*ADD THE PROCEDURE TITLE HERE*

## Lab-Specific Information

| **Department:** | Biology |
| --- | --- |
| **Date SOP was written:** | 10/3/2019 |
| **Date SOP was approved by PI/lab supervisor:** | Click here to enter a date. |
| **Principal Investigator:** | Vincent Martin |
| **Internal Lab Safety Coordinator/Lab Manager:** | Smita Amarnath & Nicholas Gold |
| **Lab Phone:** | Click here to enter text. |
| **Office Phone:** | Click here to enter text. |
| **Emergency Contact:** | Click here to enter text. |
| *(Name and Phone Number)* |
| **Location(s) covered by this SOP:**  **Biohazardous Agents covered by this SOP*:*** | *GE S126.00 GENOME FOUNDRY / Mammalian SIDE* |
| *(Building/Room Number)*  *Click here to enter text.* |

# General information

## Location

The procedures involving use of [Mammalian Cells] will be performed in the GE building, in the following rooms:

* Room 1 – *S126.00*

## Accessing location

Locations listed in paragraph 1.1 are under [Dr. Vincent Martin]’s responsibility. Anyone wishing to use the locations must have:

* Successfully completed appropriate training
* Receive authorization from [Smita Amarnath or Nicholas Gold];
* Been added to the biohazard permit;
* Be oriented on the room procedures by [Smita Amarnath or Nicholas Gold] and
* Read the current SOP and understand all requirements.

**Maintenance personnel** (electrician, plumbers, etc.) and visitors must be accompanied when entering and should not be allowed into the room by those inside without authorization.

**Visitors** must wear required PPEs and be accompanied at all time by an authorized user.

**Cleaning personnel** should not enter unless their services are requested for.

## Required training

The following training courses are mandatory to perform operations described in this SOP:

* Biosafety
* WHMIS for laboratory personnel
* Hazardous Waste disposal for laboratory personnel
* *Safe Use of Biological Safety Cabinets for anyone using the BSC*
* *Safe Handling of Blood for anyone handling blood, body fluids or any material potentially containing blood borne pathogens.*
* Being knowledgeable of the emergency procedures described into this SOP.

*Annual test of emergency procedures will be performed according to the Canadian Biosafety Standards.*

More information can be found in the [Biosafety Manual](http://www.concordia.ca/content/dam/concordia/services/safety/docs/BiosafetyManual.pdf) available online[[1]](#footnote-1) and in Public Health Agency of Canada’s *Canadian Biosafety Standards*.[[2]](#footnote-2)

# Biological material involved

*Induced Pluripotent stem cells (iPSCs) and mammalian cell lines like HeLa, HEK293T and Jurkat..*

# Description of procedures

All procedures below are derived from the *Canadian Biosafety Standards* of the Public Health Agency of Canada, and also compliant with requirements of the HPTA and HPTR.

Safety Data Sheet specifically for mammalian cells are available/posted in the room. Please read it.

## Entering the room

No one may enter the room without the authorization of Dr. Vincent Martin.

**Maintenance personnel** (electrician, plumbers, etc.) and visitors must be accompanied when entering and should not be allowed into the room by those inside without authorization.

**Visitors** must wear required PPEs and be accompanied at all time.

**Cleaning personnel** should not enter unless their services are requested for.

## Personal protective equipment:

1. Gloves, lab coat and safety glasses are mandatory and must be worn at all times.
2. Gloves are to be changed routinely and hand-washing is required upon completing any work.
3. Gloves must be inspected routinely and replaced whenever they are soiled, torn, punctured, or contaminated.
4. All gloves that may have come into contact with biohazards are to be discarded in biohazard bags (not regular lab waste).
5. Gloves must never come in contact with surfaces outside the laboratory e.g. door handles, elevator buttons, telephones, personal items.
6. Wearing gloves outside the lab should be minimized, except to move hazardous materials between laboratories. The same gloves should not be worn in the lab and out; if gloves are needed for transport, they should be carried to the storage site and then placed on.
7. Lab coats should not be worn outside the lab. If a coat is required for transport, this should be a separate coat from the one used to handle material while working inside the lab.
8. The doors to the tissue culture rooms are to be kept shut at all times. When in the tissue culture room, you should wear the disposable lab coat kept in your designated storage space. This coat MUST NOT be used for general lab work; conversely, your general lab coats cannot be used in the tissue culture room.

