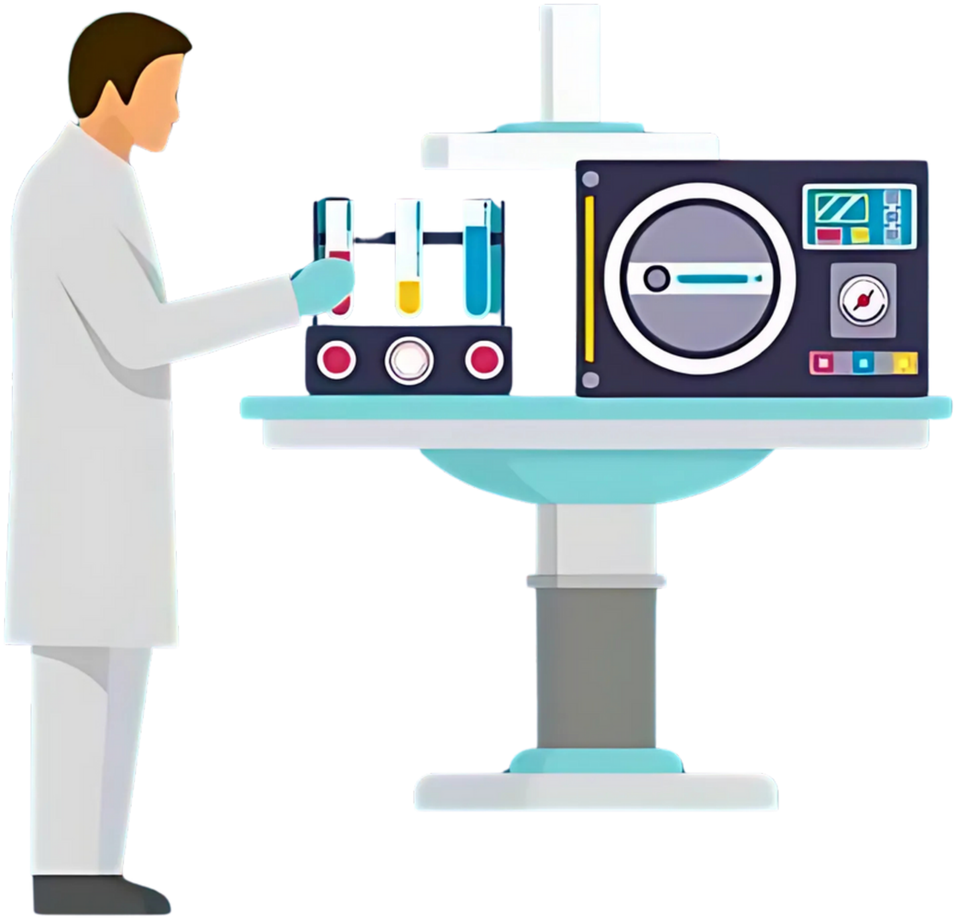
PRINCIPLES AND METHODS OF STERILIZATION



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EDU SERUMX

COLLEGE OF PARAMEDICAL

BMLT- 1ST YEAR

MICROBIOLOGY

PRINCIPLES OF STERILIZATION

Sterilization is the process of complete destruction or removal of all forms of microbial life, including bacteria, viruses, fungi, protozoa, and bacterial spores, from any object or surface.

1. Complete Destruction of All Microorganisms

· Goal: To destroy all living and dormant microbial forms.

· Includes:

- Bacteria (both vegetative and spores)
- Fungi
- Viruses
- Protozoa
- Mycobacteria

Spores are the toughest to kill and need special methods (e.g., autoclaving or chemical sterilants).

Example: Bacillus and Clostridium spores require high pressure steam or strong chemicals like glutaraldehyde.

2. Proper Time and Temperature

· Sterilization is effective only when correct temperature is maintained for a specific time.

· Time and temperature vary depending on the method:

- Autoclave: 121°C for 15–20 minutes at 15 psi pressure.
- Dry heat oven: 160–180°C for 1–2 hours.

Why important? Shorter time or low temperature may not kill all microbes, especially spores.

3. Penetration Ability

· The sterilizing agent must penetrate deep into the item:

- Inside lumens of instruments
- Tubes, joints, folds, porous materials (like cotton, gauze)

· Steam, gas (e.g., ETO), or radiation must reach all surfaces of the object.

◆ Example: ETO gas can sterilize narrow plastic tubing which steam cannot reach.

4. Material Compatibility

• Sterilization should not damage the item.

