Standard operating procedures (SOP) for the Tissue Culture Room in the ITDD-HTS Lab

The Tissue Culture Room (TCR) is in room 519B of 717 Delaware at the University of Minnesota. It is housed within the High Throughput Screening Lab (HTS) of the Institute for Therapeutics Discovery and Development (ITDD). The TCR provides a commons space for University of Minnesota (UoM) faculty, staff, students, and postdocs in the drug development field to safely conduct *in vitro* research using several human and animal cell lines in a controlled environment.

This document will serve as a guide for users to perform complex routine operations while following safe practices to uphold the integrity of the research performed in the lab.

This document is intended for UoM faculty, staff, postdocs, graduate, and undergraduate students who have completed all safety and operational training required prior to accessing the TCR.

The TCR is a shared research space that provides the tools to conduct tissue culture experimentation. All TCR users are expected to be competent in aseptic techniques and knowledgeable in equipment operation to work independently.

This document discusses facility operations and can be used as reference for general tissue culture practices, but it does not provide specific methods for cell line experimentation.

Preparation for access

- Prior to beginning any work in the TCR, fill in the *Tissue Culture Room Use Request* and schedule an orientation with the TCR manager.

Mandatory training

Users of the TCR must complete certain prerequisites before obtaining privileges to work in the lab. All users must demonstrate competency in Tissue Culture techniques. Training is available and can be coordinated with the HTS-ITDD staff.

NOTE: If it has been proven that a user is incompetent in aseptic technique or is not abiding by the guidelines of the lab, privileges will be suspended, and retraining will be required.

Safety resources training

The following mandatory training is available online from the web page of the University of Minnesota.

- Biological Safety in the Laboratory
- Biohazardous and Infectious Waste

Additional mandatory requirements include:

- Familiarity with the SOP-TCR document (this document).
- Confirmation of Biosafety Cabinet Training and applicable tissue culture techniques.
- The principal investigator must have an updated IBC protocol with “room 519B, 717 Delaware” and all names of workers listed on it.
- Certificates of online training modules must be sent electronically to the lab manager.

Emergency response plans

Detailed emergency procedures are provided in the **717 Delaware Street SE Building Emergency Plan**

Facility safety, dress code and safety equipment

All the spaces in the HTS are classified as Biohazard Level 2 (BSL2) and work conducted has the potential risk of harboring human pathogens. Doors are locked 24/7 with restricted access.

TCR users are required to provide their own PPE. This includes a designated clean lab coat, eye protection, long pants, and shoes covering the entire foot.

Please refer to the safety data sheets for specific cell lines and practice universal precautions when handling all cell lines.

Dress code and personal belongings

- Approved users must wear—and supply their own—clean designated lab coat, eye protection, and other PPE related to the nature of their work.
- Long pants, socks, and shoes that cover the entire foot must always be worn.
- Wet/dirty footwear, backpacks, coats, purses, water bottles, and food are prohibited.

Safety equipment

To ensure a safe work environment, the Core is equipped with the following:

- Biosafety Cabinets (BSCs)
    - Specialized bins for generated biological and glass waste are located near each BSC workstation.
    - BSCs must be re-certified every 12 months by an external partner.
        - BSC users are expected to accommodate this annual service.
- Eye wash and safety shower (tested regularly)

Safety protocols for power failures and spills

Power failure in biosafety cabinet (BSC)

1) Immediately stop any work. Cap all flasks, tubes, plates, bottles, and any other open container.
    a. Work slowly to avoid generating hazardous aerosols.
2) Turn off the power switch to the BSC.
3) Remove PPE and thoroughly wash hands.
4) If the power outage is widespread, vacate the area and return only when power is restored, or 30 minutes have passed to allow any aerosols to settle.
5) If the power outage is only to the BSC, post a “DO NOT USE” warning sign and vacate the area.
    a. Contact the facility lab manager and wait at least 30 minutes for any aerosols to settle before returning to the BSC to remove items and clean up.
6) If power has been restored, turn on the BSC switch and allow it to run for 30 minutes.

7) After donning lab coat and gloves, proceed with decontaminating equipment and surfaces in the BSC and remove all contaminated waste.

