

## **STERILIZATION**

Sterilization is a process by which the biological or non-biological material is made free from living micro-organisms. It is a complete destruction of all living organisms by physical or chemical agents. The material needed in a research laboratory, particularly glassware and other accessories such as forceps, inoculating needles, filter papers, and so on, need sterilization to make them microbe-free. The common methods of sterilization include dry heat sterilization, steam under pressure sterilization, filter sterilization, and UV radiation.

**STERILIZATION OF MEDIUM** For most types of media, cloth, rubber, and other material that would be destroyed by dry heat, steam under pressure sterilization is used. Such material is autoclaved at 121°C for 30 minutes using steam under 15-pound pressure in an autoclave.

**BY AUTOCLAVE** Media, water blank, empty tubes with cotton plugs, and pipettes with cotton plugs (wrapped in butter papers) are sterilized in steam under pressure. Certain sugars are destroyed in steam under pressure and therefore should not be sterilized in an autoclave under pressure.

**Procedure** Prepare media and any other materials to be sterilized. Keep the material in the autoclave, close the lid, and tighten the screws. Start the autoclave. Keep the steam release cap open to release the steam. When the steam is formed in the autoclave and released through the steam release cap, close the steam release knob/cap so as to get the steam pressure. When the pressure reaches 15 pounds, adjust

The pressure cap to maintain the pressure for 30 minutes. After this, turn off the autoclave button so as to bring the autoclave to normal pressure. When the pressure reaches zero, open the lid and remove the sterilized material.

**Precautions** Check the water level before running the autoclave. Maintain the proper water level in the autoclave. Do not close the steam release cap in the beginning. Let some steam get released and then close the steam cap so as to avoid the escape of cotton plugs from the media flasks. Fill the media up to three fourths capacity of the flask. Filling more than this capacity will touch your cotton plugs during sterilization and spoil the media. Do not open the autoclave unless the pressure reaches zero after sterilization. After opening the lid, do not put your hand in immediately to remove the sterilized material. Let the remaining steam come out.

## **BY FILTRATION**

Materials such as certain sugars are destroyed by heating at temperatures normally used for sterilization. To sterilize such heat-labile materials that are liquid or substances dissolved in solutions, filtration is used. The filters during filtration remove bacteria via the mechanical sieve-like action of the minute pores of the filter and via the adsorption of the microbes in the filter because of the differences in their electric charges. A filter widely used is a membrane filter. The membrane filter is a cellulose or plastic membrane with a pore size sufficiently small (usually 0.45  $\mu\text{m}$ ) to trap and thereby remove bacteria from a liquid. Other filters used in sterilization include sintered glass filters.

**Procedure** Sterilize the filter attached to the flask in the autoclave under steam pressure. Put the solutions to be sterilized in the filter cup. Attach the filtration unit to vacuum under pressure to start filtration. Complete the filtration and remove the filtered material in sterilized bottles or test tubes as required

**Precaution** Do not forget to put the cotton plug in the beak of the flask attached to the filter cup. This will reduce the risk of leakage of oil from the vacuum pump tube into the filter flask. This is also necessary to keep the filter flask sterilized. When a few drops are left to be filtered, remove the vacuum tube attached to the beak of the filter flask in such a way that the cotton plug of the flask beak is retained in the beak itself. This will avoid the entry of outside air into the flask and keep the sterile condition in the flask