EXPERIMENT 5 STERILIZATION OF LABORATORY GLASSWARE AND EQUIPMENTS

Structure

5.0 OBJECTIVES

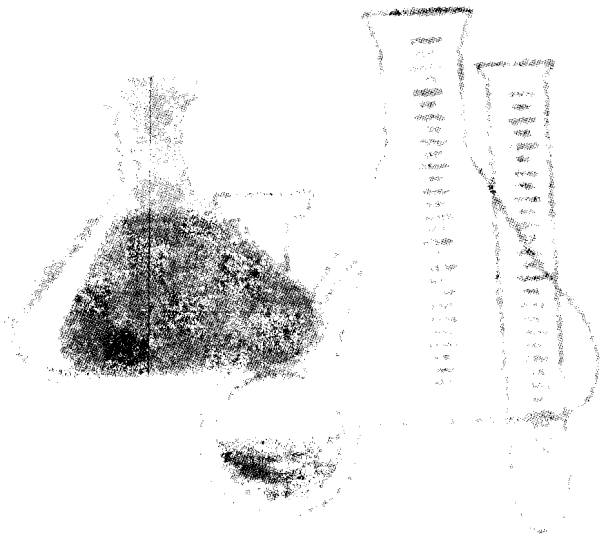
After studying and performing this practical exercise, you will be able to:

- sterilize the glasswares and equipments used in laboratory for handling meat and meat products; and
- maintain the hygienic quality of food through the use of sterilized glassware and equipments.

5.1 INTRODUCTION

You all have read earlier that sterilization is the process by which all vegetative cells as well as spores of organisms are killed to attain germ free state. There are various means of sterilization such as heat, chemical or irradiation etc. The method of sterilization depends on the nature of substance. For example glasswares are usually sterilized in hot air oven.

The sterilization of laboratory glassware/ media is the first and uppermost requirement of a microbiological analysis. The sterilization is mostly done by following methods employing either dry heat or moist heat.



5.2 METHODS OF STERILIZATION

Sterilization is the use of a physical or chemical procedure to destroy all microbial life, including highly resistant bacterial spores. This can be done either by use of dry heat or by moist heat.

5.2.1 Dry Heat

i) **Flaming:** Inoculating loops or wires, points of forceps and searing spatulas are held in a Bunsen flame till they become red hot, for sterilizing them. If the loops contain infective proteinaceous material, they should be first dipped in chemical disinfectants before flaming to prevent spattering.

Scalpels, needles, mouths of culture tubes, glass slides, cover slips, etc. could be passed a few times through the Bunsen flame without allowing them to become red hot. The bacteria get destroyed. Needles, basins and scalpels are sometimes immersed in methylated spirit and the spirit burnt off them. This is, however, unsatisfactory.

ii) **Hot air oven:** This is the most widely used method of sterilization by dry heat at 160^{0}C -170^{0}C for 2-4 hours in a hot air oven. It is used to sterilize glassware like test tubes, conical flask, Petridishes, beakers, measuring cylinders etc; forceps, scissors, scalpel, syringes, swabs, some pharmaceutical products such as liquid paraffin, dusting powder etc. Hot air is bad conductor of heat and its penetrating power is low. The oven is usually heated by electricity, with heating elements in the wall of the chamber and it must be fitted with a fan to ensure even distribution of air and elimination of air pockets. It should not be overloaded. Dry heat sterilizers should be monitored on a regular basis using appropriate indicators. Rubber materials- except silicon rubber- will not stand the temperature.

- The material should be arranged in a manner, which allows free circulation of air in between.
- Glassware should be perfectly washed and dried before being placed in the oven.
- Test tube, flask, measuring cylinder etc. should be plugged with cotton wool and covered with foil.
- Other glassware such as Petridishes and pipettes should be wrapped in kraft paper. A heavy duty foil pouch can be used to cover glass pipettes.
- Glassware can be placed in metal tray and finally in the hot air oven.
- All these can be heated at 160^{0}C -170^{0}C for 2-4 hours.
- These are then removed from the oven and cooled.
- Paper or cotton should not be kept directly in the oven as these may be charred.
- Plastic material such as pipette, plastic basket or trays to hold glassware should not be placed in the oven.

iii) **Incineration:** This is an excellent method for rapidly destroying materials such as soiled dressings, animal carcasses, bedding and pathological material. Plastics such as poly vinyl chloride (PVC) and polythene can be dealt with similarly but polystyrene materials emit clouds of dense black smoke and hence should be autoclaved.

5.2.2 Moist Heat

Moist heat is the most dependable method of sterilization. Different temperature ranges can be used for sterilization by moist heat method.

i) **Temperature below 100°C:** Clothing, eating utensils and some equipment may be disinfected by washing in water at 70°C to 80°C for several minutes.

ii) **Temperature at 100°C (Boiling):** Vegetative bacteria are killed almost immediately at 90°C to100°C but bacterial spores require considerable periods of boiling. Boiling should be regarded only as a means of disinfections. Sterilization may be promoted by the addition of 2% sodium bicarbonate to the water.

iii) **Steam at atmospheric pressure (100°C):** This is cheap method in which free steam is used to sterilize culture media, which may decompose if subjected to higher temperature. A kotch or Arnold steamer is usually used.

iv) **Steam under pressure:** The principle of autoclave or steam sterilizer is that water boils when its vapour pressure equals that of the surrounding atmosphere. Hence, when pressure inside a closed vessels increases, the temperature at which water boils also increases. Saturated steam has greater penetrating power. Autoclaving is the most convenient method of rapidly achieving destruction of all forms of microbial life.

Sterilization by steam under pressure is carried out at temperature between 108°C and 147°C {commonly 121°C at 15 pounds pressure per square inch (psi) for 20 minutes}. By using the appropriate temperature and time, a variety of materials such as media and pharmaceuticals products, dressings, instruments and laboratory wares can be sterilized. In addition to proper temperature and time, prevention of entrapment of air is critical to achieve sterility. Material to be sterilized must come in contact with steam and heat. Aqueous solutions are sterilized between 108°C and 126°C. Heat is conducted through the walls of the sealed container until the temperature of the fluid inside is in equilibrium with the steam outside.

An autoclave should not be packed tightly. There should be room for steam to circulate freely and to remove air, which would otherwise depress the temperature, even though the pressure readings are satisfactory.

Discarded laboratory waste for autoclaving should be placed in wide shallow containers. These should not leak at the base because infected material may leak out and contaminate the floor or bench before the contents are disinfected. Tall containers do not allow adequate steam penetration. In case plastic bags are used, these should not be sealed for the same reason.

5.3 ACTIVITY

After reading this experiment, you sterilize the following items:

1. Petridishes	**2. Pipette – glass and plastic**	**3. Test tubes**
4. Forceps	**5. Scissors**	**6. Culture Media**
7. Apron	**8. Test tube rack (plastic)**	**9. Cotton swabs**

10. Glass measuring cylinder.