# Working in the Hood

1. As stated above, **everything** that goes into the hood must be wiped with 70% EtOH before putting it onto the bench in the hood. This means your hands, new boxes of tips, new beakers of tubes, the exterior of bottle-top filter units, etc. **Everything**.
2. Keep the air intake (the vent in the bottom of sash, underneath your elbows) clear of **all** items at all times. If this vent is blocked, it allows room air to enter the hood, which is great source of contamination! Also, keep items from directly blocking the vent in the back of the hood.
3. Bring only the items you need for a particular procedure into the hood to prevent cluttering your working space. Having a clear working space will significantly reduce the chance of contamination! Ensure easy access to items in the hood and maintain plenty of clear space in the center of the hood to work in.
4. This is a vertical laminar flow hood, which means that air flows straight down from the top of the hood. Do not work directly over any open vessels, or contaminants from your hands could be blown into your vessel. Always work at an angle, off to one side.
5. Watch what you are pipetting! The replaceable filters in the air pipettors are expensive. Ensure that you don’t suck fluid up inside them. Especially watch the 1 mL pipettes...they fill really fast!
6. If you spill anything in the hood, clean up immediately to prevent cross-contamination and damage to working surface. Stop what you are doing and wipe up the spill – salts in particular can corrode the metal if left. Wipe the area with 70% EtOH before returning to work.
7. Styrofoam is not allowed in the hood – it often flakes and is difficult to keep out of the vents. Use plastic racks instead (if you need more racks, be sure to clean with EtOH before putting into the hood).

Incubators and Microscope

These are shared equipment, so they present a great method to spread contamination!

1. Before using the microscope, spray a Kimwipe with 70% EtOH and wipe down the stage. Do this also when you are done to prevent media spills from spreading between plates.
2. If you need counter space to set plates down on by the microscope, clean area with 70% EtOH.
3. The incubators are not technically sterile- however, every effort must be made to maintain their cleanliness to prevent contamination from spreading. If media has spilled in the incubator, clean the spill with EtOH. Spills into the water bath **MUST** be immediately taken care of- talk to either the lab manager or the tissue culture czars.
4. Put plates carefully into incubator- be sure not to bump other people’s plates, minimize stacking as much as possible, and keep your own plates set up to allow you easy access to what you need next.

## Material storage

Cell storage in liquid nitrogen allows for long term storage and use of stock cell cultures.

Cells are stored in Freezing mix (FBS: DMSO 90:10 ratio). They are aliquoted into labelled (cell name, passage number and date) cryovials. Put at -80 C o/n. Put into liquid nitrogen the following day.

## Procedure 1

1. *Cell thaw and recovery b) Cell passage and maintainence*

**Purpose**

To prepare frozen cell culture stock for long term storage and to remove cells from frozen stock.

**Scope**

This procedure is used to prepare cell stock to be stored in liquid nitrogen and for the recovery of cells from cryogenic storage.

**Description**

Storing cells in liquid nitrogen allows for long term storage and use of stock cell cultures.

**Procedure for Freezing Cells:**

1. Obtain desired cells and count to determine the number of cells you have to freeze.
2. Spin cells down at 1000 rpm for 5 minutes, and resuspend in Freexing mix (FBS: DMSO 90:10 ratio)
3. Aliquot amount calculated into 2 mL Corning cryovials, labeled with cell name, passage number, and date on each cryovial.
4. Place vials in Cryo Freezing container and store at –80°C for 24 hours.
5. 24 hours later, remove cells from freezer and store in liquid nitrogen tank. Update cryotank binder with where they are located.

**Procedure for Thawing Cells:**

1. Obtain cells from liquid nitrogen freezer.
2. Agitate tube in 37°C water bath for approximately 2 min, or until cells are thawed.
3. Wipe top of tube with alcohol, unscrew, and pipet contents into 15ml falcon tube containing a few mL of proper cell media to 15 mL tube
4. Spin cells down, and resuspend in cell media. Plate cells at desired density in a plate or flask

**Passaging cells:**

**Before splitting:** (Note: volumes may vary depending on culture equipment used--adjust as necessary)

 Take out trypsin, DMEM and other medium components, place in water bath and warm to 37° C

 Label new culture equipment with cell line, passage #, medium type and date

 Add 15 mL of medium to each fresh flask/plate.

