

- Checking of reagents and media for sterility before use.
- Checking of cultures by eyes, and microscopes (phase contrast) every time they are used.
- Use of media and separate bottles for each cell line is advised.
- Maintenance of clean and tidy conditions at work places.
- Personal hygiene of the staff is very important.

**STERILIZATION**

The sterilization procedures are designed to kill the microorganisms, besides destroying the spores. There are three major devices for sterilization.

1. Dry heat
2. Moist heat (autoclave)
3. Filters.

In the **Table 33.2**, the sterilization of major equipment, apparatus and liquids is given.

**Sterilization by dry heat :** This is carried out at a minimum temperature of 160°C for about one hour.

**Sterilization by moist heat :** Certain fluids and perishable items can be sterilized in an autoclave at 121°C for 15–20 minutes. For effective moist heat sterilization, it is necessary that the steam penetrates to all the parts of the sterilizing materials.

**Sterilization by filters :** The use of filters for sterilization of liquids often becomes necessary, since the constituents of these liquids may get destroyed at higher temperatures (dry heat or moist heat).

**Sterile filtration** is a novel technique for heat-labile solutions. The size of micropores of the filters is 0.1-0.2 µm. Filters, made from several materials are in use. These materials include nylon, cellulose acetate, cellulose nitrate, polycarbonate, polyethersulfone (PES) and ceramics. The filters are made in different designs-disc filters, cartridges and hollow fiber.

In fact, many commercial companies (e.g. Millipore, Durapore) supply **reusable** and **disposable filters**, designed for different purposes of sterilization.

**TABLE 33.2 Sterilization of major equipment, apparatus and liquids used in tissue culture**

<i>Sterilization device</i>	<i>Items sterilized</i>
<b>I For equipment and apparatus</b>	
<b>Dry heat</b>	Glass slides Pipettes Ampoules (glass) Pasteur pipettes Instruments Test tubes
<b>Autoclave</b>	Ampoules (plastic) Apparatus with silicone tubing Filters (reusable) Glass bottles with screw caps Glass syringes Magnetic stirrer bases Screw caps Stoppers (rubber silicone)
<b>II For liquids and nutrients</b>	
<b>Autoclave</b>	Salt solutions Glucose–20% Agar Bacto–peptone Glycerol Lactalbumin hydrolysate Phenol red Tryptose HEPES EDTA Water
<b>Filter</b>	Serum Amino acids Vitamins Antibiotics Bovine serum albumin Collagenase Glutamine Drugs NaOH Trypsin Transferrin