

## Lab – 6



# Sterilization and Disinfection

- **Sterilization**: is the killing or removal of all microorganisms, including bacterial spores, which are highly resistant.

e.g.:

- Sterilization by autoclave (121°C , 15Klb).
- Ethyl oxide gas for surgical instruments.
- Filtration for intravenous solution.

- **Disinfection**: is the killing of most, but not all microorganisms, mainly the **pathogenic ones**. For adequate disinfection, pathogens must be killed but some organisms and bacterial spores may survive.

- Disinfectants **vary in their tissue-damaging** from the **phenol**, which should be used only on inanimate objects, to less toxic materials such as **ethanol** and **iodine** which can be used on skin surface.

- Chemicals used to kill microorganisms on the surface of skin and mucous membrane are called **antiseptics**.

# *Classification of Sterilization Methods*

## **Physical Sterilization**

## **Chemical Sterilization**

### **Radiation**

### **Filtration**

### **Heat**

- **Direct Sun Light**
- **Non Ionizing Radiation**
- **Ionizing Radiation**
- **Infrared Radiation**

### **Moist Heat**

- **Sterilization at a temp. below 100C°**
- **Sterilization at 100C°**
- **Sterilization at temp. above 100C°**

### **Dry Heat**

- **Flaming (Red heat)**
- **Hot air oven**
- **Incineration**

# **Classification of sterilizing agents:**

## **1-physical agents:**

A. heat

B. Filtration

C. Radiation.

## **2- Chemical agents.**

# Physical sterilization

**1. Heat:** Most effective, cause stop cellular activities by coagulate proteins or oxidize cell components.

## A. Dry Heat

**1. Direct flame:** the simplest method is direct flaming. Used for rapid and repeated sterilization. This Type of sterilization is used for metal instruments such as **platinum wire loop**, **forceps** and **scissors** etc.

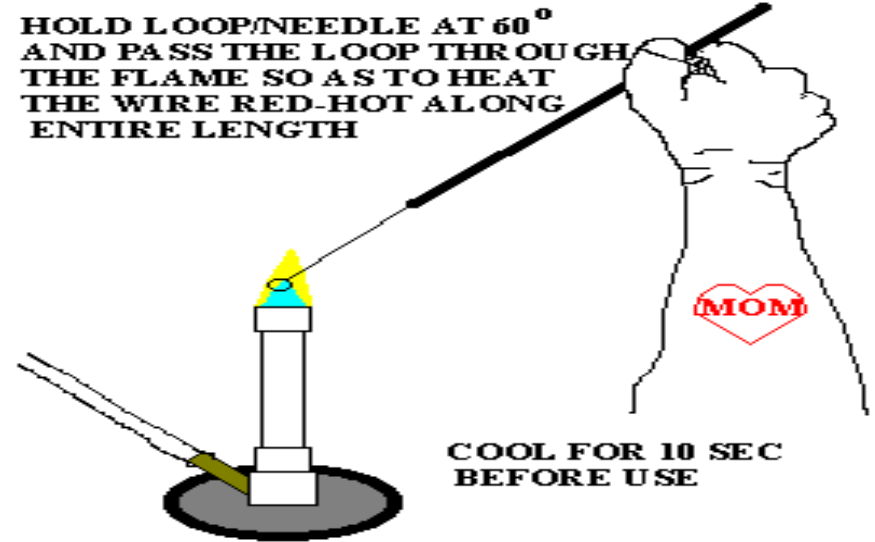
**Bunsen burner** is commonly used for sterilization by dry heat (direct flame).

**2. Hot air:** All **glassware** and **metal instrument** are usually sterilized by this method. Generally, the instruments are left in a **Hot Air Oven** on a **temperature of 160-180°C** for **one hour**.

**3. Incineration:** The use of **electrically heated** or **gas-fired** incineration filled with forced air blower units, provide an excellent means of rapidly destroying articles such as **solid dressings**, **pathological material**, **animal caresses** and **bedding**.

# FLAMING

- A simple & effective method.
- Loops or wires, needle.
- Instruments held on a Bunsen flame till red-hot.