**Splitting**:

 Aspirate medium from the cells to be split

 Rinse flask with 5-10 mL DMEM, aspirate it out.

 Add 3 mL of warm 0.25%Trypsin/EDTA and place flasks/plates in incubator for

approximately 5-10mins.

⮚*This step is to detach the cells from the bottom, if not completely detached, whack the sides of flask firmly with the palm of your hand.*

Check that all cells have been detached by viewing them under a microscope.

 When complete, add 10mL of DMEM with 2%FBS mixture to the flask. This will stop the

trypsin from further digestion.

 At this point the cells should be moving and rolling off of the dish, pipette the media up and

down, thus ensuring complete detachment of all cells.

 After cells are detached, pipette entire contents of flask into a 15 mL centrifuge tube.

 Place tube in centrifuge and spin at 1000xG for 5mins. A pellet should develop, discard

supernatant and resuspend in approx 4mL of DMEM (This amount will vary depending on the

volume of the cell pellet). Split this mixture equally amongst the new flasks.

 Plate cells according to the split ratios.

## Procedure 2

*Transfection*

Plan to do at least two electroporations per construct. The condition of the cells is critical. You don't want them to grow too dense. First thing in the morning, "feed" the cells with antibiotic free medium and let it go for 1-3 hours.

 Harvest plate of cells by washing cells with PBS,

 Add 1ml trypsin put at 37 C, for 5 min, disperse add media

 Collect suspension , pellet cells at setting 4 for 5 min.

 Wash with Ca/Mg -free PBS and spin down a second time

 Resuspend in 0.9 ml/ 6 cm plate room temperature Nucleofector solution

 Count cells and adjust to 7 X 10E6 cells/ml

Note Avoid leaving the cells in Nucleofector® Solution for extended periods of time (longer than 15 minutes), as this may reduce cell viability and gene transfer efficiency.

100 μl of each aliquot into certified cuvettes according to the experimental setup. Sample must cover the bottom of the cuvette without air bubbles). Close the cuvette with the cap.

Select appropriate Nucleofector®Programs according to the experimental setup.

Insert the cuvette with cell/DNA suspension into the Nucleofector® Cuvette Holder and apply the

selected program by pressing the X-button.

Take the cuvette out of the holder once the program is finished.

Immediately add ~500 μl of the pre-equilibrated culture medium to the cuvette and gently transfer the sample into the prepared 6-well plates (for adherent cells; final volume 1.5 ml media per well). Use the supplied pipettes and avoid repeated aspiration of the sample

 Start selection the following day, change media every day, should see cell death beginning at day 2 of selection. Usually takes 7-9 days to clear.

**Mycoplasma detection and testing:**

All new cultures coming into the foundry must be tested and cleared for mycoplasma.

**Material:**

ABMgood Mycoplasma detection kit.

**Culturing Cells for Mycoplasma Detection**

1. Thaw cells according to lab protocols (section 4.2).

2. When confluent (or about 90%), seed new plate to at least 50% density.

3. Allow cells to grow to confluency.

4. After reaching 100% confluency, let cells sit in unchanged media for at

least 3-5 days.

5. Remove 100ul – 1mL of media from flask and place in clean micro

centrifuge tube or cryo vial.

6.Place sample in box labeled “Mycoplasma Test Box”on top shelf of -20°C freezer.

**Mycoplasma PCR Test**

1. Make master mix (as per manufacturer’s instructions)

Total Volume (per sample) 45uL

2. Spin media at high speed for 10 min to pellet any cells.

3. In a PCR plate, add 45 ul of master mix to each well.

4. Add 5 ul of media to each well.

5. Make a positive control by mixing 5 ul of positive control template with 45ul of

master mix.

6. Mix well.

**Thermal cycler program**

1. 94◦ 2:00 minutes

2. 94◦ 0:30

3. 55◦ 2:00

4. 72◦ 1:00

Repeat steps 2-4 for 34 cycles, then hold at 4°C.

# Hazards

The following materials and/or equipment associated with these procedures may present exposure hazards, and/or physical hazards.

