90% die per unit time)**, not the same number



Time (min)	Number of cells
0	$10^{10}$
5	$10^9$
10	$10^8$
15	$10^7$
20	$10^6$
25	$10^5$
30	$10^4$
35	$10^3$
40	$10^2$
45	$10^1$
50	$10^0$

# Methods of Sterilization



# Physical Methods: Heat Sterilization

- Heat is the most reliable, economical, and widely used method of sterilization
- Heat kills microorganisms by denaturing proteins, disrupting membranes, and damaging nucleic acids

## **Two major types of heat sterilization**

1. Moist heat (steam under pressure)
2. Dry heat (high temperature without moisture)

# Physical Methods: Heat Sterilization- **Moist Heat**

- Uses **saturated steam** at high temperature and pressure
- More efficient than dry heat → lower temperature and shorter exposure time required
- **Gold standard** for sterilization
- **Temperature Range:** 121-134°C requires pressure above atmospheric
- **Primary Method:** autoclaving (most common)

# Physical Methods: Heat Sterilization- Moist Heat



## How does an autoclave work?

- Steam is generated under pressure in a sealed chamber
- Pressure allows steam temperature to exceed 100°C (boiling point)
  - At atmospheric pressure (sea level): Water boils at 100°C
  - Under 15 psi (1 bar) pressure: Water boils at 121°C
  - Under 30 psi (2 bar) pressure: Water boils at 134°C
- Steam penetrates materials and condenses on cool surfaces
- Condensation releases latent heat, killing microorganisms

# Physical Methods: Heat Sterilization- **Dry Heat**

- Uses **hot air** without moisture
- Less efficient than moist heat → requires higher temperature and requires longer exposure time
- Mechanism: Oxidation rather than denaturation
- **Temperature Range:** 160-180°C at atmospheric pressure
- **Primary Methods:** hot air oven, incineration, flaming

# Physical Methods: Heat Sterilization- **Dry Heat**

## Types of dry heat sterilization

### 1. **Hot air oven** (Most Common):

- Electrically heated chamber with air circulation.
- Temperature range: 160-180°C.
- Time: 30 minutes to 2 hours.



# Physical Methods: Heat Sterilization- **Dry Heat**

## 2. Incineration:

Burning items at high temperature ( $>600^{\circ}\text{C}$ )  $\rightarrow$  Complete destruction by burning  $\rightarrow$  Reduces items to ash

