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Tissue Culture Core Facility
Standard Operating Procedures (SOP)

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Document ownership, title, author, and authorization

College/unit

University of Saskatchewan (USask) Health Sciences

Procedure title

Tissue Culture Core Facility Standard Operating Procedures (SOP)

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Procedure review and revision history

Revisions to this document will be performed as needed or, at minimum, every three years. All revisions should be reviewed and approved by the person having overall authority over the procedure.

Revisions to this procedure are to be documented in *Table 1: Revision History*.

Table 1: Revision History

Document Section	Details of Amendments	Date	Author (Initials)

Purpose

Located in Room B307 of the University of Saskatchewan (USask) Health Sciences Building, the Tissue Culture Core Facility exists to provide a common facility for USask faculty, staff, and students in health science fields to safely and properly conduct in vitro research using various types of animal and human cell lines in a controlled growth environment.

This document has been developed to help approved facility users perform complex routine operations while ensuring that best practices are followed wherever possible in order to maintain safe operations at all times and uphold the integrity of the research conducted by all involved.

Audience

Tissue Culture Core Facility Standard Operating Procedures (SOP) is intended for University of Saskatchewan faculty, staff, post doctorate fellows, graduate and undergraduate students who have completed all safety and operational training required by the Tissue Culture Core Facility.

Scope

The Tissue Culture Core Facility is a shared research and teaching centre that provides the infrastructure to conduct tissue culture maintenance and experimentation. **All facility users are expected to be competent in aseptic technique and knowledgeable in equipment operation in order to work independently.**

This manual discusses facility operations and can be used as a guide in other tissue culture space but *does not* provide methods for specific cell line experimentation.

In this photo from the first University of Saskatchewan tissue culture course in August 1963, Joseph F. Morgan is seated next to the "father of tissue culture"—Sergey Federoff (standing). Photo courtesy of University Archives & Special Collections, A-3952. (Patrick Hayes)

Preparation for access

- Prior to beginning any work in the Tissue Culture Core Facility, please make an appointment with the lab manager to arrange an orientation to the room.

Access restrictions

- Researchers must accommodate restricted access to the Tissue Culture Core Facility in Term 1 and occasionally in Term 2 when it is used for approximately two weeks as a teaching lab for Anatomy and Cell Biology 331.

Mandatory prerequisite training

Users of the Tissue Culture Core Facility must complete mandatory prerequisite training before being granted access to work in the facility.

NOTE: If it has been proven that a facility user is incompetent in aseptic technique, access will be suspended and retraining will be required.

Safety Resources training

The following mandatory training is available online from USask Safety Resources. To access these materials, please visit https://safetyresources.usask.ca/services/training/index.php.

- Biosafety
- Biowaste Training
- COVID-19 Health and Safety
- Laboratory Safety
- Safety Orientation for Employees
- WHMIS 2015

Additional mandatory training requirements are located on the Tissue Culture Core Facility website: http://healthsciences.usask.ca/facility-services/tissue-culture-core-facility.php.

This includes:

- *Tissue Culture Core Facility Standard Operating Procedures*
- Mandatory training videos
- Confirmation (via *Tissue Culture Core Facility Site-specific Training Record*) of aseptic technique training and applicable tissue culture procedures from the supervisor or designate.
- Confirmation (via *Tissue Culture Core Facility Site-specific Training Record*) of facility orientation from the lab manager.

Permits and training documentation

- The principal investigator must have an updated biosafety permit with “Room B307 Health Sciences” and the names of all workers listed on it.
- Permit holders’ Biosafety Permits are located in a binder above the handwashing sink.
- Certificates from USask Safety Resources online training must be sent electronically to the lab manager.
- The *Tissue Culture Core Facility Site-specific Training Record* must be signed by the supervisor and kept in the facility manager’s file.

Emergency response plans

In the event of a building or campus-wide emergency/incident, please refer to the *Health Sciences Emergency Response Plan* located in the binder above the handwashing sink.

This document is also available online by visiting https://safetyresources.usask.ca/fire%20and%20life/emergency-response.php and selecting Health Sciences A, B, C, D Wings from the listed options.

Facility safety, dress code, and safety equipment

The Tissue Culture Core Facility is classed as *Biohazard Level 2* work with the potential risk of human pathogens. Doors are locked 24/7 with restricted access.

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

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

- For more information on safety data sheets for various cell lines, visit https://www.atcc.org/support/faqs/85d48/Safety%20Data%20Sheet%20SDS-263.aspx

Dress code and personal belongings

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

Safety equipment

To help ensure the safety of all involved, the Tissue Culture Core Facility is equipped with the following:

- Biosafety cabinets (BSCs)
    - Specialized receptacles for generated biological and glass waste are located at each BSC workstation.
    - BSCs must be re-certified every 12 months by an external partner.

