

STERILIZATION



Presented By

CH.VAMSI ANIL KRISHNA

(10AB1R0026)

Under the Guidance of

Mrs. P. SOWJANYA B.PHARM.,M.TECH

Assistant Professor

Department of Bio-Technology



VIGNAN PHARMACY COLLEGE

(Approved by AICTE & PCI Affiliated to JNTU KAKINADA)

VADLAMUDI, GUNTUR DIST, ANDHRA PRADESH, INDIA, PIN: 522 213

CONTENTS

➤ **INTRODUCTION**

➤ **DEFINITION**

➤ **METHODS**

PHYSICAL METHODS

CHEMICAL METHODS

➤ **VALIDATION**

➤ **PHARMACEUTICAL APPLICATIONS**

➤ **CONCLUSION**

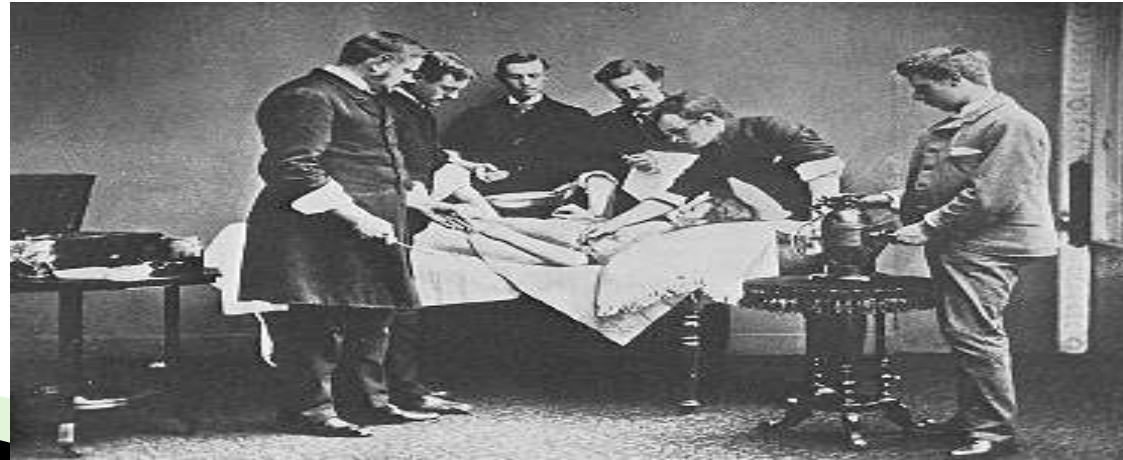
➤ **REFERENCES**

➤ **ACKNOWLEDGMENT**



INTRODUCTION

- Early civilizations practiced salting, smoking, pickling, drying, and exposure of food and clothing to sunlight to control microbial growth.
- The ancient Greeks and Romans used silver vessels to store fresh liquids and wine.
- Hypochlorite and iodine were introduced as a treatment for open wounds in 1825 and 1839.
- In 1861 Louis Pasteur proved that microorganisms caused spoilage and could be transported via the air.



DEFINITION

“Sterilization is a process used to destroy or eliminate viable micro organisms that may be present in or a particular product or package including **spores**”



TERMINOLOGY

The following terms relating to sterilization should be understood by those carrying out sterilization process or handling sterile products are

ANTISEPTIC

“A substance that arrest or prevents the growth of micro organisms by inhibiting their activity without necessarily destroying them.”

ASEPTIC

“It refer to areas and practices where the intent is to be sterile.”

ASEPTIC PROCESSING

“Those operations performed between the sterilization of an object or preparation and the final sealing of its package. These operations are, by definition, carried out in the complete absence of microorganisms.”

BACTERICIDE

“Any agent that destroys/ kills the micro organisms is called as bactericidal agent.”

BACTERIOSTAT

“Any agent that arrest the growth of the micro organisms is called as bacteriostatic agent.”



SANITIZATION

“A process that reduces the level of micro organisms count on eating and drinking utensils.”

STERILE

“The absolute absence of viable micro organisms.”

VALIDATION

“The act of verifying that a procedure is capable of producing the intended result under prescribed circumstance.”

