Lab)

Sterilization and Disinfection

In microbiology and medicine the sterilization of instruments, drugs and other supplies is important for prevention of infection

Sterilization: is the destruction of microorganisms (vegetative and spores). Physical agents are used such as heat, radiation, filtration, ultrasonic vibration.

Disinfection: is the inactivation of vegetative bacteria, and not the spores, by chemical agents (disinfectants or antiseptics). They may kill microorganisms (microbiocidal effect) or prevent their growth (microbiostatic effect). Many factors influence the activity of disinfectants:

Concentration of the disinfectant.

Time of exposure.

The degree of temperature.

Number of organisms.

Presence of spores.

These agents are used in dentistry and medicine for treatment of local infection.

Why is Infection Control Important in Dentistry?

- Both patients and dental health care personnel (DHCP) can be exposed to pathogens.
- Contact with blood, oral and a respiratory secretion, mucosa of the eyes, nose, and contaminated equipment occurs.
- Proper procedures can prevent transmission of infections among patients and DHCP.

1-Physical method:

A- Heat

B- Filtration

C- Radiation

Heating: Kills microorganisms by denaturing their enzymes and other proteins.

Thermal Death Point (TDP): Lowest temperature at which all of the microbes in a liquid suspension will be killed in ten minutes.

Thermal Death Time (TDT): Minimal length of time in which all bacteria will be killed at a given temperature.

Heat can be divided to:

A-Heat

a-Dry heat

a_1-Direct flaming: for inoculating loops and needles forceps and spreaders, and the open end of culture tubes, flasks and bottles. (fig.2)

Procedure:

For inoculating loops and needles.

1- Flame the inoculating loop or needle over a Bunsen burner until the wire becomes red –hot.

2- Cool the hot loop.

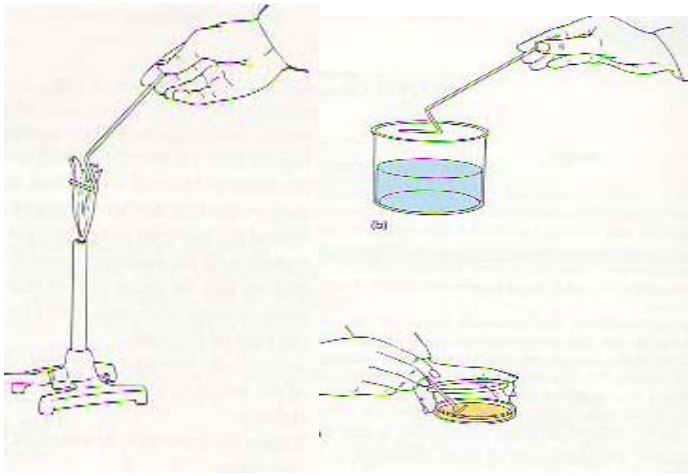
3- Use the loop or needle before it becomes contaminated.

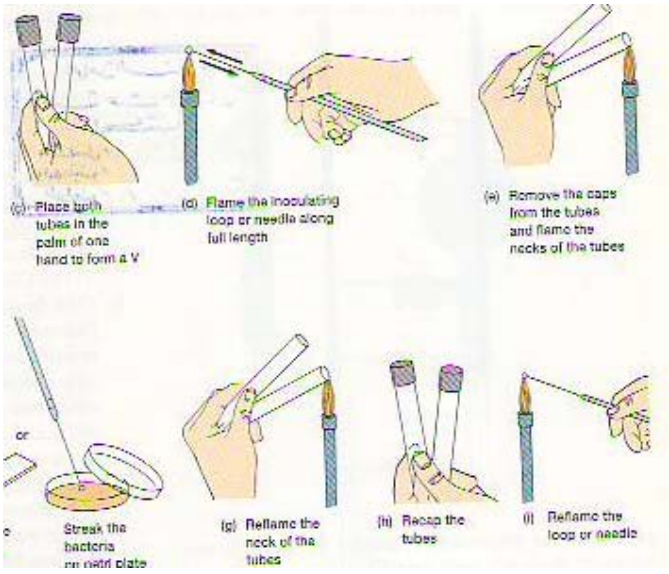
forceps and spreaders.

