TISSUE CULTURE PROTOCOL

A. TISSUE CULTURE LABORATORY

1. A WASHING AREA

- Large sink
- Running hot and cold tap water
- Brushes of various sizes
- Detergent
- Single distilled water
- Plastic buckets
- Plastic bucket with lid

2. HOT AIR OVEN

- Necessary for drying the washed glass goods.
- Number of enameled trays of different sizes to keep glass goods inside the oven

3. REFRIGERATOR

- For storing various chemical like vitamins, hormones, amino acids, yeast extract, coconut milk, casein-hydrolysate, Stock solutions of salts, etc.

4. DISTILLATION PLANT

- Single distillation and a double distillation water plant.
- Plastic containers for storing the distilled water

5. WEIGHING BALANCE

- Pan balance
- Chemical balance
- Electronic balance

- For weighing chemicals, sugars,agar-agar, etc.

6. pH METER

- For adjusting pH of the nutrient medium

7. AUTOCLAVE

- Important for sterilization of nutrient media, glass goods, instruments, etc.

8. Other Requirements

- Vacuum Pump
- Working Tables
- Heater
- Microscopes – simple, compound, inverted binocular, camera attachment for photomicrograph
- Microtome
- Wooden Racks

9. LABORATORY FOR ASEPTIC INOCULATION

- No windows, no ventilator to make it dust-free.
- Double doors
- Automatic door closer
- Inner door - Rubber mat for cleaning
- Simple inoculating hood

9. LABORATORY FOR ASEPTIC INOCULATION

LAMINAR AIR FLOW

- Number of small blower motors
- Air passes through number of HEPA (High Efficiency Particulate Air) filters
- 0.3µm
- Ultra clean air free from fungal/ bacterial contaminers
- Flows at a velocity of 27±3m/minute
- Put on for 10-15 min before starting work

10. CULTURE ROOM

- For incubating the culture under controlled temperature, light and humidity
- Double doors
- Dust free
- Constant room temperature 25±2°C
- Shelves made of glass, plywood
- LED lamps with timer
- Thermometer and Hygrometer (relative humidity should be above 50%)
- Shaker for suspension culture

B. GLASS GOODS AND INSTRUMENTS

1. Glass goods

- Should be of Corning or Pyrex or similar Boro-silicate glass
- Measuring cylinders
- Conical flasks
- Pipettes
- Beakers
- Culture tubes
- Screw capped universal bottles
- Washing and cleaning – soap water, single distilled water, hot air oven dry, dust-proof cupboard/ drawer
- Cotton plug, aluminium foil
- Flame sterilization of instruments

2. Instruments

- Various sizes of scalpel
- Forceps
- Spatula
- Scissors, etc
- Should be of stainless steel