VIRICIDE

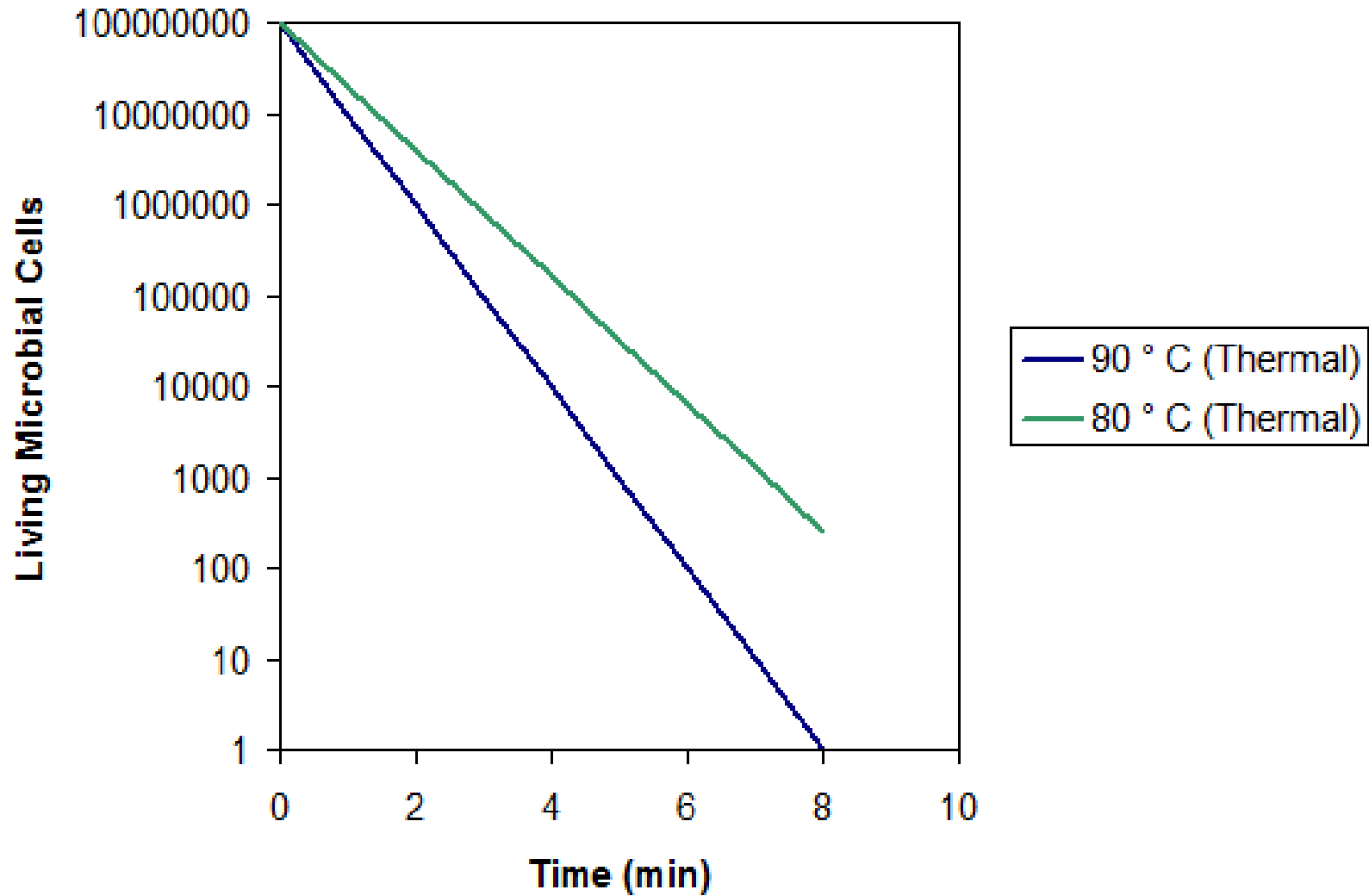
“An agent that will destroys viruses.”



RATE OF MICROBIAL DEATH

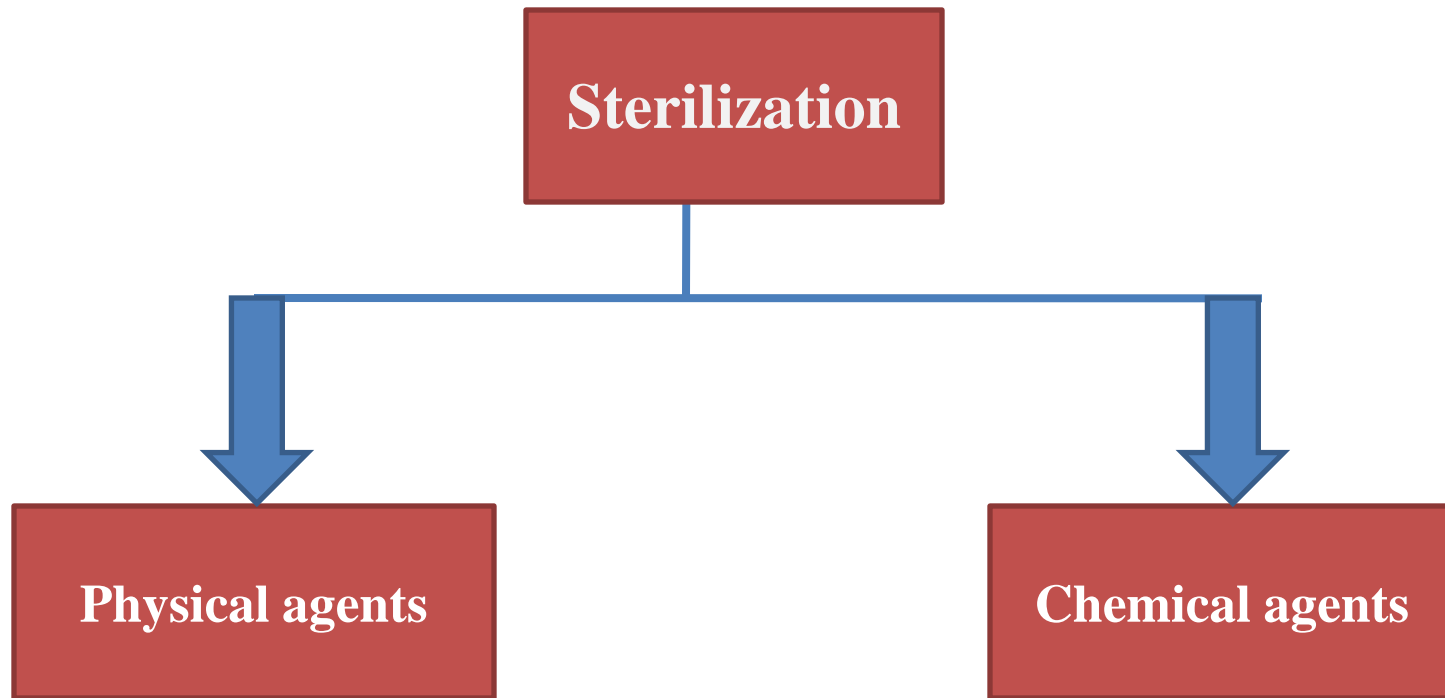
S.No	Time	Death Per Min	Number Of Survivors
1.	0 min	0	1000000
2.	1 min	9000000	100000
3.	2 min	90000	10000
4.	3 min	9000	1000
5.	4 min	900	100
6.	5 min	90	10
7.	6 min	9	1

