
General Tissue Culture Labs (Category I)

General Aspects of Safety in the Laboratory

It is not only your responsibility to “be safe”, but it is your responsibility to ensure those working around you “are safe too”.

Make sure you know and *understand* the procedures for the use and disposal of reagents, chemicals, cells and any potentially hazard materials.

If you are unsure of anything, ask a designated safety officer, first.

Detailed operating procedures for using the communal TTL tissue culture rooms on level 4 (which are class II rooms) are displayed in those rooms; the details below are applicable to the non-communal tissue/cell culture AB group areas on levels 1 and 4.

General Safety Procedures

- A clean laboratory coat must be worn at all times (when working in the communal TTL labs on level 4, this should be a green coloured lab coat, stored outside the tissue culture room). The coat should be fully buttoned for personal protection and to avoid disturbing air currents around safety cabinets.
- Disinfect tissue culture cabinets and all surfaces before and after use by swabbing with 70% ethanol.
- Have only the bottles, pipettes etc. in the hood if required - avoid clutter. Anything in the hood disturbs the integrity of the air flow so the fewer objects there, the better.
- Incubate dishes in plastic boxes (with holes for CO₂ equilibration, if required). Swab inside and outside of boxes before use with 70% ethanol. Swab outside of boxes with 70% ethanol each time they are removed from the incubator. Do not seal the boxes with paper masking tape (or sterilisation indicator tape) - use plastic tape.
- Tins of broken or badly chipped glass pipettes discovered before or during use must be labelled as such and placed on the trolley. Remember that someone has to remove pipettes by hand from the disinfectant solution in the hoods - a broken pipette can cause serious hand injuries.
- All spillages must be disinfected and cleared immediately, inside the cabinets with 70% alcohol and outside the cabinets with Virkon.

Disinfectants used in Tissue Culture

70% (v/v) alcohol: 70% by volume absolute ethanol in water.

Virkon: 1% by volume in water.

Sodium hypochlorite: 1% by volume in water.

Waste Procedures:

Glassware that has been in contact with cells must be disinfected after use by complete immersion (or completely filling large bottles and fully swabbing outsides) in Virkon for a minimum of 2h prior to washing. It should then be taken to the sterilisation washroom on level 1, labelled with an autoclave tape with AB Group written on it, logged in the washroom folder, and left there to be sterilised (and collected on the day when it is ready).

Broken glassware & Sharps must be disinfected with Virkon before being discarded in the red Broken Glass bins.

- **Plasticware** must be disinfected with Virkon. After disinfection, all the plasticware can be placed in the biohazard waste bin for disposal.

Sharps (including all needles, glass pasteur pipettes) must be placed into **white-topped Sharps Bin**.

Cultures and used medium must be disinfected by mixing with Virkon for at least 24h before placing them in biohazard bags for autoclaving.

Disposal of contaminated cultures or media:

- **Flasks** – disinfect with Virkon for at least 24h. Pour Virkon down the sink with running tap water, tighten the flask cap and discard into a biohazard bag.
- **Dishes** - remove immediately from the tissue culture area in a closed box to a fume hood. Liquid cultures - add Virkon (1% by volume in water) directly to the box, leaving the dishes to soak for 24 hours - empty the box down the fume hood drain and transfer the drained plates to a biohazard bag.

Virkon solutions must be replenished every week.