

Tissue cell culture room VBC5 4th floor – General information

- The contact persons for the TC room are Amr Abouelezz (Ries group) and Aswini Kumar Panda (Falk group). Every new user should contact them for an introduction to SOPs and cleaning duties.
- The responsible person for the TC room is Irma Querques.
- The two hoods can be used in parallel by two users. Hoods need to be booked using the PPMS system. Please, do not leave the hoods open while not working.
- Everyone teaches mammalian cell culture work and procedures to new users from their respective labs
- General rules:
 - everyone should take care of the TC room, take trash out, keep it clean etc.
 - everyone should label their stuff properly
 - bin under the hood must be changed every week (trashed by last user on Fridays).

TC VBC5 mailing list

We have a mailing list for all the VBC5 TC users that can be used for communications (e.g. report contaminations etc.)

tcvbc5@lists.maxperutzlabs.ac.at

Raffael Lichtenberger (raffael.lichtenberger@maxperutzlabs.ac.at) and Irma Querques (irma.querques@univie.ac.at) are the owners. If another user wants to use the TC, please let Raffael or Irma know so we can add him/her to the list. Every new user should also contact Amr Abouelezz (Ries group) or Aswini Kumar Panda (Falk group) for an introduction to SOPs and cleaning duties.

- General stuff to order with departmental budget (done by Julia Westermayer – Leonard/Querques group)
 - 70% EtOH
 - glass Pasteur
 - small waste bags
 - filter tips
 - mycoplasma test labels
- Apart from these general items, other consumables in the TC room belong to each lab/group. So, hands off! ☺
- Shelves in the fridges and freezers are dedicated to each lab/group as indicated by the labels.
- Hood filters and incubators checking is done once a year by the company Lindner (next appointment December 2024).
- A general cleaning day is organized once a year before Christmas by the contact person in accordance with other users.

Tissue cell culture room VBC5 4th floor – General rules

Don't bring unnecessary stuff into this room, no contamination risks, no gloves, no lab coats. We use specific TC equipment and consumables for TC, extra tips as well. Use disposable plastic pipets only.

Avoid contamination from outside

After entering TC you immediately have to wash your hands with soap and then disinfect them with EtOH. Put on your gloves and disinfect them thoroughly.

You must wear a TC room dedicated lab coat with long sleeves and cuffs (ask Julia Westermayer for purchasing one) that can be found inside the room. We have two sets of lab coats, A and B, which we share. Each set is washed every month (part of cleaning duties). Tie your hair.

Do not use watches and phone in the TC room. Calculators and timers are available in the TC room.

ANY contamination, large or small, or any unexplained morphology in your cells **MUST** immediately be reported to the TC room user mailing list.

Mycoplasma

is a genus of tiny bacteria (~0.1µm in diameter) and it is a serious threat to cell culture experiments. To assure that our cultures are safe, every TC room user is preparing samples to be subjected to Eurofins mycoplasma contamination tests once month (samples can be taken at any point throughout the month and been frozen). Samples are sent for testing to Eurofins by Raffael Lichtenberger (raffael.lichtenberger@maxperutzlabs.ac.at) the first week of each month. Results will be available at VBCShare/MAMMALIAN CELL CULTURE/MYCOPLASMA TEST/Mycoplasma test results.xlsx. Raffael will contact you by e-mail in case of positive results **ONLY**.

Please, ask other users in your lab, Amr Abouelezz (Ries group) or Aswini Kumar Panda (Falk group) how this can be done as soon as you start using the TC room. Mycoplasma test will need to be done every time a new cell line/aliquot is used. Eurofins tests are compatible with the usage of Penicillin/Streptomycin in the cultures.

For that purpose, just pool your cells and let them grow for at least 3 days without exchanging media, e.g., over the weekend.

Laminar flow hoods

We have 2 hoods, hence please **empty the laminar flow hood ASAP** so that others can use it. **Don't leave stuff in the hoods if you're not actively using it or taking a break**. Book the hoods in advance via the booking system (PPMS). Please, do not leave the hoods open while not working.

