STERILIZATION AND DISINFECTION

Sterilization

Sterilization is the process of killing or elimination of all forms of micro-organisms by physical means (including heat, radiation, and filtration) and/or chemical agents (acids, alkalis, heavy metals, salts, halogens, etc). The equipment or material treated then becomes **"Sterile"**.

In medical laboratories, materials and equipment are sterilized for the following main purposes:

1. In preparation for taking specimens, such as needles, syringes, test tubes, etc.
2. To sterilize contaminated materials and equipment.
3. To prepare apparatus used for bacteriological cultures, such as petri dishes, Pasteur pipettes, and others.

METHODS OF STERILIZATION

I. Physical method

1. Dry heat (hot air oven, flaming and red - hot)
2. Moist heat (autoclave or steam under pressure and boiling).
3. Radiation

1. Dry heat

A- ***Hot Air oven***

Higher temperatures are needed with a hot- air oven than with an autoclave due to the low penetrating power of dry heat and great resistance of bacteria to it. A temperature of 180°C for 30 minutes will kill most resistant spores. The material to be sterilized is

placed in an oven and the temperature is raised and maintained at 180°C for 30 minutes. The sterilized material should not be removed until the oven is cold. This is important particularly with petri dishes, as cold air will be sucked in to them, causing contamination, if they are removed before the oven is cold. This is due to the contraction of hot air as it cools.

This method is used only for glass or metal objects such as test tubes, petri dishes, all glass syringes, and instruments.

B- ***Flaming***

Metal spatula, glass slides, and cover slips may be sterilized by passing them through a Bunsen flame, without letting them become red hot. Alternatively, they may be dipped in spirit, and the alcohol burned off. This procedure should be repeated two or three times.

C- ***Red - hot***

Wire loops and tips of forceps may be sterilized by heating them in a Bunsen flame until they become red hot and allow the materials (instruments) to cool before using them.

2. Moist heat

A-*Boiling water*

Moist heat may be applied using boiling water or steam. Boiling water is generally used for sterilizing instruments and syringes. These are boiled for 10 minutes in a water bath. This will kill all non-sporing organisms but certain spore forming organisms can resist the temperature of boiling water for 1-2 hours.

The addition of 2% sodium carbonate increases the disinfecting power of boiling water. Spores, which resist boiling water for 10 hours, have been killed within 30 minutes by the addition of sodium carbonate. Sodium carbonate also prevents the rusting of metal instruments.

B- ***Steam under pressure (autoclave)***

Autoclave is an instrument that operates by creating high temperature under steam pressure. Autoclaving is the most common, effective, reliable and practical method of sterilizing laboratory materials.

The principle of autoclave is that steam is kept at a pressure of 15 pound (lb) per square inch to give a temperature of 121 °C, which will kill spores within 15 minutes. At this particular temperature, pressure and time, all forms of lives are destroyed.

Steam is more penetrating than hot air and will give up its heat on striking a colder object; there by raising the temperature of the object rapidly. It is used to sterilize syringes, needles, glass wares, culture media, etc. For most purposes, the following cycles will ensure sterilization of correctly loaded autoclaves correctly loaded:

• Three minute holding time at 134C°;

• Ten minute holding time at 126 C°;

• Fifteen minute holding at 121 C°;

• Twenty holding time at 115 C°.

Types of Autoclaves

1. Gravity displacement autoclaves

In gravity displacement autoclave, steam enters the chamber under pressure and displaces the heavier air downwards and through the valve in the chamber drain, fitted with a HEPA (High efficiency particulate air) filter.

2. Pre- vacuum autoclaves

This autoclave allows the removal of air from the chamber before steam is admitted. The exhaust air is evacuated through a valve fitted with a HEPA filter. At the end of the cycle, the steam is automatically exhausted. These autoclaves can operate at 134 °C and the sterilization cycle can therefore be reduced to 3 minutes. They cannot be used to process liquid because of the vacuum.

3. Fuel heated pressure cooker autoclaves

Fuel heated pressure cooker autoclaves should be used if a gravity displacement autoclave is not available. They are loaded from the top and heated by gas or electricity. Steam is generated by heating water in the base of the vessel and air is displaced upwards through a relief vent. When all the air has been removed, the valve on the relief vent is closed and the heat is reduced. The pressure and temperature rise until the safety valve operates at a preset level, which is the start of holding time. At the end of the cycle, the heat is turned off and the temperature allowed to fall to 80 °C or below before the lid is opened.

Precautions in the use of autoclaves

The following guidelines can help to minimize risks while working with autoclaves:

1. Regular inspection of the chamber, door seals and gauges.

2. The steam should be saturated and free from chemicals that could contaminate the items being sterilized.

3. Materials to be autoclaved should be in containers that allow ready removal of air and permit good heat penetration.

4. The chamber of the autoclave should be loosely packed so that steam will reach the load evenly.

5. Slow exhaust setting should be used when autoclaving liquids, as they may boil over when removed due to superheating.

6. Operator should wear protective gloves for protection when opening the autoclave.

7. Thermocouples should be placed at the center of each load in order to determine proper operating cycles.

8. Ensure that the relief valves of pressure cooker autoclaves do not blocked.

3. Radiation (ultra - violet ray)

UV radiation is lethal to certain microorganisms by inactivating the DNA of the organisms. It is effective and valuable in sterilization of air in a given room or place. E.g: Pulmonary tuberculosis Laboratory

II- Mechanical method

Filtration is a mechanical method for eliminating bacteria from biological fluids and from the laminar flow systems, which are used to ventilate operating rooms, laboratories and areas having immune suppressed and burn patients. Filtered air is pumped into the space at a pressure required to displace regular circulating air.

III. Chemical methods

Generally, many chemicals are lethal to microorganisms. Some of the commonly used chemicals are hypochlorite solution, phenolic derivatives and ethylene oxide.