The human cells are considered to pose **blood borne pathogen** risks:

|  |  |  |  |
| --- | --- | --- | --- |
|  |  | **Tissue** | **Specifics** |
| HeLa | Human cell line | Cervix | Cells contain HPV according to ATCC  [www.atcc.org/products/all/CCL-2.aspx](file:///C:\Users\audremor\AppData\Local\Microsoft\Windows\Temporary%20Internet%20Files\Content.Outlook\8MUMGSL7\www.atcc.org\products\all\CCL-2.aspx) |
| HEK-293 | Human cell line | Embryonic kidney | Cells contain adenovirus according to ATCC  [www.atcc.org/Products/All/CRL-1573.aspx](http://www.atcc.org/Products/All/CRL-1573.aspx) |
|  |  |  |  |

## Illnesses / Allergies.

Anyone must immediately notify [Dr. Vincent Martin] of any illness caused by, or that may have been caused by, the infectious material or toxin(s) being handled or stored in one of the locations mentioned in chapter 1.1.

# General rules:

1. All persons handling biohazards must read the Concordia Biosafety Manual available at: [concordia.ca/content/dam/concordia/services/safety/docs/BiosafetyManual.pdf](http://www.concordia.ca/content/dam/concordia/services/safety/docs/BiosafetyManual.pdf)
2. Authorization from Dr. Vincent Martin is required before working with biohazardous materials.
3. Stock solutions and aliquots should be stored:

10% FBS, stock solutions bought or made up as 100X

10mM MEM Non Essential A.A., store at 4C

    100x nucleosides, store at –20C

 200mM L-Glutamine, store at -20C

 5mg/ml Pen and Strep, store at -20C

10mM B- Mercaptoethanol, store at 4C

 DMEM, High Glucose without Sodium pyruvate (store at 4C).

1. Wear gloves – but note that these are to protect you, and are not inherently clean!

Keep the work surface clean (clean with 70% ethanol and keep the work area clear enough to allow work without reaching over an item and preventing inadvertent brushing of a sterile tip against another object.)

1. Use sterile reagents and media and work to keep them that way (do not reuse tips, clean the outside of reagent bottles with 70% ethanol, autoclave or sterile filter as appropriate)
2. Keep bottles, flasks and tubes covered as much as possible.
3. Remove liquid from containers at an angle.
4. Clean with 70% ethanol often (surfaces and gloves).
5. Remember that it is the inside of containers (autoclaved bottles, centrifuge tubes, etc) that is sterile treat the outside as dirty and clean with EtOH
6. Although most cell lines are reasonably safe to work with, be aware that you are working with or around potentially dangerous materials (you should have already taken Blood Borne Pathogen training since we use human lines in our facilities). To protect everyone’s safety, follow these guidelines:
7. Label **EVERYTHING**. Minimal labeling includes your initials, the date, and the cell line nomenclature. For cell passages, it is usually best to include passage # and split ratio for your own information.
8. For long term experiments update the plate with the last date you changed media, etc. In general, cells that haven’t been observed in several days should be disposed of to minimize contamination risks.
9. If you have a lot of tubes, etc that are not for long term use (falcon tubes etc), you can avoid labeling them all, but ONLY IF you dispose of properly immediately after you are done. At no time can you leave unlabeled materials in the lab when you are not in the lab.
10. Anything that has touched cells should be bleached and placed in autoclave waste, not general waste. Media/serum that has not been used on cells can be disposed of in the sink.
11. Minimizing aerosol generation is of paramount importance. Any sample manipulation that might generate an aerosol, such as preparation of aliquots from stock solution, should be carried out in the Biosafety Level 2 cabinet (Class II/type A2) in [GE-S126.00].
12. If reusable laboratory equipment comes into contact with [mammalian cells] must be disinfected by being wiped down or rinsed with a 10% bleach solution or 70% ethanol prior to being returned to general storage.
13. Wherever possible, plastic ware should be used as a substitute for glassware.
14. Disposable pipettes should be used.
15. Gloves, lab coat and safety glasses are mandatory while working with [mammalian cells], as is suitable footwear with closed toes and heels.
16. Long hair should be tied back or restrained to prevent contact with specimens, containers, equipment or hands.
17. Any open wound should be covered with a waterproof dressing.
18. Absolutely no food or drink is allowed in the laboratory.
19. Pipetting by mouth is strictly forbidden.
20. The use of needles and other sharps that may come into contact with [mammalian cells] should be restricted.
21. Sharps that may have come into contact with [mammalian cells] should be disposed of in biohazard leak- and puncture-resistant containers provided by EH&S.
22. The storage of materials that cannot be easily decontaminated should be kept to a minimum.
23. The bench area and the interior of the biosafety cabinet where [mammalian cells] samples have been handled must be washed before and after use with soap and water, followed by sterilization using 70% ethanol solution.
24. Lab users MUST wash hands before exiting the lab.