- Most extreme form of sterilization



# Physical Methods: Heat Sterilization- **Dry Heat**

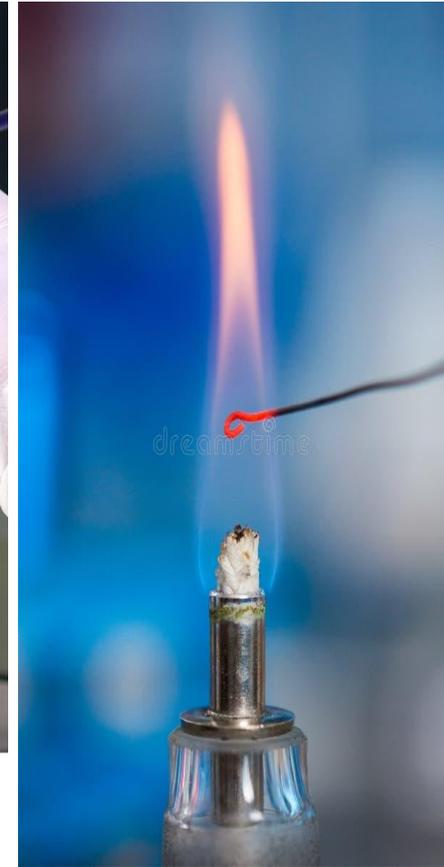
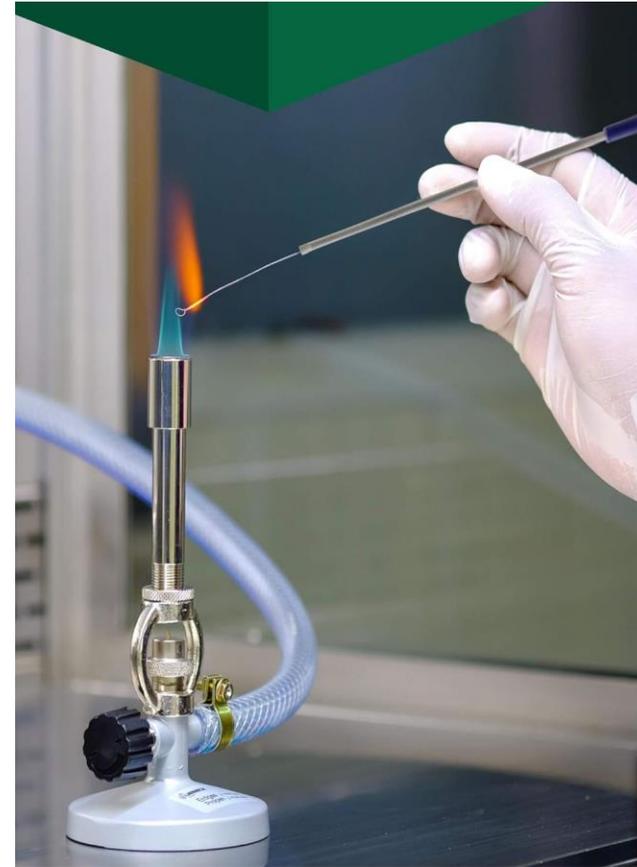
## Types of dry heat sterilization

**3. Flaming:** direct exposure to flame (bunsen burner)

- Item held in flame until red hot
- Very rapid

## Applications:

- Inoculating loops in microbiology lab
- Needles (in emergency/field situations)



# Physical Methods: Other Heat Methods

1. **Pasteurization:** controlled heating to reduce microbial load in liquids - typically at 63-72°C for 15 seconds to 30 minutes

- **NOT sterilization** - only reduces pathogens
- Named after Louis Pasteur

**Applications:** Food Industry: milk and dairy products

2. **Boiling (100°C):** Heat water to boiling point (100°C at sea level)

- **Disinfection, NOT sterilization**
- **Standard Protocol:** 100°C for 10-30 minutes

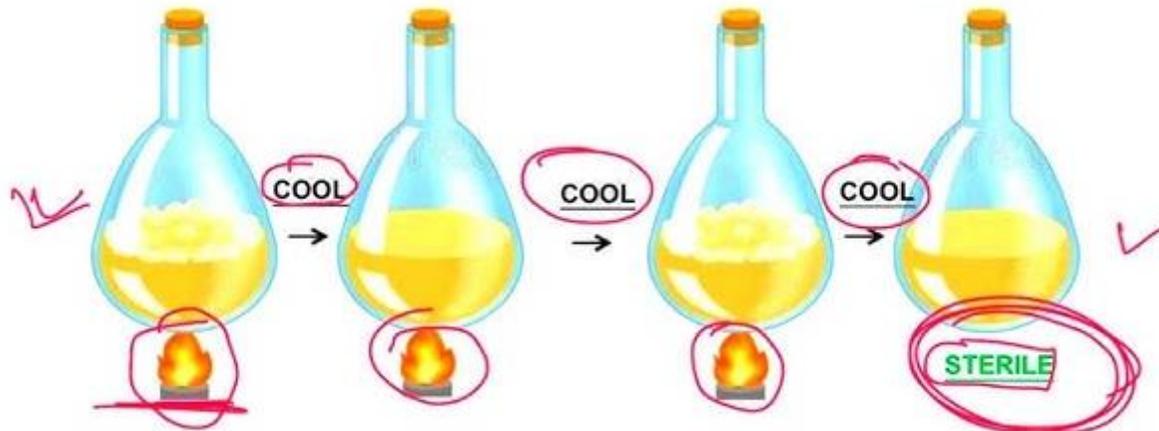
**Applications: Home/Community:** Baby bottles

