

TC room working protocols

Personal precautions

These precautions are for protecting your cultures from contamination

- Use separate pair of slippers while entering the tissue culture facility.
- Just prior to aseptic manipulations, tie long hair back and use a head cap.
- Vigorously scrub hands and arms at least 2 min with an antibacterial soap.
- Wear clean gloves.

These precautions are for protecting yourself from biohazard material in your cultures

- Thoroughly wash hands after removing protective gloves.
- Caution should be taken when handling sharp instruments such as needles, scalpels, scissors and glass pipettes.

Working in the biosafety cabinet

- Understand how the biosafety cabinet works! This will be taught to you during the training session.

Before starting work:

- Turn on the AC in your room if it is not on already.
- Turn on the UV light for 30 mins.
- Turn on the bio safety cabinet blower and lights and turn off the UV light on before use. Allow the blower to be on for a few minutes before starting your work as the air flow takes this much time to stabilize.
- Raise the front view window as needed to bring necessary items into the cabinet.
- Wipe the working surface of the cabinet with 70% ethanol before starting your work.
- Wipe media bottle, pipettes, discard beaker, etc. before taking them in and out of the hoods.

During your work:

- Organize the work surface for a clean-to-dirty work flow. Place clean pipettes, flasks, and sterile media bottles at one side of the cabinet; place discard pans, spent cultures, and other wastes on the other side.

- While working, keep all material and perform work ≥ 4 in. back from the front opening of the cabinet, and minimize rapid movements or activity. Keep the view window opening as close to 8 in. as allows reasonable access to the work surface and equipment. (can be altered according to personal comfort levels)
- If flame sterilization is needed in the hood for a particular application, use a burner that can be activated by a pilot light when needed, rather than one that burns constantly.
- Immediately dispose of any small contaminated items into a discard beaker.
- Frequently disinfect gloved hands with 70% ethanol while doing aseptic work.
- Whilst working do not contaminate gloves by touching anything outside the cabinet (especially face and hair). If gloves become contaminated re-spray with 70% ethanol as above before proceeding.

After completing your work:

- When work is completed, remove all material from the bio-safety cabinet each time, clean any spills, and disinfect the cabinet working surface by wiping with 70% ethanol. You are not allowed to keep your stuff such as tips or tubes etc inside the hood. It creates problem for other users and is not a good practice (we will tell you about a strategy to remove your stuff from the bio-safety cabinet without getting contamination).
- Turn off biosafety cabinet blower and lights and turn on the UV light for 20 mins.
- Once you are done with your work, switch off the ACs. Do not leave this for the last user. The next person can switch on the AC when he/she is in.
- If you find the biosafety cabinets in your room are being used, you can use any available cabinet in the TC room for your work.

Maintaining the CO₂ incubators

- Understand how the CO₂ incubator works. This will be taught to you in the training session.
- Each lab is assigned half an incubator; know which one is yours.

- There are 3 possible alarms that could be set off on the incubators: temperature, CO₂ and water. The first two are usually because someone has just opened the incubator. If this is the case then the incubators will stabilize and alarms will shut off after 5 minutes.
- Sometimes the CO₂ alarm is set off due to the CO₂ cylinder being empty. If this happens, then the incubator will automatically switch the cylinder to the back-up cylinder.
- You need to call Med-Gas Equipments (phone number is on the wall of the TC room, near the entry to the main cubicles) and order the empty cylinder to be filled. Med-Gas takes 2-3 days to take away the empty cylinder and return it full so do not delay this step! If both cylinders of your incubator run out, nobody will loan you their full cylinders (you are responsible for your own laziness).
- When the cylinder is delivered, the company usually attaches it and takes away the empty cylinder, however if they cannot do so, call one of the TAs to help you attach the new cylinder.
- If the water level of the incubator falls, an alarm will flash. You need to clean the water trough and refill it with autoclaved de-ionized water immediately.

Maintenance

Liquid nitrogen filling

- Mr. Khairnar and Babu take care of filling the liquid nitrogen cylinders.
- They have time to do this on Wednesdays (usually afternoon but sometimes even in the morning).
- They need to be reminded to do this and so the TAs have made a rotation of the students who are responsible for calling the office and reminding them to fill the liquid nitrogen cylinders.

Lab cleaning schedule

Daily cleaning by the department cleaning staff

- The four labs on the fifth floor that use the TC room will have a rotation for catching hold of the cleaning staff and taking them to the TC room for daily sweeping and mopping.
- The student who is doing this job will sign on the form indicating that the work has been done.

Bi-monthly cleaning by the users of the TC room

- Wipe down insides and outsides of hoods with 70% ethanol. This includes base, sides, back, sashes (inside and outside) etc.
- Use 70% ethanol for wiping of non-sterile equipment and surfaces.
- Wipe down all surfaces in the TC room: tables, centrifuge body, fridge handles, door handles, microscope stage and focus knobs, etc. There is a schedule for this cleaning on the wall of your cubicle.

The maintenance will be done on rotation and a hard copy of the schedules will be on a wall of the TC room, near the main door. The soft copies of the schedules will be on our department website, under TC facility.

Discard

- Know the Biosafety Level (BSL) of the organism you are culturing.
- The TC room is set up for BSL1 organisms and BSL2 organisms that may cause disease by pin-prick.
- The TC room is not set up for BSL2 organisms that may cause disease and are spread through aerosol, BSL3 or BSL4 organisms.
- Discard sharp instruments such as needles and scalpel blades in sharp proof container.
- Disinfect BSL1 liquid waste with sodium hypochlorite solution for 30 mins prior to discarding to the drain with copious amounts of water.
- Take the BSL1 solid waste to your own lab and disinfect by autoclaving. Discard in regular waste.
- Discard BSL2 waste in separate biohazard bags (red) in the red PHO waste disposal trash can in the TC room. Alternatively, take it to your lab and store it there in the red biohazard bags. Do not discard and mix it with the normal waste.
- Clearly label your BSL2 waste with the name of your lab. This is due to the procedure used by the PHO that is described below.
- Note that for BSL2 waste, your advisor needs to send an official letter (through Head) to the PHO informing them of the weight in kg of BSL2 your lab expects to generate. This is to help the PHO negotiate payments with SMS Envocare who charge for their BSL2 pick-up by weight.
- If the PHO does not come regularly to pick up the biohazard waste, call them. The number is on the list of useful numbers pasted on the wall near the main door.