Power failure of centrifuge

1) Turn off the switch located on the right-hand side and unplug the main power cord from the wall outlet.
2) Wait at least several minutes for the rotor to come to a complete stop.
    a. NOTE: Without power, there will be no brakes and the rotor will take longer to stop.
3) Confirm the rotor has stopped by viewing through the small round window in the center of the lid.
4) Without power, the lid must now be opened manually.
    a. Locate and pull out the string underneath the centrifuge to activate the manual opening override.
        i. Pulling the string will open the lid to provide access for removal of centrifuge tubes.
    b. NOTE: The lid cannot be closed or locked again until power is restored.

Biological spills in BSC

1) Contain the spill with absorbent tissues and spray/saturate the tissues with 70% ethanol or 10% bleach, as required.
    a. Allow appropriate contact time. Dispose of the tissues as biowaste.
2) Follow up with a final cleaning using 70% ethanol and tissue wipes.
3) If the spill is under the grate, locate a sturdy object to act as a brace.
    a. Lift the metal work plate and the grate.
    b. Brace up the metal surface with the sturdy object.
        i. NOTE: Be aware that the plate is heavy and could cause injury if not properly manipulated.

Biological spills in centrifuge

1) Turn off the power switch and unplug it from the outlet.
2) Remove tubes, inserts, and buckets. Spray everything with 70% ethanol for appropriate contact time wipe with absorbent tissues and dispose of tissues as biowaste.
    a. NOTE: If the spill is extensive throughout the chamber, the rotor may need to be removed for more extensive cleaning with 70% ethanol and absorbent wipes.

Biological spills in incubator

1) If the spill is restricted to only the shelves, spray 70% ethanol onto a tissue and wipe up the spill.
    a. If using a small tray, clean the tray and leave it by the sink for autoclaving.
2) If the contaminated liquid has reached the bottom water reservoir, inform the lab manager ASAP, and notify other users of the incubator.

a. All users will remove and relocate the cell material and the lab manager will decontaminate and restart the incubator. If users cannot be contacted, the lab manager will remove/relocate their material.

Biological spill on microscope

1) Spray 70% ethanol onto a Kimwipe and wipe up spill.
2) Repeat.
   NOTE: Never spray ethanol directly onto a microscope.

Equipment and materials

Core-provided equipment*:*

- Two Class II certified Biosafety Cabinets (BSC)
  - 70% ethanol spray bottles and Kimwipes are provided at each BSC.
  - Electric pipettors and micropipettes are also available at each BSC.
- Two shared incubators with three shelves each, 5% CO2, and a 37°C humidity-controlled chamber with sterilized deionized water
- Tabletop centrifuge with protective caps for infectious material
  - NOTE: There is only one rotor available with swinging buckets. It is limited to spinning 15ml and 50ml centrifuge tubes.
- Inverted compound microscopes
- Automated cell counter
  - NOTE: Glass hemocytometer slides are also available.
- Vacuum trap system for liquid waste
- Dry bead bath and/or water bath at 37°C
- Upright standard fridge (4°C) to store cell medium
- Freezer (-20°C) to store assay reagents.
- Bins for biohazardous waste and sharps containers for any glass pipettes or vials
- Small biohazard waste bags for pipette tips

Core-provided materials:

- Aspiration pipettes
- Serological pipettes (5, 10 and 25 mL)
- Micropipette tips (10, 200, and 1000 uL)
- Microcentrifuge tubes (0.5 and 1.5 mL)
- Centrifuge tubes (15 and 50 mL)
- Culture flasks (T75 and T175)
- Filter bottles for media preparation (500 mL)
- Reagent Reservoirs
- Gloves (S, M, L)
- PBS
- Bleach
- Trypsin 0.25%

- Fetal Bovine Serum (when available)
- Pen/strep
- Cell counting slides / Trypan Blue for automated cell counter
- Liquid nitrogen storage

The user needs to provide any other material not mentioned in the lists above (this includes, but is not limited to: Cell lines, cell media, assay plates, assay reagents, etc.)

Best practices

Advance preparation

1) Label all your items with your name.
    a. Be sure to label your lab coat, any consumables, media, or equipment you are storing in the facility.
2) Familiarize yourself with the facility's storage.
    a. Due to limited storage space, large volumes of supplies cannot be stored in the facility.
    b. Long term/permanent storage of supplies is prohibited.
    c. Storage of cardboard cases is prohibited.
    d. Contact the lab manager for storage of supplies that will be used for experiments in the coming weeks.