1- Dip the points of forceps or the L-shaped glass rod spreader into a beaker of 70% ethanol.

2- Pass the ethanol-soaked forceps or spreader through the flame to burn off the alcohol .Allow it to cool.

3- Use the forceps or spreader before it becomes contaminated.





Figure(2)

a_2 Hot air :(by oven)

This method is used by exposure at temperature 160° to 170° for two hours. This way is used for sterilization of glassware, such as glass petridishes, test tubes, pipettes, flasks et.c.

Procedure:

Set the electric oven to operate between 160° and 170°c.

Place the clean glassware inside the oven for 2 hours.

Note: pipettes and petridishes are placed inside special cans or folded with foil paper, test tubes, flasks and other containers must be closed with cover or cotton plugs before they are placed in the oven.

a_2 Hot air :(by oven)

This method is used by exposure at temperature 160° to 170° for two hours. This way is used for sterilization of glassware, such as glass petridishes, test tubes, pipettes, flasks et.c.

Procedure:

Set the electric oven to operate between 160° and 170°c.

Place the clean glassware inside the oven for 2 hours.

Note: pipettes and petridishes are placed inside special cans or folded with foil paper, test tubes, flasks and other containers must be closed with cover or cotton plugs before they are placed in the oven.

b) Moist heat: Kills microorganisms by coagulating their proteins.
- In general, moist heat is much more effective than dry heat.
b1-Sterilization at a temp. Below 100 c^o : This method is used to sterilize serum, body fluid containing coagulable proteins. Temperature of 56 Co for 1hr. used to sterilize vaccines in special water bath.

Pasteurization: This method discovered by Louis Pasteur to use for preservation of milk and fruit juices.
-Classic Method of Pasteurization: Milk was exposed to 63°-66°c for 30 minutes.
-High Temperature Short Time Pasteurization (HTST): Milk is exposed to 71-72 °c or 15 minutes

- Ultra High Temperature Pasteurization (UHT): Milk is treated at 140°c for 3 seconds and then cooled very quickly in a vacuum chamber. Advantage: Milk can be stored at room temperature for several months. This method will destroy non spore forming pathogenic bacteria. This way will kill all the non-spore forming pathogens such as *Mycobacterium tuberculosis, Brucella abortus* and various types of Salmonella

Procedure:

1- Set the water bath at required temperature.

2- Put the materials that to be preserved in the water bath for the required time.

b_2- temperature 100°c for 10 minutes. This is sufficient to kill all non-spore-forming. It is used for dental and medical instruments such as syringes, needles, sutures and others

Tyndalization: is the method that used for the removing of spores. This method involves steaming for 30 minutes on each time, for three successive days.

Procedure: (for tyndalization*)*

1- Prepare broth culture of spore forming bacteria.

2- Prepare water bath (100°c).

3- Put the broth culture in the water bath for 30 minutes.

4- Put the treated broth culture in the incubator, to allow spores to germinate and vegetative bacteria to grow.

5- Examine the presence of the vegetative bacteria by inoculating of loobful broth culture on the nutrient agar surface.

6- Repeat these steps for three times.

b_3 Heating more than 100°c under pressure

Autoclaving: By using the autoclave, in which items are sterilized by exposure to steam at 121°c and 15 lbs of pressure for 15 minutes (or 126°c and 20 lbs for 3 minutes).Air has been expelled and only steam is present in the autoclave chamber. All forms of microbial life will be destroyed by this method. (fig.4)

Horizontal autoclave vertical autoclave

Figure (3)

Procedure:

1- Put appropriate amount of water inside the chamber of the autoclave.

2- Adjust the temperature to 121°c.

3- Set the autoclave time for 15 minutes.

4- Place the culture media or other materials that to sterilized inside the autoclave.

5- Close and lock the autoclave door.

6- Start the autoclave by pushing the start button.

