

RAMA  
UNIVERSITY

[www.ramauniversity.ac.in](http://www.ramauniversity.ac.in)


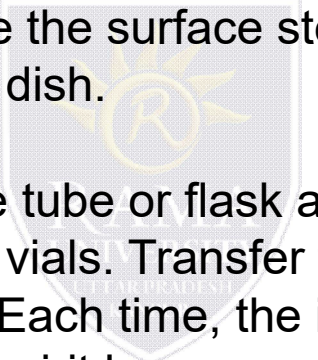
FACULTY OF ENGINEERING &  
TECHNOLOGY

**Course: B. Tech Biotechnology**  
**Sub Code: BBT-515**

**Semester: 5th**  
**Sub Name: Plant Biotechnology**

# LECTURE 8

**Dr. NIHARIKA SINGH**  
**Assistant Professor**  
**Dept. of Biotechnology**

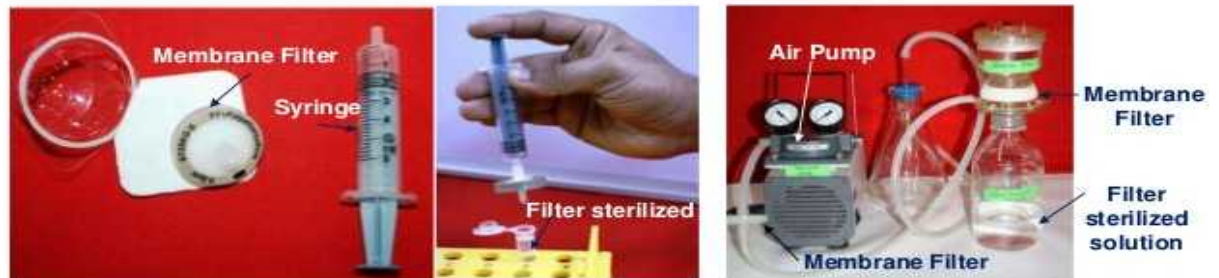
- 
- ✓ Wear a clean apron and use a mask. Clean the hands with alcohol and dry it.
  - ✓ Pour alcohol in a clean coupling jar and dip all instruments into it. Light the spirit lamp. Take the surface sterilized or aseptic plant material in a, sterile petri dish.
  - ✓ Flame the neck of culture tube or flask and in quick succession remove the plug of glass vials. Transfer the tissue onto the medium and replace the closure. Each time, the in-struments are passed through the flame of the spirit lamp.
- 
-

## STERILIZATION OF MEDIA

- ✓ Sterilization of media is routinely achieved by autoclaving at the temperature 121 ° C. (steam pressure of 1.05kg/cm<sup>2</sup>) for 15- 45 minutes
- ✓ Over autoclaving should be avoided
- ✓ Advantages of autoclaving are: the method is quick and simple, whereas disadvantages are the media pH changes and some components may decompose and so to loose their effectiveness.



- ✓ Filtration through microporous filters (0.22- 0.45) is also used for thermolabile organic constituents such as vitamins, growth regulators and amino acids.
- ✓ Filter assemblies of different sizes are available.
- ✓ Once the component is filter sterilized, it is collected in a sterile container which can be used immediately or dispensed in smaller amounts to be used later
- ✓ These filter sterilized components can be stored at 40C or -200C depending on the frequency of their usage



## INCUBATION OF CULTURE

- ✓ Cultures are incubated in a culture room where light, temperature and humidity are controlled.
- ✓ For some tissues dark is essential while for some both dark and light conditions are required.
- ✓ Humidity has also some effect.
- ✓ The cultures are incubated on culture rack at constant temperature of 25-28°C. Culture tubes are placed at 30-45° inclined position.
- ✓ Illumination is provided by cool-white fluo-rescent light placed about 18 inches above the culture to give a light intensity of  $4 - 10 \times 10^3$  lux for 16 hours.







# QUIZ