It is compulsory to keep the UV lamp ON for 30 minutes after you are done with your work.

Keep extremely clean. Clean incubators with EtOH. If you spill something on the shelves, please clean them immediately with EtOH. If you spill something into the incubator water, the water has to be exchanged immediately and the basin cleaned with EtOH. The **water** is (only) **one time distilled** (MQ can harm the metal).

Tissue cell culture room VBC5 4th floor – General cleaning and maintenance

Rubbish

Please empty the rubbish bins whenever they get full.

For the small bins inside the hood, just take out the plastic bag and put it into the black autoclave bins, then replace the bin liner. You can find new bin small bin liners in a rectangular box (like a tissue box) on the shelf above the microscope.

For the autoclave bins, take out the plastic bag and take it to the washing kitchen on the 1st floor. Pinch the opening of the bag and wrap some autoclave tape around it, then leave it there – it will get autoclaved! There are replacements for the bin liners in the cupboard next to the insect cell room, please take a new one back upstairs to line the bin.

When the metal bucket for used glass discard is full, place the lid on it and take it down to the washing kitchen on level 1. Apply autoclave tape to the bucket and leave it on the table. A new bucket can be found either in the TC room or the washing kitchen—retrieve it and use it until it's full again.

Ethanol bottles

Please refill the 70% ethanol bottles when they are empty.

There is a cannister of 70% ethanol sitting in the fume hood behind the cell counter. You can just pour this straight into the spray bottles to refill. Once that cannister is empty, you need to take a new one out of the flammables cupboard underneath the hood. For this, you need to get the key that is in the drawer next to Martin's desk in the Falk lab, labelled toxic with red tape. Take out a new cannister of ethanol, lock the cupboard again and return the key. Now there is a Key in the TC room, in the drawer named TAL. (Remember the key has to stay hidden there)

Glass pipettes

Once you use up all the glass pipettes in one of the metal containers, you can get a new one from the shelves above the water bath. It should still have autoclave tape on it to show that it's sterile. If the autoclaved ones are not in the TC room, they should be in the washing kitchen, already autoclaved. You just need to bring them to the TC room.

Once you've removed the empty container from the hood, refill it with new glass pipettes at the very top of the shelves above the water bath. New of these boxes can be found in the top self above the wash basin in TC room. If the new boxes are running out please inform Julia Westermayer. Fill up the container as much as possible and take it to the washing kitchen on level 1. Stick some autoclave tape onto the opening (please use the metal specific autoclave tape – it is always sitting somewhere in the washing kitchen, it is in a black tape dispenser), and leave the container on the table. It will get autoclaved, and brought back to the Leonard or Querques lab, so someone can bring it back upstairs.

Vacuum pump

Please keep an eye on the liquid level in the vacuum pumps. When the bottle is starting to get full, disconnect it from the pump, put the lid on, and take it to the washing kitchen on the 1st floor. Put some autoclave tape on the lid and leave it there, it will get autoclaved.

There should be a spare clean bottle somewhere next to the vacuum pump, connect this back to the vacuum pump.

Incubator water tray

Please check the water level in the incubators regularly and refill them whenever you notice that the water level is getting low!

Use the sterile water in the 2L Schott bottle next to the sink, it is labelled ddH₂O on the lid. Open the incubator, move the water tray to the front of the incubator (don't take it out), and pour some more water in. Move the tray back to the back of the incubator. Try not to keep the lid off the Schott bottle for too long (just remove it quickly to pour in the water and put it straight back on).

When you run out of water in the bottle, take a new one from the cold room on the 1st floor.

CO₂ cylinders

The photo attached is from the cell culture room showing the relevant parts of the CO₂ cylinders:



1. The cylinder valve: once the cylinder is installed, this should be fully opened and left open. You generally don't need to do anything here unless you are changing the cylinders.
2. The cylinder pressure gauge: this shows how much gas is in the cylinder. When the cylinder is empty, this will read 0 (as shown in the photo).
3. The output pressure gauge: this shows the pressure of the gas going to the incubator. Optimally, this should read 1.
4. The output pressure knob (regulator knob): this allows you to adjust the output pressure.