Contamination prevention

Contamination in tissue culture is very costly. Those who do not practice proper aseptic technique or good personal and environmental hygiene risk loss of productivity, loss of entire projects, and suspicious data resulting from the presence of undetected low levels of microbes. Follow the suggestions below to avoid contamination.

<u>Personal hygiene</u>

- Tie back long hair.
    - Long hair can introduce mycoplasma and compromise incubator doors, microscopes, culture flasks or plates, and hands.
- Wrist watches and cell phones are contamination hazards. Please keep your cell phone in your pocket.
    - Use designated lab timers and counters—not your watch or cell phone—for timing or counting cells.
- Use a clean designated lab coat.
    - As well as protecting you, a lab coat protects your work from environmental contamination that may be on your clothes such as outside pollen, spores, and pet dander or hair.
- Clean your hands. Thoroughly wash your hands before and after your work for at least 20 seconds with soap and water. Lather the backs of your hands, between your fingers, and under your nails.

- Put on a clean pair of gloves and spray your gloved hands—including fingers, thumbs and in between—with 70% ethanol.
- Once gloved hands are sprayed, do not touch your face, hair, clothes, furniture, cell phone, or other contamination hazards.

Environmental hygiene

- Spray all items that are loaded into the biosafety cabinet with 70% ethanol.
- Do not store cardboard boxes in the Tissue Culture Room.
- Do not store items on the floor.
  - Everything that is kept off the floor reduces the chances of dust balls floating into the lower incubators.
- Keep the areas around the incubators free of items and clutter.
- Inspect the incubators and biosafety cabinets for contamination and report any issues immediately.
- Clean all spills and residues.
  - Media for tissue culture is nutrient rich. Spilled media on any surface in the facility and media left in vacuum flasks encourages growth of micro-organisms.

Working in the Tissue Culture Room

1) Practice mindfulness while performing all work in the TCR.
   a. Do not rush.
   b. Avoid unnecessary distractions from your colleagues.
2) Always wear PPE – including lab coat, gloves, and eye protection – upon entering the facility.
3) Turn on BSC by pressing the fan and light buttons.
   a. Let the fan run for about 10-15 minutes.
4) Use the vacuum set up to aspirate media and any other liquid waste.
   a. if the vacuum bottle is more than half full, empty it as follows:
      i. Empty the bottle down the sink with a running faucet.
      ii. Add bleach enough to cover the bottom of the bottle.
      iii. Place bottle back in the vacuum setup after spraying with 70% ethanol.
5) Keep the electric pipettors in the BSC charged.
6) Place any media into the water or bead bath.
7) Spray your gloves with 70% ethanol and load the BSC with the necessary supplies.
   a. Spray each item.
   b. Arrange clean supplies on the left and waste on the right.
8) Wipe microscope stage and focus knob with a Kimwipe saturated with 70% ethanol.
   a. NOTE: Never spray ethanol directly on a microscope
9) Spray your gloves again – including between the fingers – before accessing the incubators.
   a. NOTE: Be mindful not to touch anything after this point or your hands will no longer be sterile.
10) Ensure that the temperature and CO_2 on the incubator are reading 37°C and 5%, respectively.
11) View your cells with the microscope and proceed to the BSC.
12) Carry out your cell culture procedures with proper aseptic techniques.

   a. Ensure that you work from left to right.
   b. NOTE: Do not reach back over the work area from right to left or over top of flasks or plates.
13) Label all flasks and plates with your name along with the cell type and date of plating.
14) Return cultures to incubator.

Upon completion of work in the facility

1) Upon completion, remove all contents from the BSC and return them to storage.
2) Handle your waste as follows:
   a. Discard any plastic/solid waste in the biohazardous waste bins by the side of the BSC.
   b. Discard any glass/sharp waste into the sharp container.
   c. Aspirate any liquid waste into the vacuum flask with bleach.
   d. NOTE: Do not place liquids in the biowaste bins.
3) Wipe down the BSC with 70% and turn the fan and light off.
4) Remove PPE and wash your hands.

NOTE: If you no longer need to access the TCR, clean out all your materials including media, consumables, and PPE. Abandoned or unlabeled materials will be discarded.