7- When the period of sterilization is completed and the pressure in the chamber read O, carefully open the door and remove the containers using heat-proof gloves

b-Filtration:

Seitz filter (asbestos-paper disc), sintered glass and cellulose membrane filter are commonly used .The pore size of these filters ranges from 0.22-10 μm.These filters are used for the separation of bacteria from viruses (bacterial size 2-10 μm). Filtration used for sterilization of gases and liquids that would be affected by heat such as serum, antibiotic solutions and the soluble products of bacterial growth, e.g. toxins. (Fig.4)

High Efficiency Air Filters (HEPA): Used in operating rooms to remove bacteria from air**.**

c-Radiation: Three types of radiation kill microbes**:**

c1. Ionizing Radiation: Gamma rays, x rays, electron beams, or higher energy rays. Used to sterilize pharmaceuticals, disposable medical supplies.

Disadvantages: Penetrates human tissues and might be caused mutation in human.

c_2. Ultraviolet light (Nonionizing Radiation): Wavelength is longer than 1 nanometer. Damages DNA by producing thymine dimers, which cause mutations. Used to disinfect operating rooms, nurseries, cafeterias. Disadvantages: Damages skin, eyes. Doesn't penetrate paper, glass, and cloth.

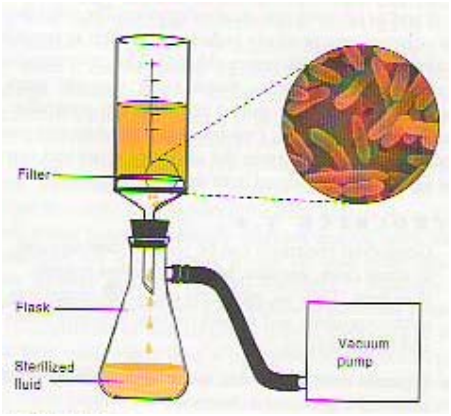


Figure (4)

2) Chemical methods: It act on lipid contents of the cell membrane denaturation of protein, and on nucleic acid, it either kill or stop the growing of microorganism.
a) Potent disinfectant: It is toxic and corrosive for living tissue, it's used for nonliving

1) Phenol group: contain benzene ring e.g. bathrooms, hospital, floor …ect.

2) Chlorine is most often used in form of sodium hypochloride (house hold bleach) in dilution (1:10) treatment of swimming pools etc.

3) Strong alkaline and acids e.g. NAOH Used for treating sputum for detecting TB and decrease viscosity

b) Mild: Antiseptics: It is less toxic and can be applied to living tissue like skin.
eg. 1) Detole

2) Chlorhexiden (Hibitane): very good as skin antiseptics used to treat surgical wounds because it work on both gram-positive and gram-negative bacteria.

It is widely in dentistry as an antiseptic and plaque controlling agent. It is used highly basic (cationic molecule) very active in

3) Iodine: used in surgery to sterile skin pre-operation.

4) H2O2 (hydrogen peroxide) can be used for sterilization of deep wound or gangrene infected by anaerobic bacteria

5) Soap and detergent.

6) Alcohol at conc.70% because it can penetrate tissue easily at this concentration better than 99 % (absolute) and kill microorganisms by denaturation and dehydration.

Growth Inhibition: the purpose of this method is to study the activity of some disinfectants and to learn the importance of time and microbial species in disinfection

1- Select one of the chemical agents provided. Add 0. 5ml of the solution in to sterile test tube.
2-To 0.5ml of disinfectant, add 0.0.5ml of Esch. coli or Staph. aureus culture Gently shake the tube. Note the time.
3- Divide Nutrient agar plate in to 4 sections with a marking permanent pen (2, 5, 10, 15) minutes.
4-Transfer one loopful from the mixture of disinfectant culture to a section of the N.A. plate. Lable each plate with the name of m.o., time and the disinfectant, concentration.
5- Incubate at 37o for 48 hours.

Phenol Coefficient (PC): The microbiocidal efficiency of a chemical is determined with respect to phenol and is called the phenol coefficient (PC) .The PC

is calculated by dividing the highest dilution of chemical (disinfectant) that kills all microorganisms after incubation for 10 minutes, by the highest dilution of phenol that has the same characteristics. Chemicals that have a phenol coefficient greater than 1 are more effective that phenol and those that have a phenol coefficient less than 1 are less effective than phenol