

Tissue culture room etiquette

Groups/individuals will be assigned principal tissue culture rooms for CL1 clean, CL1 primary, CL2 clean and CL2 primary work. In the assigned room there will be plasticware storage provided in designated areas. It is up to the group to decide what plastics are stored in their area. NO CARDBOARD is to be stored in TC rooms except glove boxes. All plasticware should be stored in linbins or useful boxes supplied by the group.

MSC booking

All MSC use must be recorded on the hood sheet.

Clean rooms – Mycoplasma free

In the principal room the MSCs can be booked up to 1 week in advance in blocks of up to 2 hours. If a user has not commenced work within 10 minutes of a booking slot starting the booking becomes void and other users can over write the booking for the whole period.

Primary rooms

In primary rooms when tissue is scheduled to arrive, a MSC can be booked for the duration of the isolation procedure, but this must be clearly labelled on the hood sheet. If there is a delay in the work starting other users may use the MSC, but must be willing to stop working as soon as the tissue samples arrive.

MSC cleaning

Each MSC will be stripped cleaned on a fortnightly basis between the hours of 20:00 - 22:00. MSCs should be left switched off with no personal items or plates left in. All items in the MSC will be removed for the cleaning process and sprayed with 70% ethanol before being returned to the hood.

In the event of an MSC being left with cells, plates or bottles in or appearing to be in use during the designated cleaning time a notice will be attached to the MSC and it will not be cleaned.

Incubator Use

TC rooms routinely have 2 incubators per MSC, mainly CO₂, but every room has some with hypoxic capabilities. Hypoxic incubators will only be set up for low oxygen work if requested. Low oxygen incubators should only be used where necessary to avoid opening of the incubator door. Incubator use is not regulated, but each user of an incubator is required to fill in their contact details on the front of the incubator.

Cleaning and Watering

Only autoclaved DI water should be added to incubators.

Users are responsible for maintaining the water in the incubator they are using. Incubator water should be changed completely every 2 weeks to avoid fungal contamination. Incubator shelves should be wiped with 70% ethanol daily as samples are added and removed from incubators.

Decontamination cycles

Users can run decontamination cycles when needed on incubators that are equipped with this function. CO₂ incubators with no stacking plate between the incubators must be decontaminated in pairs. Running the decontamination cycle on only one incubator can result in the other incubator overheating.

Infections

Any bacterial, fungal or mycoplasma infections found in an incubator should be reported to the other users of the lab.

If you have an infection in your cell culture please:

- Place a note on the incubator you were using with the date, type of infection and shelf location.
- CL2 infected samples –add concentrated chemgene to give a concentration of not less than 10% in an MSC. Leave for 30 minutes before discarding down the sink. Run the tap for 5 minutes.
- CL1 infected samples - remove to the main lab. Add concentrated Chemgene to give a concentration of not less than 10%. Leave for 5 minutes before discarding down the sink. Run the tap for 5 minutes.
- Remove the shelf where the infection was located, thoroughly clean with detergent (1 % chemgene), clean with ethanol and replace.

Pipettes

Each room is assigned enough pipettes for a set per MSC. Only filter tips should be used with TC pipettes. Tissue culture pipettes must not be removed from their designated room.

Pipette boys

Each room is assigned enough pipette boys for one per MSC. Tissue culture pipette boys must not be removed from their designated room.

Water Baths

Each TC room will have 1 waterbath. The waterbath will be emptied and fresh DI water and detergent will be added on a monthly basis. Users should top up the waterbaths with DI water only.

Microscopes

Each TC room will have at least one microscope with phase contrast x4, 10, 20 and 40. If a group wishes to keep a florescent microscope in a TC lab it will be treated as core equipment and can be used by all users of the TC room. No priority will be given to the group that it belongs to.

Aspirator

Only solutions that can be discarded down the sink should be aspirated into the aspirator bottle. Solutions that have toxic chemical that need to be treated as chemical waste should be collected in an appropriate container and sent out as chemical waste.

Users are responsible for emptying aspirators when they are $\frac{3}{4}$ full. Aspirators must be changed at least every 7 days. To change an aspirator:

- Check the filter to make sure the bottle has not been over filled
- Ensure the solution has been in the appropriate detergent for the required contact time (10% chemgene - CL1 5 mins, CL2 30 mins)
- Remove the bottle from the container
- Take to the TC sink and pour down, run the tap for 5 minutes
- Wearing safety glasses add 300ml of Chemgene – or the appropriate volume that allows for the full container to have a 10% Chemgene concentration.
- Connect the bottle back to the pump

For CL2 blood room

- Add Virkon tablets to the correct concentration to the aspirator to give a final concentration of 1% (30g to a 3000ml bottle)
- Leave the bottle for 10 minutes once work is complete
- Remove bottle from the aspiration and transfer into a MSC
- Carefully pour into a sweetie jar
- Add 150ml (3 scoops) amounts of Vernagen and tape closed with hazard tape
- Solidified sweetie Jars should be disposed of in chemical waste bags

Labelling

Every flask, tube, plate, dish and sample in the TC rooms must have an owner's name, substance name and date clearly marked on it.

Cleaning

Every user must clean the MSC and working area after each session. All waste should be cleared away following the correct disposal routes. The lab must be left in a clean condition ready for the next user.