# Hot air oven



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## Dental Instruments



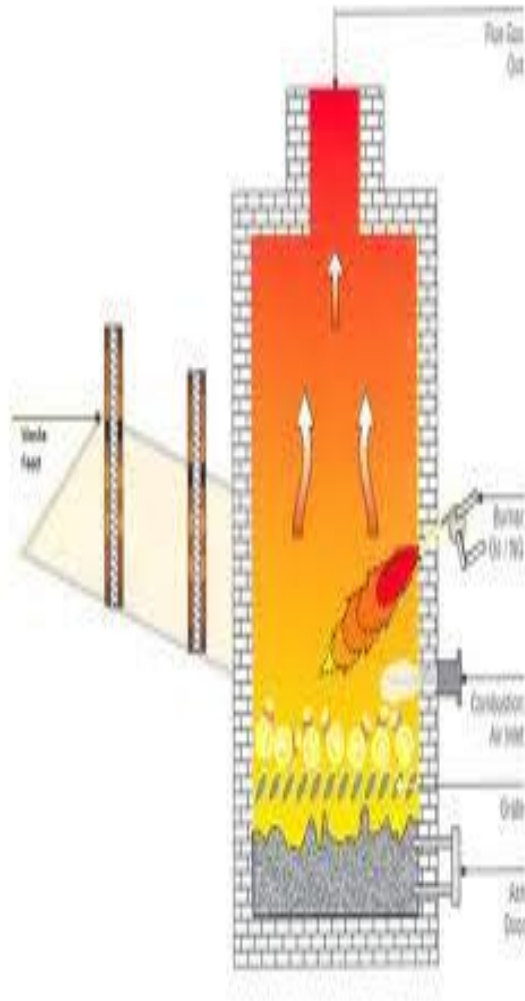
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## Glassware



# Incinerator





# B-moist heat

**1.pasteurization:** uses heat  $63^{\circ}\text{C}$  at  $30^{\circ}\text{C}$  minutes.

The organisms such as *Brucella* or *Salmonella* and tubercle bacilli which contribute to milk born-disease are readily killed by this process.

**N.T:** bacterial spores are not killed by this method.

**2.Boiling water:** A temperature at  $100^{\circ}\text{C}$  will kill all non-sporing and vegetative organisms within 10 minutes. Most spores will be killed in 30 minutes at this temperature, but some spores will resist boiling for several hours.

- The addition of **2% sodium carbonate** increases the disinfecting power of the water, and spores resistant to boiling water for 10 hours have been killed in 30 minutes by this addition.

**This method is suitable for infected instruments or small pieces of infected glassware.**

**3. Tyndallization:** the process of sterilization by intermittent steaming., is a form of sterilization that involves boiling the goods to be sterilized in their cans or jars at 100 °C for about 15 to 20 minutes a day, for three days in a row. Sterilized **canned food**.

**4. Steam under pressure (high pressure steam):** The **autoclave** or pressure cooker is the instruments used for high pressure steam sterilization. **Bacteriological media, surgical instruments are sterilized in the autoclave at 121°C (15 lbs) for 15 minutes.**

Autoclave



# MOIST HEAT

Sterilization  
By  
Moist heat

Moist heat at  
Below 100°C

e.g..  
pasteurization

Moist heat  
At 100°C

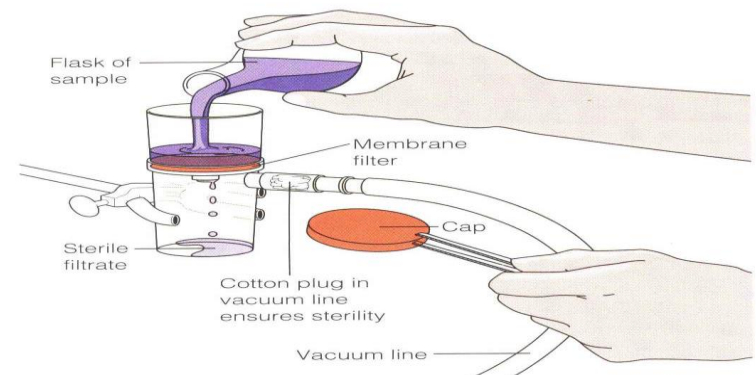
Boiling  
Tyndallization

Moist heat  
At above 100°C

Autoclave

**2. Filtration:** The principle of this method is to pass the material to be sterilized through special bacterial filters which hold back any bacteria present.