        - BSC users are expected to accommodate this annual service.
        - BSC log sheets are kept for one year and certification documents are posted on each BSC.
- Designated flammables cabinet for storing ethanol stocks.
- Chemical and biological spills kits for large spills.
- First aid kit.
- Eye wash and safety shower (tested regularly).
- Hands-free hand washing sink.
- Landline phone with emergency contact list.

Safety protocols for power failures and spills

Power failure in biosafety cabinet (BSC)

1) **Immediately cease work and cap all flasks, plates, tubes, bottles and anything opened.**
    a. Work slowly to avoid creating any biohazardous aerosols that could be present.
2) **Turn off the power switch to the BSC and remove PPE**, being sure to thoroughly wash hands.
3) **If the power outage is widespread,** vacate the area and return only when power is restored or after 30 minutes to allow any aerosols to settle.
4) **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.
5) If power has been restored, turn on the BSC switch and allow it to run 30 minutes.
6) **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 socket.
2) **Wait** at least several minutes for the rotor to come to a complete stop.
    a. **NOTE:** Without power, there will be no brake and the rotor will take longer to stop.
3) **Confirm the rotor has stopped** by viewing through the small round window in the centre of the lid.
4) Without power, the lid must now be opened manually.
    a. **Locate and remove the plastic stopper** found underneath the left-hand side of the front of the centrifuge.

b. **Locate and pull out the string** found inside the hole covered by the stopper to activate the manual opening override.
    i. Pulling the string will open the lid to provide access for removal of centrifuge tubes.
c. **NOTE:** The lid cannot be closed and locked again until power is restored.

Biological spills in biosafety cabinet

1) **Contain the spill with absorbent tissues** and spray/saturate the tissues with 70% ethanol.
    a. Allow appropriate contact time and 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 (such as one of the plastic storage boxes from the adjacent roll-out cart) 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 power switch and unplug from outlet**.
2) Remove tubes, inserts, and buckets. Spray everything with 70% ethanol for appropriate contact time (documented in the permit holder's biosafety plan), 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.
        i. For removal of rotor, please refer to the centrifuge manual located in the cupboard above the sink.

Biological spills in incubator

NOTE: It is highly recommended to use the small trays provided when using plates to contain any potential spills.

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.
    a. **NOTE: Never spray ethanol directly onto a microscope.**

Equipment and materials

Facility-provided items

The Tissue Culture Core Facility provides the following equipment:

- Three 6 ft. and two 4 ft. Class II certified biosafety cabinets
    - 70% ethanol spray bottles and Kimwipes are provided at each biosafety cabinet.
    - **NOTE:** Electric pipettors are only available at the 6 ft. biosafety cabinets.
- Six shared incubators with three shelves each, 5% CO_2, and a 37°C humidity-controlled chamber with sterilized deionized water
- Refrigerated 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.
- Olympus inverted compound microscopes
- Glass hemocytometer slides and counters
- Vacuum flasks with pump to aspirate supernatant
    - Beakers for pipetting supernatant off are also available
- Dry bead bath and/or water bath at 37°C
- Upright standard fridge to store media
- Waste receptacles (including plastic bin lined with bag for biological-contaminated consumables waste) and sharps containers for any glass pipettes or vials
- Small biohazard waste bags
- Bleach (6% stock)

User-provided items

Users of the Tissue Culture Core Facility must supply the items listed below.

Consumables such as:

- Flasks or plates
- Tips
- Serological pipettes
- Media

- Trypan blue
- Other liquids or items that have been sterilized by an autoclave if not commercially sterile

Additional items:

- Micropipetters
- PPE including lab coat, gloves, eye protection
- Stationary such as lab books and pens

Methods and best practices

Advance preparations

Before beginning any work in the Tissue Culture Core Facility, review the advance preparations and facility information below.

1) Label all of 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 *Contamination Prevention at the Tissue Culture Core Facility* PDF located on the facility's website.
    a. This document includes essential information covering personal and environmental hygiene, reminders to not use a cell phone while working in the biosafety cabinets, and more.
3) Familiarize yourself with the facility's storage rules:
    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.

Working in the Tissue Culture Core Facility

1) Practice mindfulness while performing all work in the Tissue Culture Core Facility.
    a. Do not rush.
    b. Avoid distractions from your colleagues.
2) Don PPE — including lab coat, gloves, and eye protection — upon entering the facility.
3) Turn on a biosafety cabinet (BSC) by pressing the fan and light buttons.
    a. Let the fan run for about 10 minutes.
    b. Sign-in using the log sheets posted by the control panel located at approximately eye level on the right side of the front panel.

4) If using a vacuum flask setup, select a set stored on the cart by the main entrance.
    a. Place the bucket on the floor by the vacuum pump and connect.
        i. Please refer to the diagram posted on the BSC.
5) Turn on electric pipettors if supplied at the BSC.
6) Place any media into the water or bead bath.
7) Spray your gloves with 70% ethanol and load the BSC with necessary supplies.
    a. Spray each item.
    b. Arrange clean supplies on the left and waste collection 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) Use the provided lens paper to clean the optics, including eyepieces and objective lenses.
10) 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.
11) Ensure that the temperature and CO_2 on the incubator is reading 37°C and 5% CO_2.
12) View your cells at the microscope and proceed to the BSC.
13) Carry out your cell culture procedures *with proper aseptic technique.*
    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.
14) Label all flasks and plates with your name along with the cell type and date of plating.
15) Return cultures to incubator.
16) Indicate which incubator you are using on the attached magnetic board.
    a. **NOTE:** This is mandatory for all users.

