Tissue Culture Facility

User Handbook for TC room working protocols

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Chapter 1

Personal precautions

1.1 These precautions are for protecting your cultures from contamination

1. Wearing a full-sleeved lab coat is mandatory for working inside the tissue culture facility.
2. Remove your footwear while entering the tissue culture facility. The footwear needs to be placed in the rack provided besides the main door.
3. Prior to aseptic manipulations, tie long hair back and use a head cap.
4. Vigorously scrub hands and arms at least 2 min with an antibacterial soap.
5. Wear clean gloves.
6. Make sure to close the cubicle doors before you start to work.
7. UV sterilization of each cubicle to be done at least once a day.
8. Please ensure to lock the cubicle door with the key if the UV light is on. Before entering your cubicle, check the UV light to avoid accident exposure to UV rays.

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

1. Never bring eatables into the TC facility.
2. Thoroughly wash hands after removing protective gloves.
3. Caution should be taken when handling sharp instruments such as needles, scalpels, scissors, and glass pipettes.

Chapter 2

Equipment

2.1 Biosafety Cabinet

Understand how the biosafety cabinet works! This will be taught to you during the training session. Briefly, the biosafety cabinet works on the principles of (1) sterilization by UV followed by (2) maintaining sterility through flow of sterile (HEPA filtered) air in a vertical curtain to prevent outside air from entering.

2.1.1 Before starting work

1. ****** Do not walk away with AC remote ******
2. Turn on the AC in your room if it is not on already.

   Que:- **Why?**

   Ans:- *Because the motor in the hood heats up. If you do not keep the ambient temperature cool, the lifetime of the hoods may decrease.*

3. Turn on the UV light for 30 mins.

   Que:- **Why?**

   Ans:- *So as to create a sterile environment inside the hood.* ***E. coli*** *are killed by UV light exposure for 1 min. Keeping the UV on for 30 mins ensures that the hood will be completely sterilized.*

4. Turn on the bio safety cabinet blower and white lights. Then turn off the UV light 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.

   Que:- **Why?**

   Ans:- *After UV exposure, the environment inside the hood is sterile. To maintain sterility, the blower is kept on for few minutes before one starts the experiments. It prevents outside air from entering inside by forming an* ***air curtain****. Make sure that at any given time, to maintain sterility, either the UV light or the blower is on. This means that if you are not actively working, the UV should be on. If you are working in the hood, the blower should be on. When you complete work, both can be turned off.*

5. Raise the front view window as needed to bring necessary items into the cabinet.

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Ensure only a minimal part of the window is open for your use to ensure sterility.

6. Wipe the working surface of the cabinet with 70% ethanol before starting your work.
7. Wipe media bottle, pipettes, discard beaker, etc. before taking them in and out of the hoods.

****** IMPORTANT NOTE: Do not wipe the front plexiglass window of the hood with ethanol since the material will discolor and turn white. ******

2.1.2 During your work

1. 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 waste on the other side.
2. While working, keep all material and perform work 4 inches from the front opening of the cabinet. Minimize rapid movements or activity.

   Que:- **Why?**

   Ans:- *Making rapid actions may disrupt the airflow allowing the outside air and contaminants to enter the hood.*
3. Keep the view window opening close to 8 inches as this allows reasonable access to the work surface and equipment (can be altered according to personal comfort levels).
4. Do not use flame sterilization at all, it damages the HEPA filter inside the hood.
5. Frequently disinfect gloved hands with 70% ethanol while doing aseptic work.
6. While 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.
7. While working in the hood, do not talk and chat with your friends as this can contribute to contamination.

****** Do not discard any tips, tubes, flasks, or bottles into the dustbins that are cleaned by the cleaning staff. The waste that is taken away should be just like office waste (only paper, tissues, etc. that has no organisms/cell lines on them). ******

Note: ** Usually your cultures are contaminated by bad techniques, not the instruments ******

2.1.3 After completing your work

8. 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. Check the cubicle to make sure you have not left any flasks for bleaching, slides, used plasticware on the work benches and sink in your cubicle.

9. Turn off biosafety cabinet blower and lights and turn on the UV light for 5 mins.

10. 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. The ACs should not be left on all night!

NOTE: Although unlikely, it is possible that the biosafety cabinet could malfunction.

(a) This could be a problem with the UV light which will show as not turning on. If this happens, inform the faculty in-charge and initiate the process of getting the light replaced. Vendor information is on the TC facility wall.

(b) The blower might not work in which case the air pressure will be less than 10-15 psi (see the red pressure gauge on the side of the cabinet). This should be reported to the service company whose number is on the TC facility wall.

(c) The HEPA filter might be old or damaged. This should be tested by putting sterile bacterial plates with no antibiotics in the hood (following the same working procedure you would if you were culturing your cells). Incubate the plates and check for contamination the next day.

The information on the last replacement of UV light need to be pasted on the biosafety cabinet (each tube has around 2000 hours' lifetime).

2.2 Carbon dioxide (CO_2) incubators

1. Understand how the CO_2 incubator works. This will be taught to you in the training session. You will be trained to change the CO2 cylinder.

