Safety Operating Procedure (SOP)

Name of IBC Project

 Biosafety Level 2 Laboratory

**Name of PI(s)**

Building Location (Name) & Room #

Department Name

Rochester Institute of Technology

**All Lab users must read and understand this SOP before conducting work in the lab.**

Version # & Date

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# Chapter 1 Introduction to the Laboratory

**Biosafety level**

* This laboratory is a Biosafety Level 2 (BSL-2) laboratory. This means that only work that falls under Biosafety Levels 1 or 2 can be performed in this facility.
* Accordingly, the main doors to this laboratory are labeled with the universal symbol for biohazards
* Biosafety Levels are a means to determine the level of danger that the work done in a facility poses to its users, other users present, as well as the environment.
* Biosafety Level 2, as defined by the WHO Laboratory Biosafety Manual (Third Edition) and the CDC Biosafety in Microbiological and Biomedical Laboratories (6th Edition), describes any facility wherein work involving well-characterized materials that are pose moderate hazards to laboratory users or the environment, are carried out.
* As such, this lab is augmented with equipment for containment of aerosols generated during the course of experimental work (biosafety hoods). Furthermore, the department has access to equipment for the proper treatment of biohazardous waste prior to its disposal (see Chapter 5).

# Housekeeping

* Work areas should be kept clean and free from obstructions.
* Drinking and eating is not allowed in the laboratory.
* Walkways and passages should be kept clear of any equipment or boxes, as this may pose a fire hazard or cause accidents
* Never use any chemical found in an unlabeled container. Unlabeled containers and chemical wastes should be disposed of promptly by appropriate procedures.
* Spilt chemicals should be cleaned up immediately and disposed of properly. For further information see Chapter 6. All spillage must be reported to NAME OF PI(s).
* Cleaning up should follow the completion of any operation or take place at the end of each day.

# Safety Training

* Before the second course day (= first day in the lab), appropriate safety training has to be taken before working in the laboratory. All trainings are conducted online under **https://www.rit.edu/fa/grms/ehs/**
	+ Biosafety Awareness Training
	+ Bloodborne Pathogen Training
	+ Lab Safety Training
	+ Gas Cylinder Training
* The confirmation has to be handed to the Department Lab Manager’s Name, who will keep the records for the entire Department Name. In addition, upload your confirmations to mycourses. In addition, the document related to Hepatitis B vaccination needs to be filled out if working with human tissue, blood, cells, cell lines, feces, urine, unfixed tissues or organs.

**Responsible Person and Emergency Contact**

* This facility is under the purview of Name of PIs. In case of emergency, she can be contacted at RIT phone number, or at PI(s) email address.
* The RIT Public Safety can be reached at **585 475 3333 OR Text: (585) 205-8333.**
* RIT’s Workers’ Compensation Workplace Injury/Illness Process needs to be followed for employee injuries/illnesses that occur related to this project.

**HR Links:**

* 1. [Workers' Compensation | Human Resources (rit.edu)](https://www.rit.edu/fa/humanresources/benefits/supplemental/workerscomp.html)
	2. Process Flowchart: <https://www.rit.edu/fa/humanresources/sites/rit.edu.fa.humanresources/files/docs/workerscompbulleted.pdf>
* For student injuries/illnesses, complete the **Student Lab/Student Accident Report** form
	1. [Student Programs | Environmental, Health & Safety | RIT](https://www.rit.edu/fa/grms/ehs/content/student-labshop-accidents-injuries-or-exposures-program)

# Chapter 2 General Laboratory Safety

# Note: Specific information on chemical safety and biological safety are provided in Chapter 3 and Chapter 4. Disposal of waste is described in Chapter 5.

# Personal Protection and Hygiene

* All users should wear proper personal protective equipment including a laboratory coat, gloves and goggles. Lab coats must be worn at all times, whereas gloves and googles should be worn when appropriate. These items would minimize contact of samples with the skin and the eyes.
* During the COVID-19 pandemic, make sure to adhere to the special COVID PPE requirements.
* Be sure to wash your hands thoroughly before leaving the laboratory and to disinfect them (hand sanitizer at door). Always assume that the hands have come into contact with samples, even when handling them with gloves.
* Clothing should extend to the ankles to protect the lower limbs. This is to ensure that any sample or liquid spill will not come into direct contact with the skin of the lower limbs. Similarly, no open-toed footwear such as sandals should be used in this laboratory. Leather or polyester shoes are most appropriate.
* Never touch door handles or telephone receivers with gloved hands! These are surfaces that are commonly handled without gloves and come into contact with the unprotected face. As such, contamination of these surfaces pose a danger to unwary users. Gloves must always be assumed to be contaminated with biological material. Never consume any food or drink inside the facility. It should also be noted that the application of cosmetic products in the facility should be avoided.

# Experimental Techniques

Be familiar with the potential hazards of the techniques being used, and the appropriate response. At the end of the experiment, conduct a check to make sure all equipment is cleaned and put away and gas supply, vacuum, electrical apparatus etc. turned off. Hot plates and water baths, which are still hot, should be clearly indicated as such.

# Spills

If the spill is minor and presents no danger, clean up spill immediately. If the spill is large or potentially hazardous, evacuate all personnel from the area and inform Name of PIs. A spill kit for spill removal is available in the lab. For detailed instructions, see Chapter 6.