# Additional special handling procedures:

1. Transport of agent between labs and/or buildings requires safe-handling procedures to reduce the risk of spills or leaks (secondary containment).
2. Transport must be done using a cart that has raised rails or edges.
3. A secondary container with the ability to hold the volume of the material in the event of a leak or a spill will be used. Since very small volumes (in microliter range) would typically be transported, the secondary container would be a Pyrex dish that can be autoclaved if needed.
4. Never use passenger elevators for the transport of biological and chemical materials. Always use the freight elevators for moving chemicals and biological materials.
5. Unattended Operations:
6. Electrical instruments will be turned off.
7. All vials and containers will be sealed.
8. E**verything** that goes into the hood must be wiped with 70% EtOH before putting it onto the bench in the hood. This means your hands, new boxes of tips, new beakers of tubes, the exterior of bottle-top filter units, etc.
9. Keep the air intake (the vent in the bottom of sash, underneath your elbows) clear of **all** items at all times. If this vent is blocked, it allows room air to enter the hood, which is great source of contamination! Also, keep items from directly blocking the vent in the back of the hood.
10. Bring only the items you need for a particular procedure into the hood to prevent cluttering your working space. Having a clear working space will significantly reduce the chance of contamination! Ensure easy access to items in the hood and maintain plenty of clear space in the center of the hood to work in.
11. If you spill anything in the hood, clean up immediately to prevent cross-contamination and damage to working surface. Stop what you are doing and wipe up the spill – salts in particular can corrode the metal if left. Wipe the area with 70% EtOH before returning to work.
12. Styrofoam is not allowed in the hood – it often flakes and is difficult to keep out of the vents. Use plastic racks instead (if you need more racks, be sure to clean with EtOH before putting into the hood).

# Hazardous Waste

## Solid biological waste

### Anatomical waste

Animal carcasses animal tissues and any material contaminated with animal tissues must be placed in bags that are then stored in the designated biohazardous freezer located in CSBN. All the data concerning that waste should also be recorded at the same time in the logbook located near the freezer. Approval to access and use the freezer/room must be obtained from the AF manger.

### Non-Anatomical waste

1. Solid wastes (e.g. agar plates, serological pipettes, pipette tips, tubes, cell culture flasks, paper and gloves used while handling biohazardous material, etc.) must be put in the 20L biohazards grey bins
2. Sharps (for scalpels, needles, microscope slides) must be put in yellow sharp container. When full, these yellow containers go in the 20L biohazards grey bins.
3. Contact EHS for waste containers pick-up at [hazardouswaste@concordia.ca](mailto:hazardouswaste@concordia.ca). The closed container(s) are left inside the room, by the door, and will be picked up by EHS personnel.

Complete guidelines are available here: [concordia.ca/campus-life/safety/Waste-Disposal/bio-waste.html](http://www.concordia.ca/campus-life/safety/Waste-Disposal/bio-waste.html)

## Liquid biological waste

Liquid biological waste must be treated with a 10% dilution of household bleach (final concentration) for 30 minutes, then dispose with plenty of water in the sink. During the treatment with bleach for 30 minutes the container must be closed or kept in the fumehood.

NEVER AUTOCLAVE MATERIALS DECONTAMINATED WITH BLEACH OR SODIUM HYPOCHLORITE:

HIGHLY TOXIC FUMES WOULD BE GENERATED IN THE ROOM.

## Contaminated glassware

Glassware must be decontaminated by washing with Sodium Hypochlorite (1%) or with a 10% dilution of household bleach (final concentration), rinsed and then placed in dirty glassware bucket.