DEATH CURVE OF MICRO ORGANISMS



METHODS OF STERILIZATION

The various agents used in sterilization can be classified as follows



Physical Agents

```
graph TD; A[Physical Agents] --> B[Heat sterilization]; A --> C[Filtration sterilization]; A --> D[Radiation sterilization]; B --> E[Dry heat sterilization]; B --> F[Moist heat sterilization]; D --> G[Ionising Radiation]; D --> H[Non-Ionising Radiation];
```

The diagram is a hierarchical flowchart. At the top is a blue box labeled 'Physical Agents'. A red line descends from this box and branches into three green arrows pointing to three red boxes: 'Heat sterilization', 'Filtration sterilization', and 'Radiation sterilization'. From the 'Heat sterilization' box, a green line descends and branches into two orange arrows pointing to two white boxes: 'Dry heat sterilization' and 'Moist heat sterilization'. From the 'Radiation sterilization' box, a green line descends and branches into two orange arrows pointing to two white boxes: 'Ionising Radiation' and 'Non-Ionising Radiation'. The 'Filtration sterilization' box has no further sub-categories shown.

**Heat
sterilization**

**Filtration
sterilization**

**Radiation
sterilization**

**Dry heat
sterilization**

**Moist heat
sterilization**

**Ionising
Radiation**

**Non-Ionising
Radiation**

Equipments used in Physical Methods

➤ Heat Sterilization

Hot air oven (Dry heat)

Flaming

Incineration

Autoclaves (Moist heat)

➤ Filtration Sterilization

Candles

Asbestos pads

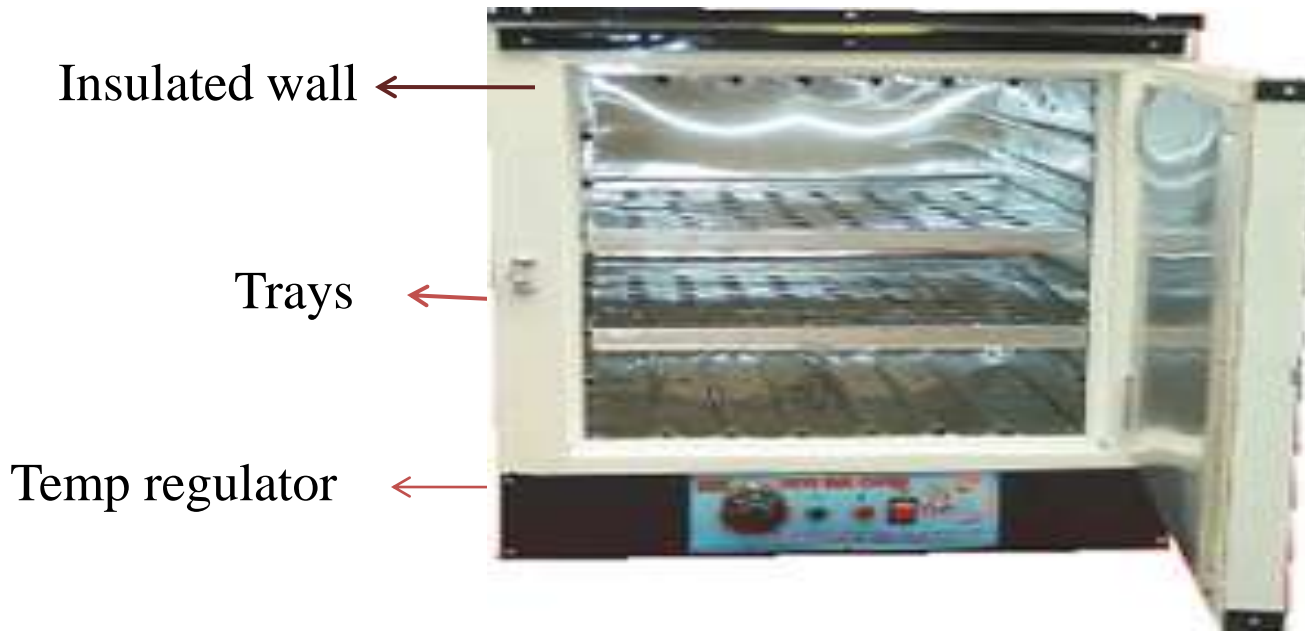
Membrane filters

➤ Radiation Sterilization

Gamma rays

X-rays & UV- rays

HOT AIR OVEN



- ✓ Mechanism involved is oxidation process.
- ✓ The temperature range is 50°C to 300°C.
- ✓ Thermostat controls temperature.
- ✓ The Double walled insulation keeps the heat in and conserves energy.

Temperature Conditions For **HOT AIR OVEN**

S.No	Temperature	Time Required
1.	160 ⁰ c	60 min
2.	170 ⁰ c	40 min
3.	180 ⁰ c	20 min

Pharmaceutical Uses

- ✓ Dry powdered drugs.
- ✓ Oils, fats, waxes.
- ✓ Oily injections.
- ✓ Ophthalmic ointments.



FLAMING

- It is the simplest method used in the Physical method.
- Source of heat is Bunsen burner.

Uses

- ✓ Inoculation loops
- ✓ Wires
- ✓ Tip of forceps
- ✓ Spatulas



INCINERATION

- Incineration means burning the substance until it becomes ash in the presence of the oxygen.
- This is an excellent method of destroying materials such as contaminated cloth, animal carcasses and pathological materials.

