

Laboratory Requirements

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Laboratory Requirements:

- (a) Cultivation should be done under aseptic conditions.
- (b) The isolated plant part should get an appropriate environment which will help to divide the cell and to get an expression of internal potential.

- (a) Washing and storage facilities;
- (b) Media preparation, sterilization and storage room;
- (c) Transfer area for aseptic manipulations;
- (d) Culture rooms or incubators for maintenance of cultures under controlled conditions of temperature, light and humidity;
- (e) Observation or data collection area;
- (f) Transplantation area.

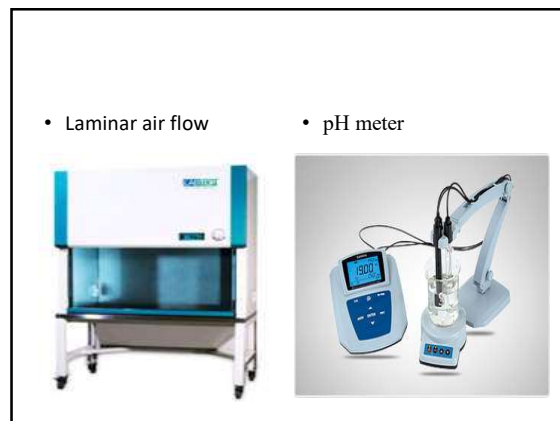
Washing and Storage Facilities:

- An area with large sink (lead lined to resist acids and alkalis) and draining area is necessary with provision for running water, draining-boards or racks and ready access to a deionized, distilled and double-distilled apparatus.
- Space should also be available to set up drying ovens, washing machines, plastic or steel buckets for soaking lab ware, acid or detergent baths, pipette washers, driers and cleaning brushes.
- For storage of washed and dried lab ware, the laboratory should be provided with dustproof cupboards or storage cabinets.

Media Preparation Room or Space:

- This part is the central section of the laboratory where most of the activities are performed i.e., media preparation and sterilization of media and glassware's needed for culture.
- There should be sufficient working bench as well as storage space.

- (i) Different types of glassware
- (ii) Different kinds of balances
- (iii) Required chemicals
- (iv) Hot plates and Stirrer
- (v) Water bath
- (vi) pH meter
- (vii) Autoclave and Hot air oven
- (viii) Microwave oven
- (ix) Vortex, Shaker
- (x) Centrifuge
- (xi) Refrigerator and Freezer
- (xii) Storage cabinet (Dust-free)



Transfer Area:

- Tissue culture techniques can only be successfully carried out in a very clean laboratory having dry atmosphere with protection against air-borne microorganisms.
- For this purpose a sterile dust-free room/cabinet is needed for routine transfer and manipulation work.
- The 'laminar air flow cabinet' is the most common accessory used for aseptic manipulations now-a-days.
- The cabinet may be designed with horizontal air flow or vertical air flow where the air is forced into the cabinet through a bacterial HEPA (High Efficiency Particulate Air) filter.
- The air flows over the working bench at a constant rate which prevents the particles (microorganisms) from settling on the bench.

- Before operation in the laminar air flow cabinet, the interior of the cabinet is sterilized with the ultraviolet (UV) germicidal light and wiping the floor of cabinet with 70% alcohol.
- Inoculation chamber, a specially designed air tight glass chamber fitted with UV light, may also be used as transfer area.

Culture Room:

- Plant tissue cultures should be incubated under conditions of well-controlled temperature, illumination, photoperiod, humidity and air circulation.
- Incubation culture rooms, commercially available incubator cabinets, large plant growth chambers and walk-in- environmental rooms satisfy these requirements.
- Culture rooms are constructed with proper air-conditioning; perforated shelves (Fig. 16.2D) to support the culture vessels, fitted with fluorescent

- tubes having a timing device to maintain the photoperiod, black curtains may be used to maintain total darkness.
- For the suspension cultures, gyratory shakers are used.
- Air conditioners and heaters are used to maintain the temperature around $25 \pm 2^\circ\text{C}$ and humidity is maintained by uniform forced air-ventilation.
- The lighting is also done in a measured amount i.e., 40-200 fc (foot-candle).

Data Collection Area:

- The growth and development of tissues cultured in vitro are generally monitored by observing cultures at regular intervals in the culture room or incubators where they have been maintained under controlled environmental conditions.
- Arrangement should be there where the observations can be done under aseptic conditions using microscope.
- Special facilities are required for germplasm conservation i.e., cryopreservation accessories should be there.

Transplantation Area:

- Plants regenerated from in vitro tissue culture are transplanted to soil in pots.
- The potted plants are ultimately transferred to greenhouse but prior to transfer the tissue culture grown plants are allowed for acclimatization under well humid condition and controlled temperature and under controlled entry of sunlight.

Thank You