• Choose the method based on material type:

Material Type -Suitable Sterilization Method

- Metal instruments -Moist heat (autoclave) or dry heat
- Plastic items -ETO gas or low-temperature plasma gas
- Glassware -Dry heat or autoclave
- Heat-sensitive liquids -Filtration sterilization
- Surgical dressings -Autoclave

🔹 Example: Autoclaving plastic can melt or deform it. Use gas instead.

5. Pre-cleaning is Essential

• Clean the instruments before sterilization to remove:

- Blood
- Pus
- Mucus
- Organic debris

• Organic matter can protect microbes by blocking sterilant penetration.

🔹 Example: Blood-stained scissors must be washed before autoclaving.

6. Monitoring and Validation

• Sterilization process should be regularly checked and verified to ensure effectiveness.

o Chemical Indicators:

 Change color when correct temp/time is achieved (e.g., autoclave tape)

o Biological Indicators:

 Use of spore strips (e.g., Bacillus stearothermophilus) to test actual sterilization.

🔹 Example: Indicator tape on an autoclaved pack turns black when sterilization is successful.

7. **Safety and Cost-effectiveness**

• Method should be:

o Safe for users and environment

o Economical

o Leave no toxic residue

• Avoid methods that can cause burns, chemical exposure, or equipment damage.

🔹 Example: Formaldehyde gas is effective but toxic, so only used with proper ventilation.

Summary Table:

Principle Key Focus
Destruction of Microbes : All forms including spores must be killed
Correct Time & Temperature: Specific values for each method (e.g., 121°C for 15 min)
Penetration Agent must reach : all parts (inner tubes, folds)
Material Compatibility : Choose method based on material to avoid damage
Pre-cleaning : Remove organic matter before sterilizing
Monitoring & Validation :Use indicators to confirm sterilization
Safety and Cost :Ensure it is human-safe and economically viable

Methods of Sterilization

Sterilization means complete destruction or removal of all microorganisms, including bacteria, viruses, fungi, and spores, from materials or surfaces.
भौतिक विधियाँ (Physical methods) में हम गर्मी, विकिरण (radiation), filtration आदि का उपयोग करते हैं।

Types of sterilisation:
1) Physical Methods (भौतिक विधियाँ) :
- Heat Sterilization : Dry heat, moist heat , tyndallization
- Radiation Sterilization : Ionizing Radiation and Non-ionizing Radiation

2) Chemical Sterilization
3) Filtration Sterilization
4) Mechanical Methods

A. Heat Sterilization

Heat kills microorganisms by:

- Protein coagulation (प्रोटीन का जमाव)
- Oxidation of cellular components (कोशिकीय अंशों का ऑक्सीकरण)

Heat sterilization is divided into :

- Moist Heat Sterilization
- Dry Heat Sterilization
- Tyndallization

1. Moist Heat Sterilization : This uses steam or water to generate heat.

It works by:

- Denaturing enzymes & proteins
- Damaging cell membranes

a) Autoclaving

Principle: Uses steam under pressure to increase the boiling point of water.

Standard Parameters:

- Temperature: 121°C
- Pressure: 15 psi
- Time: 15–20 minutes

Mechanism:Steam penetrates objects and kills even spores by protein denaturation.

Applications:

- Culture media (मीडिया)
- Glassware (काँच की वस्तुएँ)
- Surgical instruments (शल्य चिकित्सा उपकरण)

Example:

Autoclaving petri dishes, test tubes, surgical gloves before operation.

b) Boiling (उबालना)

Principle: Moist heat at 100°C kills most vegetative pathogens.

Time: 10–30 minutes

Limitation: Does not kill bacterial spores (जो बहुत कठोर होते हैं)

Uses:

- Baby bottles
- Small surgical tools in home settings

Example: Boiling water to disinfect it before drinking.

c) Tyndallization (टिण्डलाइजेशन)

Also known as: Intermittent sterilization

Method: Heat material to 100°C for 30 minutes on 3 consecutive days.

Purpose: Allows spores to germinate into vegetative forms, which are then killed in subsequent heating.

Used For: Heat-sensitive media like sugar-containing media, gelatin, etc.