- This method is used for the sterilization of **fluids** that do not withstand heating, e.g. **Sera, Plasma, Vitamins and Antibiotic solutions.**
- Several kinds of bacterial filters are available. The kinds which are most used are the **chamborlain** and **Daulton filters** which are made of unglazed porcelain, the **Mandler Filters** , **Seitz filters**, etc.



**3.Radiation:** It is employed commercially for the sterilization of large amount of **pre-packed disposable items** such as **plastic syringes** and **Catheters** that are unable to withstand heat.

- It is done by applying ultra-violet rays.



sterilized by ionizing radiation

# Chemical sterilization.

**1-Alcohols, Ethyl alcohol, Isopropyl alcohol (70% aqueous solution):**

**Uses:** Antiseptic to sterilize the **thermometer**, the **skin** before injection.

**2-Phenols:**

**Uses:** sterilization of **surgical instruments, bathroom, hospital floor.**

Chlorohexidin ; as skin disinfectants.

**3-Heavy metal ions (Mercury, silver nitrate):**

**Uses:** Mercuric salt e.g Methiolate used as preservation for sera, bacterial and viral vaccine.

Silver salt e.g  $\text{AgNO}_3$  (1%) used as eye drop for newborn infants to prevent infections by *Neisseria gonorrhoea*.



## 4-Oxidizing agent.

**Uses:**  $\text{H}_2\text{O}_2$ , for disinfection of contaminated wounds.

**5- Halogens :** chlorine and hypochlorite, iodine

**Uses:** to disinfect the swimming pools and water supplies.

## 6-Akalyting agents

a. Formaldehyde. **used** in the sterilization of instruments, lab, clothing, books.

b -Ethylene oxide **used** to sterilize heat sensitive objective such as plastic petri dishes, tubes, syringes, pipettes.

## 7-Detergents:

\*Soaps to clean the skin.

\*quaternary Compounds used as antiseptic and disinfectant.



## Chlorhexidine agents

# Iodine (Betadine): used in surgery to sterilize skin pre-operation



# Experiment:

## Materials Provided:

Tubes of nutrient broth

Sterile, empty tubes

Sterile, 1.0-ml pipettes (cotton plugged)

Sterile, 1.0-ml pipettes (cotton plugged)

1.0 per cent phenol, 2.0 per cent phenol

Absolute alcohol, 70 per cent alcohol

3.0 per cent hydrogen peroxide

1.0 per cent Lysol, 5 per cent Lysol

Tincture of iodine

Antiseptic mouthwash

24-hour nutrient broth culture of *Escherichia coli*

Three-to-six-day-old culture of *Bacillus subtilis*

# Procedures:

- 1- Select one of the chemical agents. Draw 5.0ml of the solution into a sterile test tube.
- 2- To the 5ml of disinfectant, add 0.5ml of the *E. coli* culture. Gently shake the tube to distribute the organisms uniformly. Note the time.
- 3- At intervals of 2, 5, and 10 minutes, transfer one loop full of the disinfectant-culture mixture to a tube of fresh nutrient broth. Label each broth tube with the name of the organism, the disinfectant, its concentration, and the time of exposure (for example, *E. coli*, 1 percent phenol, 2 minutes).
- 4- Using the same concentration of the same disinfectant, repeat procedures 1 to 3 with the culture of *Bacillus subtilis*.

5- Inoculate a tube of nutrient broth directly from the *E. coli* culture and another from the *B. subtilis* culture. Label each tube with the name of the organism and the word "Control".

6- Incubate all tubes at 35°C for 48 hours.

7- Record your results.



**Thank You**