Uses:

- ✓ Papers
- ✓ Clothes



→ Ash

MOIST HEAT STERILIZATION

This is carried out by the following ways depends on the temperature range. They are

- **Temperature below 100⁰c**
 - Pasteurisation
 - Hot water bath
- **Temperature at 100⁰c**
 - Boiling
- **Temperature above 100⁰c**
 - Steam under pressure: Autoclave



TEMPERATURE BELOW 100°C

PASTEURISATION

“Process of killing of pathogens in the milk but does not sterilize it.”

METHODS

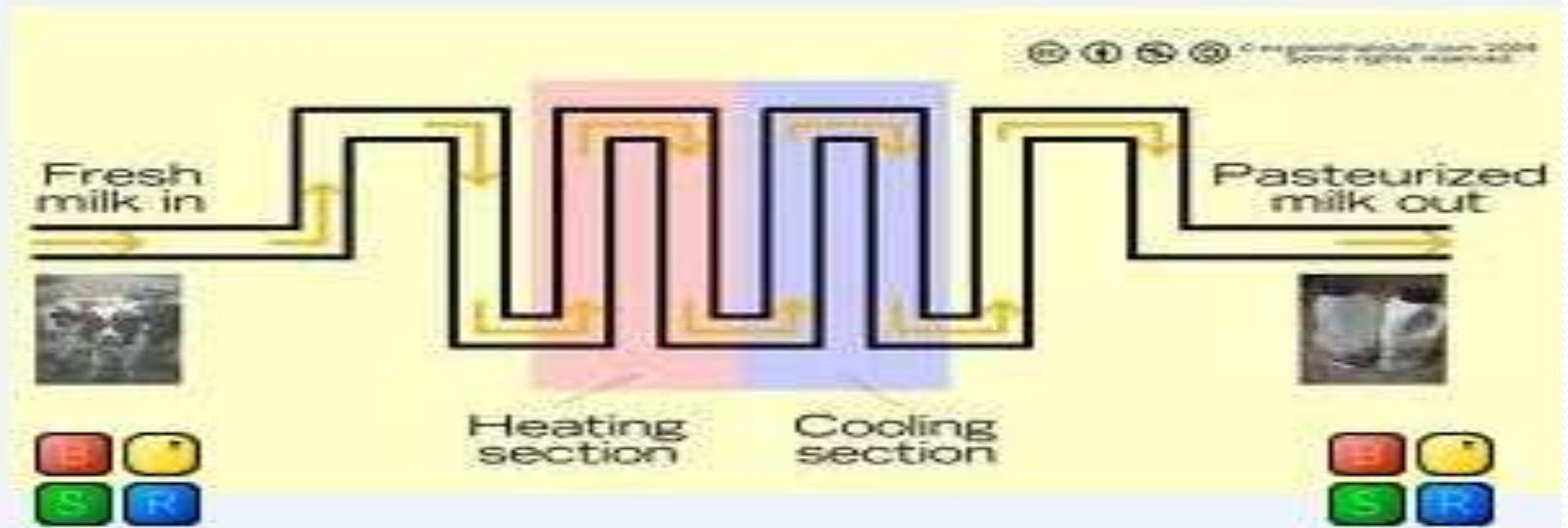
Holder Method

Milk is heated at 63°C for 30 min.

Flash Process

Heat at 72°C for 15-20 Sec then rapid cooling to 13°C.

PROCESS OF PASTEURISATION



MECHANISM



HOT WATER BATH

- For to inactivate non sporing bacteria hot water bath is helps.
- For the preparation of vaccines - **Special Vaccine Bath** at 60°C for one hour is used.
- Serum or body fluids containing coagulable proteins can be sterilized by heating for 1 hr at 56°C in a water bath for several successive days.



TEMPERATURE AT 100°C

Boiling:

- This process Kills vegetative forms of bacterial pathogens.
- **Hepatitis virus:** Can survive up to 30 minutes of boiling.
- **Endospores:** Can survive up to 20 hours or more of boiling.



TEMPERATURE ABOVE 100°C

AUTOCLAVE


- Autoclave was invented by Charles Chamberland in 1879.
- Works on the principle of **Steam under pressure**.

Types of Autoclaves

Depends up on the cylinder these are of two types. They are:

- ✓ Horizontal Autoclaves
- ✓ Vertical Autoclaves

CONSTRUCTION

- ✓ One end has an opening which is meant for keeping materials to be sterilised.
 - ✓ The lid is provided with a Pressure gauge, to measure the pressure.
 - ✓ A safety valve is present to permit the escape of steam from the chamber.
 - ✓ The stem chamber contains a perforated shelf.
 - ✓ On the perforated shelf the substances that which we want to sterilize is placed.
- 