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. Tie a small biowaste bag closed and place in biowaste bin.
    b. Discard any glass waste into the sharps containers.
    c. Decontaminate any liquid waste with bleach for a contact time of 20 minutes and then discard in the sink.
    d. **NOTE:** Do not place liquids in the biowaste bins.
3) Record your finishing time on the BSC log sheet.

4) Wipe down the BSC with 70% ethanol and turn the fan and light off.
5) Turn off pipettor and power bar.
6) Empty vacuum flask containing bleach in the sink. Rinse the flask and return to the cart.
7) Remove PPE and wash your hands.

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

References

Health Sciences Lab Safety Manual

http://safetyresources.usask.ca/

APPENDIX A: Common terms and definitions

Adherent	Cells that attach to a substrate such as a flask or plate.
Animal and human cell lines	Primary cells are harvested directly from species tissue; immortal cells have the ability to undergo cell division indefinitely. Cells are in the form of adherent monolayer or suspension.
Aseptic technique	A process of manipulation that eliminates potential contamination while using sterile materials.
Aspirate	To remove the supernatant with suction using a vacuum pump, tubing, and vacuum flasks.
Autoclave	An instrument that sterilizes items with heat, steam, and pressure.
Biohazard	Biological material that is capable of causing illness and infection.
Biohazardous	The ability to cause disease.
Biosafety cabinet (BSC)	A specialized cabinet that protects workers from transmission of infectious material and keeps the material sterile and free of contamination.
Biowaste	Discarded waste material that is contaminated with biological components.
Centrifuge	An instrument using speed and centrifugal force to separate the supernatant and cells to form a cell pellet.
Contamination	A foreign culture of cells that have been inadvertently introduced and are coexisting/competing with the intended culture by using the nutrient rich media.
Counter	A device that allows users to manually count cells in a marked grid pattern on a hemocytometer while looking through a microscope.
Flasks and plates	Specialized vented sterile plastic vessels that allow gas exchange for growing cell cultures.
Hemocytometer	A glass microscope slide with a marked grid and coverslip used to count viable cells in a given volume.
In vitro	Cells grown outside a living being in an artificial environment.
Incubator	Equipment that provides a controlled environment for growing cell cultures through temperature, gas, and humidity settings.
Inverted compound microscope	A microscope with the objective lenses located under the stage and a light source that shines down from the top to view cells in the plate or flask.
Kimwipes	Lint-free wipes.
Media	The commercially prepared liquid that contains the nutrients needed for cells to grow in culture.
Micropipetter	A device to measure, transfer, and dispense a small volume of liquid less than 1ml.

Mindfulness	Concentrating on your work, not rushing through the steps, and being aware of the surfaces you are touching.
Monolayer	A single layer of cells attached to a substrate such as a plate or flask.
Pasteur pipette	Elongated tapering glass pipettes used to transfer small volumes of liquid.
Personal protective equipment (PPE)	Safety controls worn to protect a worker from hazardous products and procedures. *Examples include lab coat, safety glasses, gloves, and respirator mask.*
Pipetter	An instrument that is used with a serological pipette to suck up and release liquid.
Refrigerated tabletop centrifuge	A centrifuge that is housed on a lab bench that has an adjustable temperature-controlled interior for spinning samples.
Rotor	The insert in a centrifuge that holds and spins the samples and is positioned on a rotating shaft.
Safety data sheet	A document from a supplier listing all hazards and safety measures of a product.
Sharps container	A hard-plastic container used to collect discarded sharp objects such as needles, scalpel blades, and glass Pasteur pipettes.
Sterile	A condition that is free of microbiological organisms achieved by heat, pressure and steam, or chemical treatment.
Supernatant	The liquid phase that lies above a layer of cells after centrifugation or on a substrate where cells have attached.
Suspension	The state achieved when cells are suspended or floating in a liquid medium.
Swinging buckets	The inserts in a rotor in a centrifuge that hold the sample tubes in place.
Trypan blue	A staining solution to determine cell viability when counting cells on a hemocytometer. Dead cells will appear dark. Opaque and live cells will be clear.
Universal precautions	To handle all biological material as being capable of transmitting infection.
Vacuum flask	A glass flask with a spout on the side and designed to withstand vacuum pressure when connected with flexible tubing to a vacuum pump.
Vacuum pump	An instrument with a negative pressure to suck the supernatant off the cell layer or pellet *or* to apply to a filtering system when filtering liquids.
Water or bead bath	Equipment that heats tubes or bottles of liquids to working temperature by using a heated basin of water or dry metallic beads.