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**Third Year B.Sc. Botany**  
**Practicals - Group-II**

**PAPER - III**  
**Molecular Biology and Biotechnology**

**Exercise 1. Introduction to tools and techniques: Laminar flow bench, autoclave and filter sterilization**

**Submitted by**

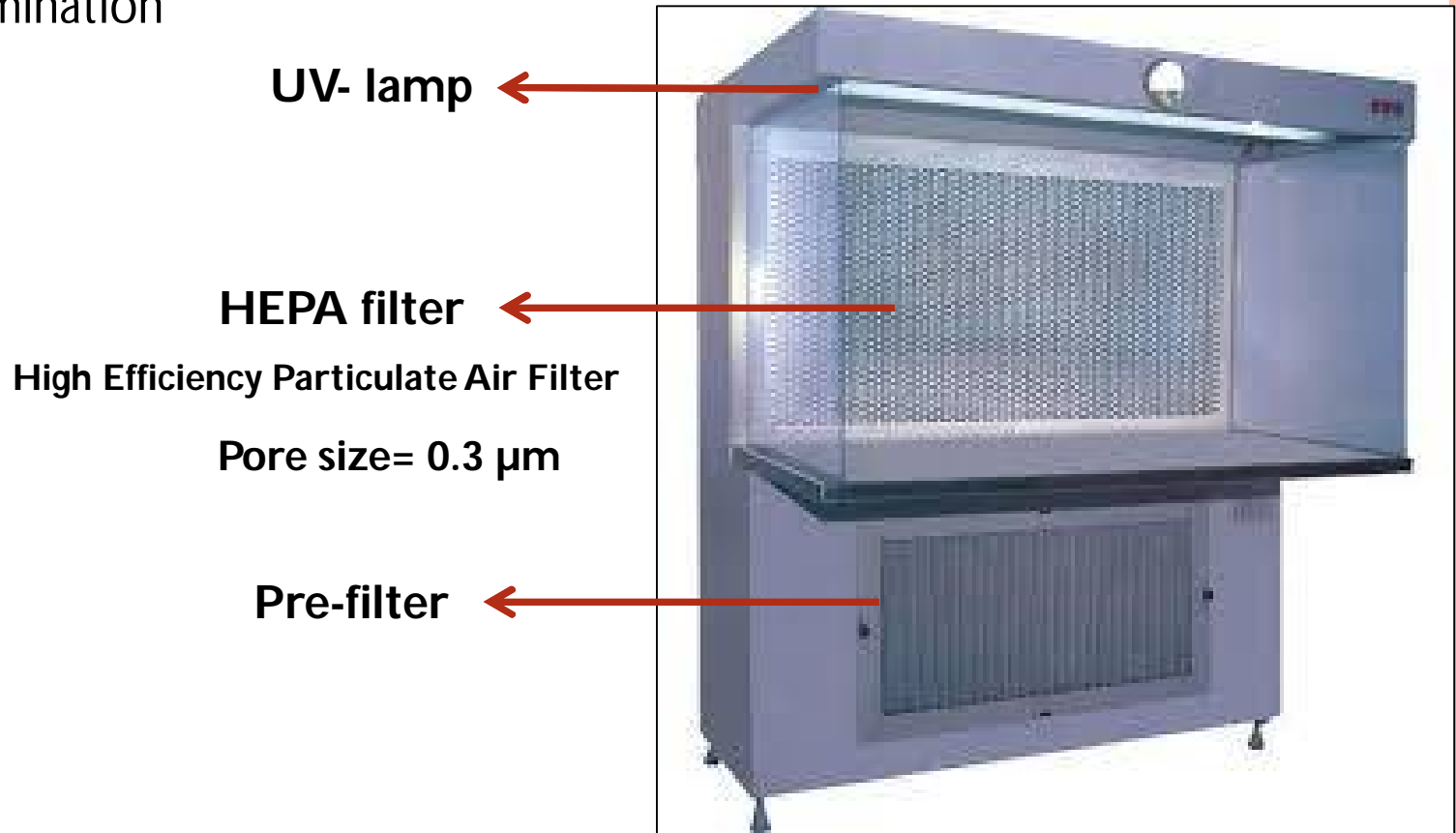
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# Introduction to tools and techniques