VERTICAL AUTOCLAVE



Doors

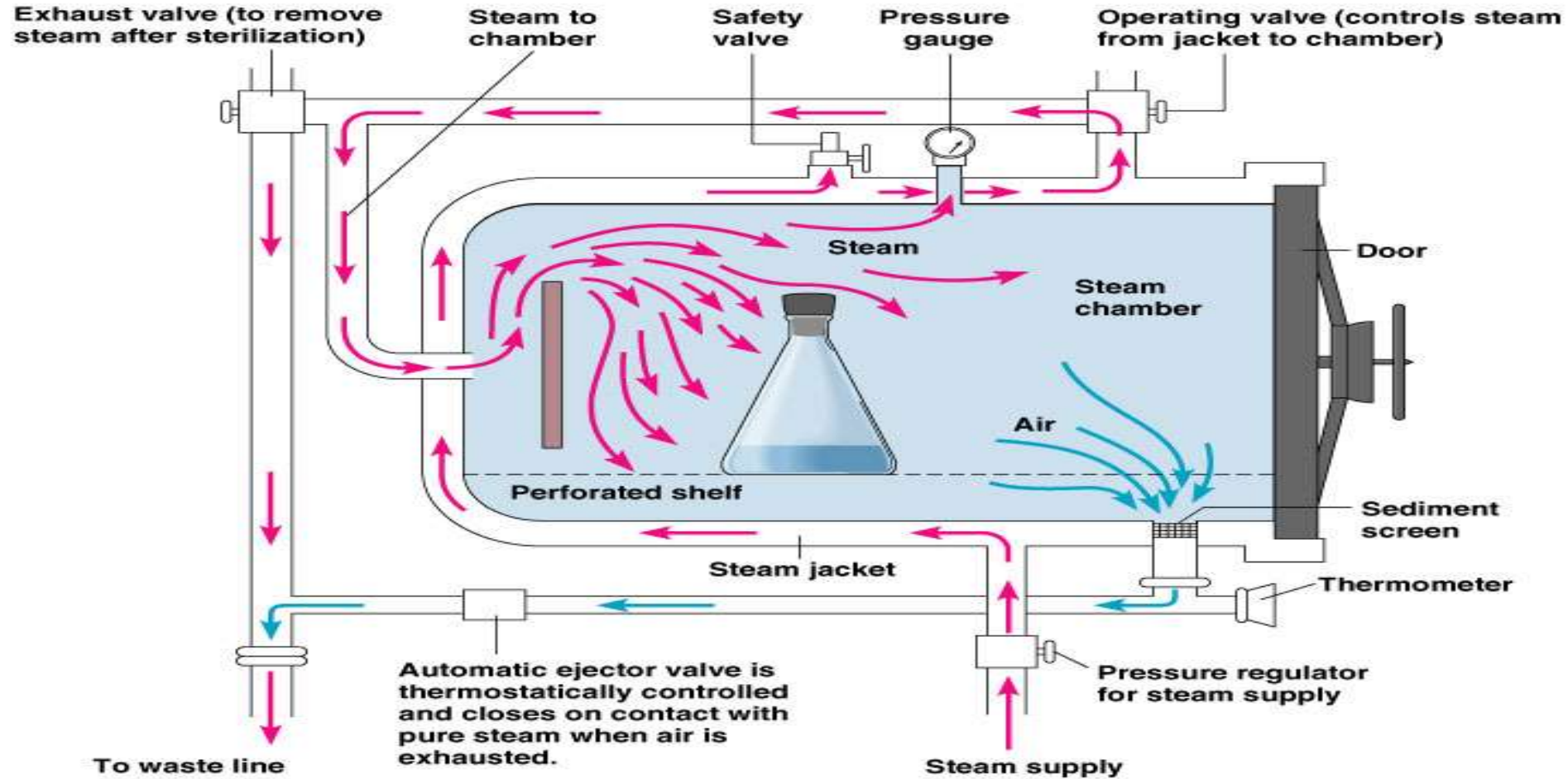
Pressure guage

Stainless steel wall

HORIZONTAL AUTOCLAVE



HORIZONTAL AUTOCLAVE



STERILIZATION CONDITIONS FOR AUTOCLAVE

S.No	Temperature	Time required	Pressure
1.	100 ⁰ c	90 min	0 lbs
2.	110 ⁰ c	60 min	5 lbs
3.	116 ⁰ c	30-40 min	10 lbs
4.	121 ⁰ c	15-20 min	15 lbs
5.	126 ⁰ c	10 min	20 lbs
6.	135 ⁰ c	3 min	30 lbs

$$\text{Temperature} \propto \frac{1}{\text{Time}}$$

MECHANISM OF HEAT STERILIZATION

Method	Mechanism of action	Use
1.)Moist Heat Sterilization		
Autoclave	Protein Denaturation	Microbiological media Solutions, dressings etc.,
Pasteurization	Protein Denaturation	Milk ,cream, alcoholic beverages
2.)Dry Heat Sterilization		
Hot air oven	Oxidation	Empty glass ware, needles and glass syringes etc.,
Incineration	Burning to ash	Paper cups ,bags etc.,

FILTRATION STERILIZATION

Filtration helps to remove bacteria from **HEAT LABILE** liquids such as

- Sera.
- Solutions of sugars.
- Antibiotics.

The following filters are used

- ✓ Candle filters.
- ✓ Asbestos filters.
- ✓ Sintered glass filter.
- ✓ Membrane filters.