2. Each lab is assigned half an incubator; know which one is yours. Check that your lab incubator is clearly labelled with the name of your lab, names of current users and phone numbers.

3. There are 3 possible alarms that could be set off on the incubators: temperature, CO_2 and water (RH pan). 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.

   Que:- **Why is the temperature at** 37°**?**

   Ans:- 37° *is body temperature and cells are usually from human sources (cancer cells, parasites, etc) and grow at* 37^oC

4. Sometimes the CO_2 alarm is set off due to the CO_2 cylinder being empty. Your lab should have a back-up cylinder in the cubicle.

   Que:- **Why are your cells cultured in 5% CO_2?**

   Ans:- *Buffer used in your media is based on exchange of bi-carbonate with atmospheric CO_2. So your flask has a membrane on its lid to allow this exchange. CO_2 incubator provides CO_2 for this buffer.*

5. If one of your cylinders is empty, you need to call "Med-Gas Equipment" (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 incubator).**

6. When the cylinder is delivered, you should be able to attach the new cylinder. You will be trained for this.

7. If the water level of the RH pan in the incubator falls, an alarm will flash. You need to clean the RH pan and refill it with autoclaved de-ionized water immediately.

   Que:- **Why?**

   Ans:- *Humidity prevents evaporation of your media, because your flasks are open to atmosphere (recall the pH buffering due to bicarbonate). If you do not fill the RH pan, your data is not reliable as the concentration of salts in your media will be different.*

8. CO_2 filling and payment will be on rotation basis and the payment schedule will be available on the incubator based on users.

2.3 Liquid nitrogen storage tanks

1. ****** This is one of the important user duties that you must do as a user of the TC facility. You will be assigned a one-week slot that comes around only once a year. This is given on the user duty chart pasted on the door of the TC facility. If you are not around during your slot, exchange with another user and write the change on the duty chart.******

2. Office staff take care of filling the liquid nitrogen storage tanks.

3. They do this once a week.

4. Users need to give them entry using their own biometric access. You need to be with them when they are filling the liquid nitrogen tanks in the TC facility.

5. The responsible person also has to check the level of the liquid nitrogen twice a week during their duty period. There is a metal ruler in the TC facility. Place this in the liquid

nitrogen container and when it is removed, you will see the level of the liquid nitrogen where evaporating fumes will be visible. This should not be less than 12 inches. If it is less, you need to call the office staff and ask them to top up the level. As we all store our cryogenic vials with important cell lines in this common storage, we must ensure that the tank is not empty/below the required level. One lab has lost Rs. 2 lakhs of cell lines due to our carelessness.

2.3.1 Liquid nitrogen usage

Always wear protective gear, lab coat, protective glasses, face shield and cryogenic gloves, when opening liquid N_2 tanks to avoid cold burns or vials rupturing on hand. These will be available in the cupboard outside the TC facility. Always wear covered shoes when you are using the tanks.

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Chapter 3

Lab cleaning schedule

3.1 Daily cleaning by the department cleaning staff

1. ****** This is another important duty that you must do as a user of the TC facility. You will be assigned a one-week slot that comes around once a year. This is given on the user duty chart pasted on the door of the TC facility. If you are not around during your slot, exchange with another user and write the change on the duty chart.******
2. The assigned weekly in-charge will be responsible to catch hold of the cleaning staff and take them to the TC room for daily sweeping and mopping.
3. The student who is doing this job will sign on the form indicating that the work has been done.
4. The student has to be present **full time** during the cleaning time with the staff. It takes 10-15 minutes only.
5. The maintenance will be done on rotation and a hard copy of the schedule will be pasted on the entry glass door of the TC room.
6. The soft copy of the schedule will be on our department website, under TC facility.

3.2 Monthly cleaning by the users of the TC room

1. Wipe down inside and outside of hoods with 70% ethanol.
2. **Very important: Do not wipe the front of the hood with ethanol! The material will turn white!**
3. Use 70% ethanol for wiping of non-sterile equipment and surfaces.
4. Wipe down all surfaces in the TC room: tables, centrifuge body, fridge handles, door handle, microscope stage and focus knobs, etc. There is a schedule for this cleaning. Take a printout and put it on the wall of your cubicle.

3.3 Cleaning of water bath

1. The assigned weekly in-charge will be responsible for providing access to the office attendants to clean the water baths in the facility on alternate Saturdays.

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Chapter 4

Discards

1. Know the Biosafety Level (BSL) of the cell line that you are culturing.
2. The TC room is set up for BSL1 cell lines and BSL2 organisms that may cause disease by pin-prick.
3. The TC room is not set up for BSL2 organisms that may cause disease and are spread through aerosol, BSL3 or BSL4 organisms.
4. The TC room is not set up for bacteria and yeast cultures with cell lines.
5. Discard sharp instruments such as needles and scalpel blades in sharp proof container.
6. Disinfect BSL1 liquid waste with sodium hypochlorite solution for 30 mins prior to discarding to the drain with copious amounts of water.
7. Take the BSL1 solid waste to your own lab and disinfect by autoclaving. Discard in regular waste.
8. 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.
9. 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.

   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.
10. 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.

FINALLY: After completing the training, you will receive an email outlining the subsequent steps required to obtain access to the facility.

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