**Lab :** 5 **Sterilization**

**Sterilization:** a physical or chemical process that completely destroy or removes all microbial life, including spores.

**Disinfection:** a physical or chemical process that kill or prevent the growth of pathogenic microorganism but not necessarily the spores.

**Sterilization methods:** there are many methods for sterilization, in general they are divided into two methods:

**A-Physical methods.** **B-Chemical methods.**

**A-Physicalmethods:**

**1-Heat.**

**2-Filteration.**

**3-Radiation.**

**١-Heat :** 1.1 Dry heat. 1.2 Moist heat.

1.1 Dry heat

1.1.2 Red heat : in this method, the tools (especially the metalic ones ) are exposed to the flame of benzene burner until reaching the red color. These tools include: loops, inoculation needles, forceps.The tools which are sterilized by using the red heat method are made of steel that resist oxidation &high temperature .

1.1.2 Flaming : in this method , the tools are exposed to the flame of benzen burner without allowing them to reach the redness point. The tools include : the mouth of the test tubes & flasks , slides , cover slips (cover slide).

Note:

For more precision, alcohol can be used by immersing the above mentioned toolsin it before the exposure to the flame. e.g. knives used in the surgery (dissection scalpel), medical syringes and spreaders.

١.١.٣ Hot air oven or electrical oven : in this method , the tools are sterilized at (160-180) C°for (1-2)hrs.. It is a perfect method for glass wares sterilization. The glass wares include: tubes, flasks, petridishes & pipettes.

This method is suitable for sterilization of metalic tools that are not affected by high temp. eg.knives (dissection scalpel)& forceps.

Note :

Moist glass wares must be dried before sterilization by using this method to prevent smashing (breaking).

1.2 Moist heat

1.2.1 Pasteurization: in this method, sterilization is done by using temp. less than (100)C°to destroy pathogenic bacteria by heating (62)C° for(30)min. (LTLT) or (72)C° for (15) sec.(HTLT) . The materials which are sterilized by this method are : serum , body liquids(whichcontain albumin) & milk .

1.2.2 Bioling : in this method , sterilization is done by using (100)C° for (10)min. . It is enough to kill all pathogenic m.o. in vegetative phase (but not their spores) this method can be used if there is no alternative method of sterilization . It is used for sterilization of materials & instruments (tools) (withermetalic or glass).

1.2.3 Tyndalization or Steaming: in this method , sterilization is done by exposing the materials to the vapour of boiling water for a couple of minutes & for (3) days in sequence.

The materials which are sterilized by this method are : sugar solutions , enzymes , vitamins , antibiotics.

Note:

- This method is used for sterilization of the material which may be destroyed by using the high temperature.
- The sterilization in this mathod is done for 3 days in sequence because:

1st day -----→ the vegetative cells are killled.

2nd day-----→ the spores convert into vegetative cells.

3rd day-----→ the coverted vegetative cells are killed , in addition the sterilization process is completed effectively.

1.2.4 Autoclave : in this method , sterilization is done by using temp. higher than (100) C°(steam +pressure). The materials & instruments (glass or plastic) which are sterilized by this method are:

cultural media , solutions & liqiuds , cottons , other materials which are usually destroyed by using dry sreilization (oven).

Note:

- The reason of using autoclave (moist heat) for the sterilization of cultural media & other liquids is the existence of the steam which prevent the vaporization of the media & other watery solutions by heating ,but if these media & solutions are sterilized by an oven (dry heat) , they will be decreased in volume because of the oven environment that leads to the vaporization of these solutions.
- The srerilizationby using an autoclave kill all kinds of pathogenic m.o. &their spores (which can not be killed by other sterilization methods).

**2.Filteration**

This method involves filtering the solutions or any other liquids through special srerile filters.

These filters allow the liquid to penetrate while the m.o. are trapped on the surface of the filter . The marerials which are sterilized are : serum, enzymes , vitamins, antibiotics & sugar solutions.

Note:

The most important filters are cellulose membrane filters which are made of cellulose nitrate or cellulose acetate e.g. millipore filters.

This kind of filters is commen because:

1. The membrane filter disc can be directly removed and placed on surface of an approperiate (suitable) solid medium in order to make the microorganism grow on that medium ,so the growth characteristics can be studied.
2. High speed of filteration.

**3.Radiation:** The ability of the sunlight to kill the m.o. is due to the ultra violet(u.v.) ray existence in it . The rays can be devided in it. The rays can be devided in to:

1) Non –ionizing ray : e.g. u.v.light which is effective in the wavelength about (240-280) nm.

**Uses:** Srerilization of hospitals , laboratories , meat package factories.

Note: The sterilization by using u.v. is more effective on a clean surface because its inability to penetrate more than some millimeters in liquids and solid surface , so it is used only to sterilize clean surface .

2) e.g. Gamma ray.

**Uses :** Sterilization of food products , penicillin , plastic items (which can be consumed).

**B. Chemical methods:**

Those methods are used for the srerilization of rooms and floor. The efficiency of these methods depends on:

1. Chemical agent concentration .
2. Time period required for sterilization .

Quantity and quality of m.o.

**Disinfcetion or antiseptic :**

It is a chemical agent or materials which have the ability to kill or prevent growth or metabolism of m.o. vegetative cells but its efficiency on their spores is not confiolent .

**Disinfectants :**

1. Volatile disinfectants → chloroform.
   Uses : serum which is added to the cultural media.
   Note: Chloroform can be used in the form of liquid or concentrated vapor state by exposing the liquid or contaminated surface to it for about (1)min.
2. Phenols → lysol , cresol , hycoline , phenol.
   Uese : surgical tools , bacterial cultures , serum , vaccines.
3. Metalic saltsor heavey organic compounds of metals → mercuric chloride.
4. Gases → formaldehyde.
   Advantages: 1) Not expensive. 2) Water soluble. 3)Effective for killing all kinds of m.o. and their spores. 4)Have no damaging effect to the surfaces to be disinfected.

   Disadvantages: This gas causes irritation of the respiratory tract when it is inhaled.
   Uses : rooms , laboratory environment , laboratory cultures (formalin).
   Note: This gas (formaldehyde) is also used in a liquid state (formalin) which disinfects by direct contact with the contaminted surfaces.
   Note:
   The gas (formaldehyde) is used for the disinfection of the materials that cannot b exposed to the liquid solutions which may damage those materials.
5. Halogenes → chlore uses: water disinfection.
   →iodine uses: wounds disinfection.

6. Alcohol →ethanol with the concentration of (70-75)% , this concentration causes melting (lysis) and damaging of lipid membranes and denaturation of the microbial cell proteins.
   Note: Alcohol is used in concentration of 70% rather than 90% because the aqueous solution (diluted solution) of alcohol is more effectivein disinfection than absolute alcohol , and this is due to the m.o. cell wall proteins which are more soluble and easily damaged when the diluted alcohol is used.
7. Detergents: they are effective on some kinds of bacteria .

Typical properties of disinfectants:

(1) Non- toxic to human.
(2) Not lossing its ability of disinfection after it is diluted.
(3) Have no damaging effect on the surface of the material to be disinfected and its efficiency acts only with m.o.