CANDLE FILTER

Candle filters are mainly works on the mechanism named as gravitational force

These are of two types

➤ Ceramic filters

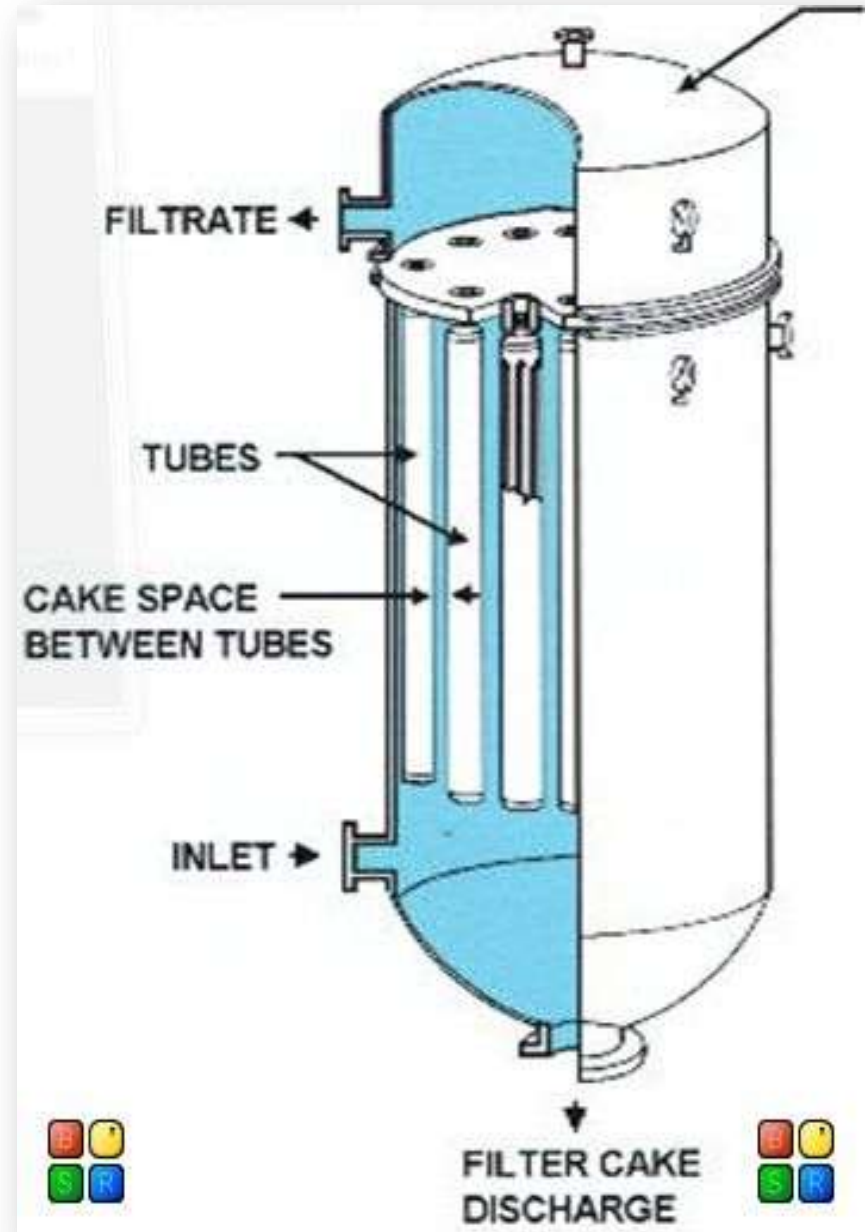
➤ Earth filters

Uses

✓ Filter water in house hold purpose.

✓ Effective- with proper use and cleaning they have been shown to reduce contaminants.

✓ Low cost.



ASBESTOS FILTERS

- Disposable single use discs
- High adsorbing tendency
- Carcinogenic
- Used in the cigarette



Sintered Glass Filter

Prepared by heat fusing powdered glass particles of graded size.

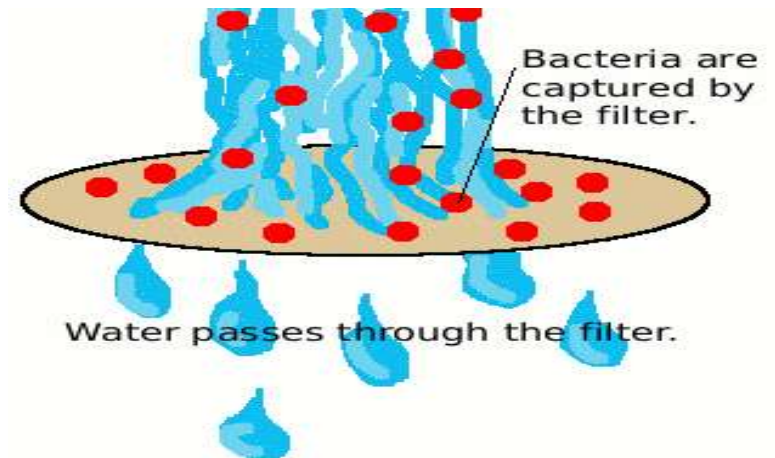
- Cleaned easily.
- Brittle.
- Expensive .