## Chemical waste

Chemical waste must be discarded in the appropriate containers (liquid or solid waste).

The chemical waste procedure ([concordia.ca/campus-life/safety/Waste-Disposal/chemical-waste.html](http://www.concordia.ca/campus-life/safety/Waste-Disposal/chemical-waste.html)) must be followed.

# Engineering Controls:

## Vacuum system

Vacuum systems are used to facilitate liquid capture from various containers (culture medium, supernatants, etc.). The use of vacuum to aspirate infectious liquids can result in generation of infectious materials or toxins and subsequent contamination of vacuum lines, pumps and centralized vacuum systems. These systems are protected by liquid disinfectant traps and an in-line HEPA or 0.2 µm filter placed between the secondary flask and the vacuum source. More details on the [EHS-DOC-097](http://www.concordia.ca/content/dam/concordia/services/safety/docs/EHS-DOC-097-VacuumPumpSystemDesign.pdf) available online. [[3]](#footnote-3)

## Biological Safety Cabinet (BSC)

Biological safety cabinets (BSCs) provide effective primary containment for work with pathogens and toxins when properly maintained and used in combination with good microbiological laboratory practices (GMLP). BSCs reduce the risk of airborne exposure by preventing the escape of aerosolized biohazardous agents into the laboratory environment. They should be used for procedures that have the potential to produce infectious aerosols and for work involving high concentrations or large volumes of infectious material.

[Safe use of the BSC procedure](http://www.concordia.ca/content/dam/concordia/services/safety/docs/EHS-DOC-036_ProcedureforSafeUseofaBiologicalSafetyCabinet.pdf)[[4]](#footnote-4) is posted on the sash of the BSC and is available online.

Before working in the BSC, please verify: the correct position of the sash, that air grilles are free from obstructions, inward airflow using a tissue, and disinfect all interior surfaces of the BSC using 70% ethanol.

Any equipment you bring into the BSC should be decontaminated.

While working in the BSC do not leave anything on or against the grates at the front or back of the BSC; if the laminar flow is blocked, the interior of the BSC will become non-sterile and air may also flow out. You should also: avoid excessive movement of hands and arms through the front access opening, keep contaminated materials to the rear of the cabinet, and ensure to always discard materials in containers inside the BSC.

When you have finished working in the BSC please ensure that:

1. all containers are closed, covered, and disinfected before removing them from the BSC;
2. area has been cleaned and disinfected with  *70% ethanol*
3. only essential equipment has been left inside it

Lab staff must have read the [Procedure for Safe Use of a Biological Safety Cabinet](http://www.concordia.ca/content/dam/concordia/services/safety/docs/EHS-DOC-036_Procedure%20for%20Safe%20Use%20of%20a%20Biological%20Safety%20Cabinet.pdf)[[5]](#footnote-5) and must have received the Safe use of BSC training offered by EHS. Additional orientation by the supervisor prior to use the equipment is required.

## Centrifuges with sealed centrifuge rotor

Sealed centrifuge rotor will prevent dispersion of aerosols containing biological material in the centrifuge and thus avoid potential exposure. After the end of the run, let the rotor closed for 30’’ and then proceed with the opening.

# Decontamination/clean-up procedures

1. Bench-top work surfaces will be cleaned daily using either a 10% solution of bleach or 70% ethanol. The work surface will be sprayed with the disinfectant solution and then wiped up with a disposable paper towel. Paper towels will be discarded in a biohazard bag stored in a secondary bucket under the bench.
2. Biological safety cabinet will be cleaned by water and soap, and further with a 70% ethanol solution.

# In Case of Injury or Exposure

1. Contact Security at x3717 (or dial 514 848 3717); note that **red telephones** allow reaching Security directly.
2. Report the accident/near miss to [Peter Ulycznyj] (514-848-2424, x [3422]).
3. Report the accident/near miss to EH&S (ext. 4877 or [ehs@concordia.ca](mailto:ehs@concordia.ca))

For any injury or exposure, an Injury/Near miss report (EHS-FORM-042) must be submitted to [Peter Ulycznyj] within 24h at maximum. Reports (French or English) are available as fillable pdf online at: [concordia.ca/campus-life/safety/injury.html](http://www.concordia.ca/campus-life/safety/injury.html).