# Physical Methods: Other Heat Methods

## 3. Tyndallization (fractional sterilization)

**Definition:** Intermittent heating method for heat-sensitive liquids

- **The Principle:** spores resist heat, but vegetative cells don't → Heat kills vegetative bacteria
- Multiple cycles of heating and incubation → Allows spore germination between cycles
- Named after John Tyndall



# Physical Methods: Radiation

## A. Ionizing radiation

- gamma rays or electron beams
- short wavelength and high energy → breaks DNA strands → creates free radicals that damage cellular components
- ionizing radiation **penetrates deeply** - you can sterilize entire of sealed, packaged items at once!
- **Application:** for the medical industry: the sterilization of disposable supplies (syringes, bandages, and gloves)

# Physical Methods: Radiation

## B. Non-ionizing

- **Type:** Ultraviolet light
- **Mechanism:** UV light absorbed by DNA → Creates thymine dimers (DNA mutation) → Prevents DNA replication → Cells cannot reproduce → death
- long wavelength and low energy
- **Penetration: POOR** - only surface sterilization
- **Application:** air disinfection in operating room air or surface disinfection like laboratory benches

# Physical Methods: Filtration

- Physical removal of microorganisms by passing liquid/gas through filter - **organisms trapped, not killed**
- **Principle of filtration**
  - Physical barrier with microscopic pores
  - Microorganisms too large to pass through
  - Liquid/gas passes, microbes retained
  - **Removal, NOT killing**
- **Application:**
  - **Liquid sterilization** (Primary Use): Heat-labile solutions
  - **Air sterilization:** HEPA Filters in Operating rooms or Isolation rooms

# Chemical Methods

- Uses chemical agents **to kill** or **inhibit** microorganisms
- **Chemical Agents Classified by:**
  1. **Function:**
    - Sterilant (Sporicides): Kill ALL organisms including spores
    - High-Level Disinfectants: Kill all organisms EXCEPT high numbers of spores
    - Intermediate-Level Disinfectants: Kill vegetative bacteria, mycobacteria, most viruses, fungi. Does NOT kill spores
    - Low-Level Disinfectants: Kill most vegetative bacteria, some fungi, enveloped viruses. Does NOT kill mycobacteria, spores, non-enveloped viruses

# Chemical Methods

## 2. By Chemical Structure:

- Alcohols
- Aldehydes (formaldehyde)
- Halogens (chlorine, iodine)
- Oxidizing agents (hydrogen peroxide)
- Phenolics
- Quaternary ammonium compounds
- Gases (ethylene oxide)

# Chemical Methods

## 3. By Application:

- **Disinfectants** = For inanimate objects ONLY (too toxic for tissue)
- **Antiseptics** = Safe for living tissue (skin, mucous membranes)
- **Preservatives** = Prevent microbial growth in products

# Chemical Methods: Mechanisms of Action

## How Chemicals Kill Microorganisms:

**1. Protein Denaturation:** Disrupts protein structure → Inactivates enzymes

- Example: Alcohols, aldehydes

**2. Membrane Damage:** Disrupts lipid bilayer → Causes leakage of cell contents

- Example: Alcohols, phenolics

# Chemical Methods: Mechanisms of Action

**3. Oxidation:** generates reactive oxygen species → damages proteins, lipids, DNA

- Example: Hydrogen peroxide, chlorine

**4. Alkylation:** binds to proteins and DNA → interferes with cellular processes

- Example: Glutaraldehyde, formaldehyde

**5. Halogenation:** oxidizes proteins and nucleic acids → disrupts cellular metabolism

- Example: Chlorine, iodine compounds

Thank you

A hand holding a red marker is shown on the right side of the image, writing the words "Thank you" in a cursive script. The text is written in a vibrant red color and is positioned to the left of the hand. The hand is wearing a dark suit jacket and a white shirt cuff is visible. The background is plain white.