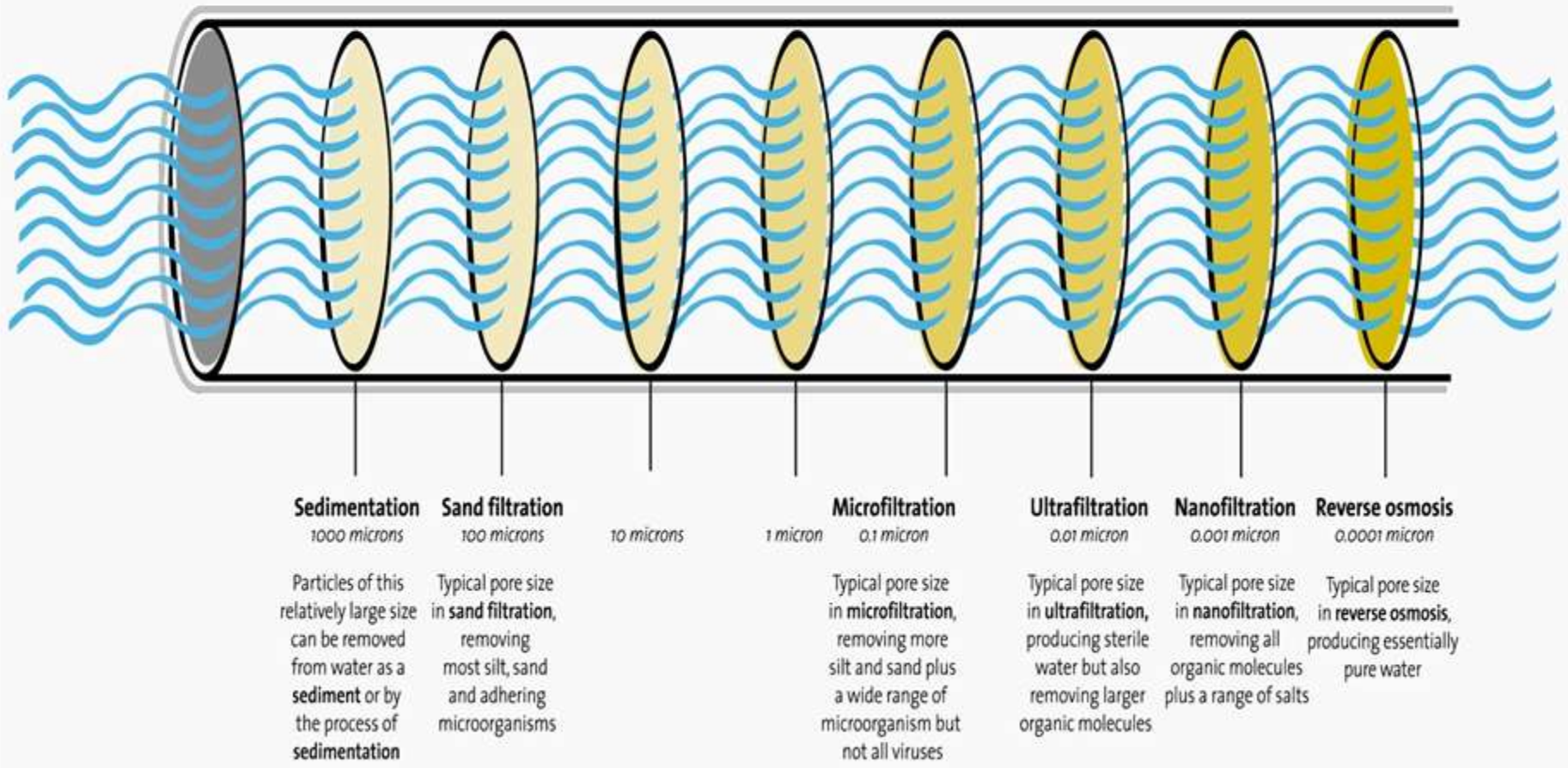
Membrane Filters

Applied pressure across a selective membrane. 3 types based on size range:

- Microfiltration – $5\ \mu\text{m}$ and up
- Microfiltration – $0.05\ \mu\text{m}$ – $5\ \mu\text{m}$
(Direct Flow and Tangential Flow)
- Ultrafiltration – $5\ \text{kD}$ to $0.05\ \mu\text{m}$
(Tangential Flow only)



Membrane Filtration



RADIATION STERILIZATION

```
graph TD; A[RADIATION STERILIZATION] --> B[Non – ionising Radiation]; A --> C[Ionising Radiation]; B --- D[✓UV-rays]; C --- E[✓X-rays]; C --- F[✓Gamma rays]; C --- G[✓Cosmic rays];
```

The diagram is a hierarchical flowchart. At the top is a purple rounded rectangle containing the text 'RADIATION STERILIZATION'. A vertical line descends from this box to a horizontal line. From the horizontal line, two thick blue arrows point downwards to two light blue rounded rectangles. The left rectangle contains the text 'Non – ionising Radiation' and below it, in pink, is '✓UV-rays'. The right rectangle contains the text 'Ionising Radiation' and below it, in pink, are three items: '✓X-rays', '✓Gamma rays', and '✓Cosmic rays'.

Non – ionising
Radiation

✓UV-rays

Ionising
Radiation

✓X-rays
✓Gamma rays
✓Cosmic rays

Difference between Ionising and Non-Ionising Radiations

Non- Ionising Radiation	Ionising Radiation
✓ Longer wavelength.	✓ Higher wavelength.
✓ Low penetration power .	✓ High penetration power.
✓ Hot sterilization .	✓ Cold sterilization .
✓ Absorbed as heat.	✓ Not absorbed as heat .
✓ Used in rapid mass sterilisation of prepacked Syringes .	✓ Used in Sterilise plastics Syringes, catheters, grease fabrics metal foils.
✓ Eg: UV-rays .	✓ Eg:Gamma rays, X-rays, cosmic rays.

MECHANISM

S.No	Method	Mechanism of action	Uses
FILTRATION STERILIZATION			
1.	Filtration	Separation of bacteria from suspending liquid	For sterilizing liquids (enzymes, vaccines) that are destroyed by heat.
RADIATION STERILIZATION			
2.	Ionising	Destruction of DNA	Sterilizing pharmaceuticals & medical & dental supplies.
3.	Non-ionising	Destruction of DNA	Control of closed environment with UV lamp

CHEMICAL AGENTS

Liquids

Alcohols

Halogens

Gases

- ✓ Ethylene oxide
- ✓ Formaldehyde
- ✓ Glutaraldehyde

- ✓ Ethyl alcohol
- ✓ Isopropyl alcohol
- ✓ Trichlorobutanol

- ✓ Iodine
- ✓ Chlorine

CHEMICAL AGENTS (DISINFECTANTS)

Definition

“The process that decreases or removes the vegetative microorganisms using chemical agents but not their spores is called as disinfection.”

LIQUID AGENTS

The disinfecting liquid substances

- Alcohols.
- Halogens.



ALCOHOLS

- Generally using alcohols are as a disinfecting agents.
- Alcohols are mainly used for the antiseptic agents.
- Mechanism involved is denaturation of bacterial proteins.
- These are efficient at optimum conditions.
- Usually 70% alcohol is more effective than the 90% alcohol.
- They have no action on spores.



Example

- ✓ Ethyl alcohol.
- ✓ Methyl alcohol.
- ✓ Isopropyl alcohol.
- ✓ Trichlorobutanol.

Uses

- Disinfection of clinical thermometer.
- Disinfection of the skin – Venupuncture



HALOGENS

Normally using halogens such as

- ✓ Iodine
- ✓ Chlorine

IODINE

- Iodine in aqueous and alcoholic solution has been used widely as a skin disinfectant.
- Actively bactericidal with moderate against spore
- Iodine has side effects like
 - ✓ **Irritation.**
 - ✓ **Staining.**
- Used for antiseptic on wounds.