# Spill response procedure

|  |  |
| --- | --- |
| **When sample volumes are less than 1 ml:** | **When volumes are larger than 1 ml:** |
| * wipe-up with disposable wipes (Kim wipes) moistened with 10% bleach solution and dispose of in biohazard bag kept inside the hood. * soak contaminated area with a 10% bleach solution, allow 20 minutes for disinfection and wipe-up puddle and dispose of in biohazard bag. * Notify Smita Amarnath or Nicholas Gold | * use dry paper towels to cover the spill. * soak towels (once they have adsorbed the spill) with 10% bleach and place in biohazard bag. * soak contaminated area with a 10% bleach solution, allow 20 minutes for disinfection and wipe-up puddle and dispose of in biohazard bag. * Notify Smita Amarnath or Nicholas Gold |

**For large spills contact Security at x3717 or 514-848-3717 and notify the supervisor.**

# Waste Disposal general procedures

* All lab members must have taken the Hazardous Waste Disposal training offered by EHS.[[6]](#footnote-6)
* All lab members must consult the [EHS Waste Disposal webage](http://www.concordia.ca/campus-life/safety/Waste-Disposal.html)[[7]](#footnote-7) and related documents.
* Spent samples and containers must not be disposed of in the regular waste.
* All pipette tips, gloves, and other disposable items must be placed into biohazard bags and autoclaved prior to disposal in regular lab waste..
* All bleach solutions may be disposed of in the regular sinks, followed by flushing with tap water.
* All animal carcases and tissues will be stored in a labelled freezer in SPS-231.05 and disposed of by incineration through EH&S.

**Standard Operating Procedure**

**ADD THE PROCEDURE TITLE HERE**

***I have read and understand the above SOP.***

***I agree to contact my Supervisor if I have any questions or if I plan to make modifications to this procedure.***

***Repeated failures to follow the guidelines can result in loss of access and privileges***.

|  |  |  |
| --- | --- | --- |
| Name | Signature | Date |
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FBS first, as the DMSO is harmful to the cells. A typical cell concentration is between 4x106-

6x106 cells/ml.

FBS first, as the DMSO is harmful to the cells. A typical cell concentration is between 4x106-

6x106 cells/5.3  Aliquot amount calculated into 2 mL Corning cryovials, labeled with cell nam

1. [concordia.ca/campus-life/safety/lab-safety/bio-safety.html](http://www.concordia.ca/campus-life/safety/lab-safety/bio-safety.html) [↑](#footnote-ref-1)
2. [canadianbiosafetystandards.collaboration.gc.ca](http://canadianbiosafetystandards.collaboration.gc.ca/cbs-ncb/index-eng.php) [↑](#footnote-ref-2)
3. [concordia.ca/content/dam/concordia/services/safety/docs/EHS-DOC-097-VacuumPumpSystemDesign.pdf](http://www.concordia.ca/content/dam/concordia/services/safety/docs/EHS-DOC-097-VacuumPumpSystemDesign.pdf) [↑](#footnote-ref-3)
4. [concordia.ca/content/dam/concordia/services/safety/docs/EHS-DOC-036\_ProcedureforSafeUseofaBiologicalSafetyCabinet.pdf](http://www.concordia.ca/content/dam/concordia/services/safety/docs/EHS-DOC-036_ProcedureforSafeUseofaBiologicalSafetyCabinet.pdf) [↑](#footnote-ref-4)
5. [concordia.ca/content/dam/concordia/services/safety/docs/EHS-DOC-036\_ProcedureforSafeUseofaBiologicalSafetyCabinet.pdf](http://www.concordia.ca/content/dam/concordia/services/safety/docs/EHS-DOC-036_ProcedureforSafeUseofaBiologicalSafetyCabinet.pdf) [↑](#footnote-ref-5)
6. Register at [www.concordia.ca/campus-life/safety/training.html#calendar](http://www.concordia.ca/campus-life/safety/training.html#calendar) [↑](#footnote-ref-6)
7. [www.concordia.ca/campus-life/safety/Waste-Disposal.html](http://www.concordia.ca/campus-life/safety/Waste-Disposal.html) [↑](#footnote-ref-7)