## ❑ Laminar flow bench

Laminar flow can produce dust free air current with uniform velocity along parallel flow lines which help in transferring the culture media bacteria free. Air is passed through these special filters into the enclosure and the filters does not allow any kind of microbes to enter into the system. Due to uniform velocity and parallel flow of air current we can perform pouring, plating, slanting, streaking without any kind of contamination



## ❑ Autoclave

The principle used here is to increase the temperature of steam (gas) in a closed system that increases its temperature. The water molecules become more aggregated that increases their penetration considerably

The autoclave is usually operated at **15 lb/sq.** inch steam pressure for 30 min., which as seen from the above table corresponds to **121.5°C**. This temperature for a period of **30 min.** is sufficient to kill all the spores and vegetative cells of microorganisms.

**Table 35.1 : Pressure-temperature relationship**

<i>Pressure</i> (Pounds/inch <sup>2</sup> )	<i>Temperature</i> °C	<i>Pressure</i> (Pounds/inch <sup>2</sup> )	<i>Temperature</i> (°C)
0	100	20	126.5
5	109	25	130.5
10	115.5	30	135.5
15	121.5	40	141.5



## Precautions:

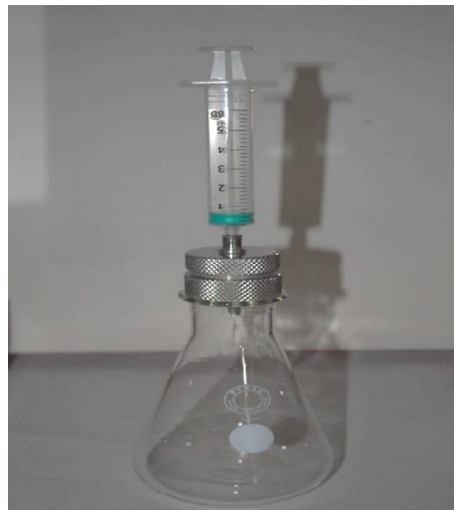
1. Check water level each time, the heating elements should remain immersed in the water
2. Check spring of safety valve frequently and clean opening whenever necessary
3. Opposite screws of the lid should be tightened simultaneously
4. Do not over tighten the screws to avoid damage to the gasket

## ❑ Filter sterilization

Filter Sterilization is used for the removal of microbes from solutions that cannot easily be treated in other fashions. Typically heat-sensitive compounds such as antibiotics and vitamins are filtered before addition to sterile cool media

### ❖ Types of Filters

- Depth filters
- Membrane filters
- Nucleopore membranes



### ➤ Depth filters

- ✓ Utility as pre-filters before final filtration
- ✓ Depth filters, are the oldest type and consist of overlapping layers of fibrous sheets of paper, asbestos or glass fibers
- ✓ The random nature of the fibers laying on one top of another creates torturous paths through the filter that trap many particles

### ➤ Membrane filters

- ✓ Membrane filter is the most common type of filtration system used in modern laboratories
- ✓ These are made from high tensile strength polymers of cellulose acetate, cellulose nitrate, polycarbonate, polyester, polypropylene or polysulfone

### ➤ Nucleopore membranes

- ✓ Nucleopore membranes are created by exposing a very thin polycarbonate film (10  $\mu\text{m}$ ) to nuclear radiation, which creates areas of weakness in the polymer. The membrane is then treated with an etching solution that degrades the weak areas creating pores in the membrane
- ✓ Nucleopore membranes are particularly useful for trapping bacteria on the surface of a membrane for subsequent microscopic examination