- If one of the cylinders is empty (the cylinder pressure gauge reads 0), check that the valve is fully open. If the valve is indeed open and the gauge still says 0 then the cylinder needs to be changed. Please let me know so I can change it, or go ahead and change it yourself.

It looks to me like each of the incubators is connected to both cylinders, so unless both cylinders are empty, there is generally no critical urgency to change the cylinder.

- If the output pressure valve reads 0, or if the incubators are showing low CO₂ levels, or if the CO₂ level takes too long to recover, then the output pressure needs to be re-adjusted using the regulator knob. Feel free to get in touch with me. If you decide to adjust it yourself, remember to turn the knob in very small steps (only a few degrees per turn). The gauge is also slow to react, so you need to wait after every step to see how much has changed. The output pressure should be set to 1.

Important: too much output pressure can explode the tubes, damage the incubator, and possibly cause injury. Be patient with turning the knob.

Ordering new CO2 cylinders

CO2 cylinders are rented from Messer Austria GmbH. If a cylinder is empty, it can be exchanged for a full cylinder.

Go to <https://www.messer.at/web/e-services>

Log-in credentials: e-mail: raffael.lichtenberger@maxperutzlabs.ac.at

 Password: -a:y_BsJTps2t"i

Go to “E-Order”

Select the type and number of bottles to order. On the next screen, specify the number of empty bottles to be returned. “Bestellnummer” can be anything, just put your initials and the date. In the comment field, provide a phone number so the delivery driver can contact you. Also, write a comment to make the delivery at Helmut Qualtinger Gasse 6 (back entrance, near the freight elevator). Delivery should take place within a few days.

Tissue cell culture room VBC5 4th floor – TC duties

TC duties by responsible person every 2 weeks

One user for each lab is assigned to do certain duties in a regular manner (once every two weeks). Ask Toni Manolova (Falk group) to be included in the user and TC duties list (available in smb://isis.msp.univie.ac.at/VBCShare/MAMMALIAN_CELL_CULTURE/CLEANING_LISTS/).

For now (July 2024), those include:

Hood cleaning

The hoods should be cleaned underneath the working surface every 2 weeks, as liquids can spill and drip through the holes in the working surface.

Remove the working surfaces from the hood. Start on the right hand side, since there is a knob that you can use to pull the first one out. You can keep the other items in the hood (glass pipettes, bin, etc), just move them to the side. Spray the hood with IncuClean, leave it for 2 minutes, then wipe it with a paper towel. Then spray the hood with 70% ethanol and wipe it with a paper towel. Put the working surfaces back in and repeat the IncuClean/ethanol cleaning on top of the surface.

Close the hood and turn on the UV for 30 minutes. If you're feeling extra helpful you can also clean the glass shield with 70% ethanol.

Water bath

The water in the water bath should be replaced every month. If this is the case, you will find 'W' in the comments close to your name in the cleaning schedule.

To replace it, pour out the water in the water bath. Spray the inside of the water bath with IncuClean, leave it for 2 minutes, then wipe it with a paper towel. Then spray it with 70% ethanol and wipe it with a paper towel.

To replace the water, you will need a few bottles of autoclaved water which you can get from the cold room on the 1st floor (~10L). If you run out, you can also get some MilliQ water from the washing kitchen. Pour them straight into the water bath until it is ~half full. Then add some Aqua Care solution (in a small bottle on the shelf above the water bath), add 1mL/1L of water. When the bottle starts to run out, please notify Julia Westermayer.

Lab coats

We have two sets of lab coats, A and B, which we share. Each set is washed every month. Please bring only one set to the washing kitchen on floor 0 for washing (Ines will take care of them). You will find either an 'A' or 'B' next to your name in the cleaning schedule comments.