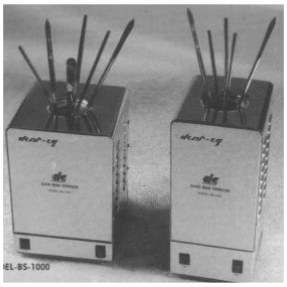
Sterilization

- **Media**:
  - The media must be sterilized using an autoclave.
  - Temperature (121 Cº), pressure (15 pascals), time (15 min).
  - Some plant growth regulators are thermolabile destroyed with autoclaving. They will be sterilized using Millipore then applied to sterilized media after cooling down to 40-50 Cº.
- **Glassware:**
  - Autoclave: more efficient
  - Dry heat: up to 160-180 ºC for 3 hrs.
  - Disadvantages: poor circulation of air and slow penetration

Instruments:

- Forceps, scissors, scalpels, spatula, needles…..
- Sterilization is done by dipping them in 95% ethanol followed by flaming till the instrument reaches a reddening level.
- Using 70% is also satisfactory, however, it's not flammable as 95% ethanol
- Why not 100% ethanol?
- Glass bead: heating glass beads up to 250 C for 15-20 mins.



Laminar air-flow (Transfer area):

- It flows clean filtered air to prevent contamination of the biological sample and the media by circulating air or the worker.
- The air-flow route should not be blocked.
- Sterilizing the laminar flow with 70% EtOH.
- Occasional sterilization of working area with UV light.

Media preparation

Media preparation is one of the primary and most essential steps in tissue culture. Media is prepared based on the type of tissue being cultured. Most media differentiate from each other based on the requirements of the growth of the specimen it supports.

Procedure

- Add approximately 150 ml of the final volume of double distilled water to a (250ml) 1/4-liter beaker.
- Slide the magnetic stir bar into the flask
- Weight the required quantities of *myo*-inositol and sucrose and add to the flask
- Weight required amount of commercially available MS media is made up with DW by using a graduated cylinder.

- Add the required amount of growth regulator and adjust the PH of the medium to the required value, by adding a drop of 1 N NaOH OR 1 N HCl with separate Pasteur pipettes
- Weight and add 7g of agar
- After adding agar, turn on the hot plate/stirrer heat and continue to heat until the medium boils vigorously, but do not allow it to boil over
- Turn off the hot plate/stirrer and remove the flask
- Dispense the medium into the desired culture vessels using an automatic pipette
- Cap the culture vessels
- The culture vessels containing medium are transferred to appropriate racks and sterilized by autoclaving at 121°C for 15 min.
- The medium is allowed to cool at room temperature and is stored at 4C° in a clean place