2. Dry Heat Sterilization (सूखी गर्मी द्वारा निष्फलीकरण): This method uses dry air and kills microorganisms through oxidation.It requires higher temperature and longer time compared to moist heat.

a) Hot Air Oven (हॉट एयर ओवन)

· Temperature: 160–180°C
· Time: 1–2 hours
· Mechanism: High dry heat destroys microbial proteins and oxidizes cellular components.
· Applications: Glass syringes, Oils and powders, Metal forceps and scalpels etc
· Precaution: Material should be heat-resistant and completely dry before putting into oven.

b) Incineration (दहन / जलाना)

· Definition: Direct burning of materials to ash
· Use: Complete destruction of infectious waste
· Examples: Burning used cotton, gauze, and dressings, Flame sterilization of inoculating loops in labs
· Common in: Hospitals and microbiology labs

1. Radiation Sterilization

Definition: Radiation sterilization is the process of using electromagnetic waves or rays to kill microorganisms by damaging their DNA and proteins. It is used especially for disposable items and air/surface disinfection where heat or chemicals are not suitable.

Types of Radiation:

A. Ionizing Radiation (आयनिक विकिरण):

Uses: Gamma rays, X-rays

Mechanism: Penetrates deep into materials and breaks DNA strands, preventing microbial reproduction.

Highly effective against: All forms including bacterial spores.

Used For: Disposable syringes, Catheters, Gloves, IV tubing, Pharmaceutical products

Example in hospital/lab: Sterilization of pre-packed surgical kits and plastic syringes using gamma rays.

B. Non-Ionizing Radiation (गैर-आयनिक विकिरण):

Uses: Ultraviolet (UV) light (254 nm wavelength)

· Mechanism: Damages DNA and RNA of microbes → mutation → death

· Limitations: Poor penetration (only surface sterilization)

· Used For: Surface sterilization in laminar air flows, Air sterilization in operation theaters and microbiology labs

Example: UV light in biosafety cabinet used during media preparation or subculturing.

2. Chemical Methods (रासायनिक विधियाँ)

Definition:

Used to sterilize heat-sensitive items (जो गर्मी सहन नहीं कर सकते). It involves using chemical agents that kill or inhibit microorganisms.

Chemical	Use/Application	Example
Ethylene Oxide (ETO)	Low-temp sterilization (gas)	Plastic instruments, catheters
Formaldehyde	Fumigation	Sterilization of operation theaters
Glutaraldehyde (2%)	High-level disinfectant	Endoscopes, metal instruments
Alcohol (70% Ethanol)	Surface sterilization	Skin disinfection before injections
Phenol (Carbolic acid)	Disinfectant for surfaces	Hospital floors, beds, equipment
Halogens (Iodine, Chlorine)	Water & skin sterilization	Betadine (iodine), Bleaching powder

Example :

· ETO gas: Sterilizing IV tubing in CSSD department of hospitals.
· Alcohol (70%): Cleaning skin before collecting blood samples.
· Formaldehyde: Room fumigation in microbiology labs.
· Glutaraldehyde: Disinfecting cystoscopes, endoscopes.
· Iodine (Betadine): Skin sterilization before injections or surgery.

3. Filtration Sterilization (छानने द्वारा निष्फलीकरण)

Definition: A method of removing microbes physically (not killing) by passing liquid or air through filters with tiny pores.

Used For:

· Heat-sensitive liquids like: Serum, Antibiotics, Vaccines, IV fluids

· Also used for air filtration in labs.

Types of Filters:

Filter Type	Used For
Seitz Filter	Asbestos filter for bacterial filtration
Membrane Filter	Thin membrane with pore size <0.2 µm
HEPA Filter	High Efficiency Particulate Air filter for **air**

Example :

· Use membrane filters to sterilize antibiotic solutions before adding to culture media.

· HEPA filters in laminar flow hoods to keep work area sterile.

4. Mechanical Methods (यांत्रिक विधियाँ)

Definition:

These are cleaning methods, not true sterilization, but help in removal of microorganisms from surfaces or instruments.

Method	Description	Where Used
Washing with Detergents	Removes dirt and microbes from tools	Labs, hospitals
Ultrasonic Vibrations	Dislodges microbes using sound waves	Cleaning surgical instruments
Air Filtration (HEPA)	Removes microbes from air	Operation theatres, ICUs, Labs

Example :

· Washing test tubes and flasks before autoclaving.

· Ultrasonic cleaners used for cleaning glassware in hospital labs.

· HEPA filters protect sterile zones in microbiology and pathology labs.

Summary Table – Sterilization Methods

Method	Examples	Use
Moist Heat	Autoclave, Boiling	Media, surgical tools
Dry Heat	Hot air oven, Incineration	Glassware, infected waste
Radiation	UV, Gamma rays	Surfaces, disposables
Chemical	Alcohol, ETO, Glutaraldehyde	Skin, plastic items, endoscopes
Filtration	Membrane, Seitz, HEPA	Serum, air, antibiotics
Mechanical	Washing, HEPA, Ultrasonics	Pre-cleaning, sterile environments