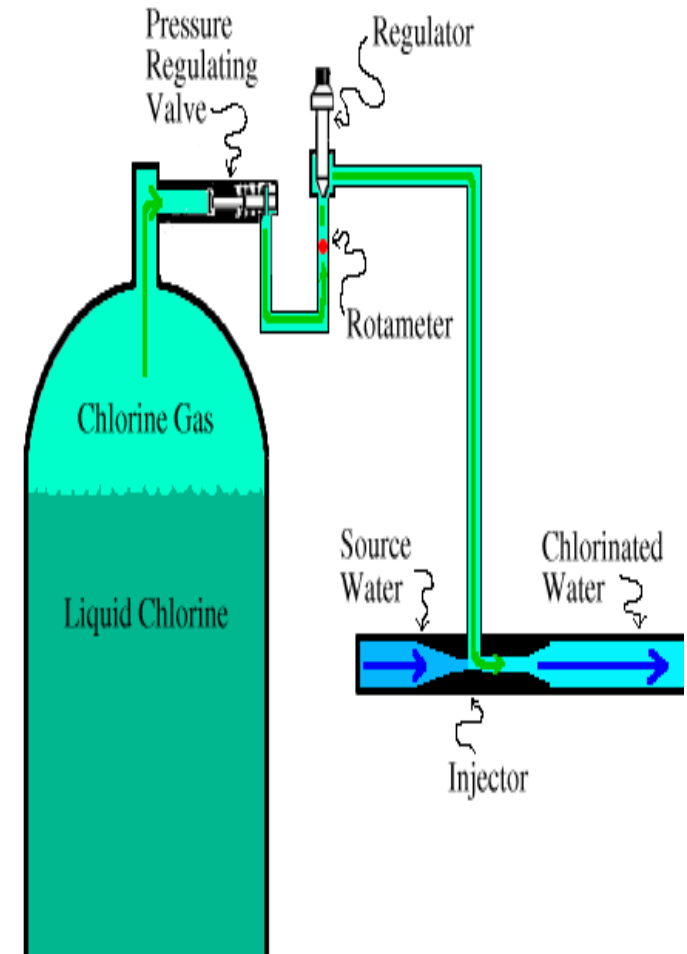


CHLORIDES

- It is the compound that have been used as a disinfectant for many years.
- Chlorine is used commonly as the hypochlorites.
- Chlorine and hypochlorites are markely bactericidal.

Uses:

- ✓ Water supply
- ✓ Swimming pools
- ✓ Food and dairy products etc.,



FORMALDEHYDE

It removes bacteria including spores

Formalin is used

Intensity ↑ when moisture ↑

FORMALDEHYDE GAS

Used in operation theaters

Heat sensitive equipment like incubator etc.,

GAS AGENTS

GLUTARALDEHYDE

Broad spectrum against microbes

Acts slowly on spores

Used for face masks

BETAPROPILACTONE(BPL)

Low penetrating power

More effective

Active against virus

PHARMACEUTICAL APPLICATIONS OF STERILIZATION

❖ Moist Heat Sterilization

- ✓ Surgical dressing
- ✓ Diagnostic equipments
- ✓ Containers
- ✓ Aqueous injections
- ✓ Ophthalmic preparation
- ✓ Irrigational fluids etc.,

❖ Dry Heat Sterilization

Mainly used for thermo stable and moisture sensitive pharmaceuticals and medicals



- ✓ Dry powdered drugs
- ✓ Oils, fats, waxes
- ✓ Soft hard paraffin
- ✓ Oily injections
- ✓ Ophthalmic ointments etc.,

❖ **Gas Sterilization**

- Hormones
- Proteins
- Heat sensitive drugs

□ **Radiation Sterilization**

- Antibiotics
- Plastics
- Sanitation of utensils

❖ Filtration Sterilization

- Heat sensitive injections
- Biological products
- Supply of gas to aseptic areas etc.,

☐ Chemical Sterilization

- ✓ Water supply
- ✓ Swimming pools
- ✓ Operation theater
- ✓ Face mask



VALIDATION

BIOLOGICAL INDICATORS

S.No	Method	Micro organism Required
1.	Moist heat (Autoclave)	Bacillus Stearothermophilus
2.	Dry heat (Hot air oven)	Clostridium Tetani or Bacillus Subtilis
3.	Filtration (Membrane)	Pseudomonas Diminuta
4.	Radiation (Non-Ionising)	Bacillus Pumulis

CONCLUSION

- ✓ It is essential in all health care facilities to avoid spread of diseases.
- ✓ The sterilization is totally depends on the ethics of instrumental users.

They should keep in mind that contaminated instruments prevent risk to patient as well as the user himself.



REFERENCE

1. Remington The science and practice of pharmacy 21st edition volume-1, Page no.776-801.
2. Ananthanarayan and Paniker's Textbook of microbiology (8th edition) page no.30-38.
3. Tortora Microbiology an introduction (9th edition) page no.188-197.
4. S. S. Purohit Microbiology fundamentals and applications (6th edition) page no.354-366.
5. Michael J. Pelzar Microbiology page no.474-491.
6. Hugo and Russell's Pharmaceutical Microbiology page no.336-345.
7. Dr. R. C. Dubey, Dr. D. K. Maheshwari A textbook of microbiology page no.546-549.
8. Roger Y. Stanier General microbiology page no.20-23.



ACKNOWLEDGMENT

My heart full thanks to my guide

Mrs.P.SOWJANYA B.PHARM.,M.TECH

&

My beloved principal sir

Dr.P.SRIVASA BABU M.PHARM.,PH.D



**Is your brain also steaming
with heat**