# Working Alone or After Office Hours

* Where the work involves potential hazard, personnel should not work alone or after office hours in the laboratory. Read the instructions on the **RIT Buddy System**:

<https://www.rit.edu/fa/grms/ehs/content/buddy-system>

* It is prudent to avoid working in a laboratory alone. If this must be done, arrangements should be made as required by the buddy system. Experiments known to be hazardous should not be undertaken by a worker who is alone in a laboratory.
* Before working alone in the laboratory, consult Name of PI(s) who has the responsibility for determining whether the work requires special safety precautions, such as having two persons in the same room or in close proximity during a particular operation.

# Electrical Equipment

* Examine all electrical cords periodically for signs of wear and damage. If damaged electrical cords are discovered, unplug the equipment and send it off for repair.
* Properly ground all electrical equipment. If sparks are noticed while plugging or unplugging equipment or if the cord feels hot, do not use the equipment until it can be serviced by an electrician.
* Do not plug too many items into a single outlet. Cords that enable you to plug more than one item in at a time should not be used. Multi-plug strips can be used if they are protected with a circuit breaker and if they are not overloaded.
* Use only carbon dioxide, halon or dry chemical fire extinguishers for electrical fires.

# Chapter 3 Chemical Safety

# Material Safety Data Sheets/Safety Data Sheets

Material Safety Data Sheets (MSDS)/ Safety Data Sheets (SDS) always accompany all hazardous chemicals that are shipped. These data sheets contain detailed safety information. (M)SDS will be filed in a binder located in the teaching lab. Workers should consult (M)SDS concerning the relevant chemicals before commencing their experiments.

# General Rules

* Do not pipette chemicals or start a siphon by mouth; a pipette bulb or an aspirator should be used.
* Never taste and avoid smelling any reagent or product.
* Wear appropriate personal protection at any time.

# Labeling and Storage

* Treat all chemicals as potentially hazardous.
* If the label on the container does not give safety information, refer to the (M)SDSs for detailed information.
* All chemicals and reagents must be clearly labeled. Post warning signs if chemicals are flammable, highly toxic, and carcinogenic or other special problems exist.
* The flammable liquids must be stored in the fire-resistant cupboard.
* Chemical which are light-sensitive should be stored in dark bottles or on bottles wrapped with aluminum foil.

# Carcinogenic and Toxic Materials

* The use of carcinogenic and toxic materials should be avoided if possible.
* If they are required, extra precautions are necessary in handling the materials:
	+ Storage in locked cabinets, according to temperature sensitivity
	+ Operations should be performed in fume hoods
	+ Gloves should be worn and immediately after use, hands should be washed with cold water
	+ It is common procedure to wear two layers of gloves for extra protection as many carcinogens can permeate glove material. Replace the outer pair as soon as any contamination is observed.
	+ Before handling chemicals that are harmful when inhaled, contact RIT’s Environmental Health & Safety for a Hazard Assessment and to determine suitable measures (e.g. respirator).

# Transport of Chemicals

When chemicals are carried by hand, they should be placed in a box or acid-carrying bucket to prevent breakage and spillage.

# Fume Hood

* Perform experiments that involve the use of chemicals that release gases, vapors or aerosols in fume hood.
* Weighing of powdered chemicals which are harmful when inhaled, e.g. acrylamide should be carried out in a fume hood.
* Fume hoods should be inspected periodically by authorized personnel for proper and optimum working conditions. Inspected hoods will have a posted RIT Laboratory Hood Air Flow & Preventative Maintenance Form showing an average flow of 80-120 fpm.

# Liquid Nitrogen

* Cryogenic fluids are usually stored in special insulated containers. When transferring liquid nitrogen from one container to another, the operator must be adequately shielded, with a face mask or goggles, heavy- duty gloves and protective clothing.
* Store and use in well-ventilated areas. Keep away from sparks and flames, which may cause explosions. Cryogenics present the hazard of asphyxiation due to displacement of oxygen, or embitterment of metals due to extreme cold.
* When liquid nitrogen is spilt on the skin, it will roll off due to its movement on a cushion of gas. If, however, it is trapped by garments, it may produce burns. Such clothing should be discarded until the liquid has vaporized.

# Chapter 4 Biological Safety

**Although this laboratory is classified as BSL-1, students in this course will learn proper techniques to also be able to work in laboratories with a higher level (BSL-2) Keep this statement if it applies. The following rules and regulations will have to be followed:**

# Always use aseptic technique when handling biological samples. Proper aseptic technique ensures that contamination of clean surfaces is minimized if not eliminated altogether. The use of this technique not only protects your sample from contamination, it also confines and prevents your sample from contaminating the environment. Using the biosafety cabinets minimize the contamination of the environment and protects the user

* Gloves and lab coats should be used at all times. Eye and face protection should be used when needed. Cover any abrasion, cut or open wound with adhesive plaster before beginning work.
* The working bench should be free of any personal items. Clean and disinfect work surfaces before and after usage with Ethanol 70%
* Biohazardous waste must not be discarded into normal disposal bins.
	+ Liquids containing living micro-organisms or tissue cultures cannot be directly poured down the sink without treatment. All liquid cultures need to be inactivated. The primary choice is autoclaving. Should the autoclave be out of service, your instructor will inform you and household bleach will be used instead, whereby the concentration required needs to be at least 10% of bleach of the total volume (e.g. 900 ml of culture supernatants and 100 ml of bleach).
	+ All plastic ware (e.g. cell culture flasks, pipettes, etc.) and other waste that was in contact with biological materials has to be discarded into the appropriate biohazard waste containers with autoclavable waste bags. The bags will be autoclaved before final disposal. Small autoclavable bags on holders or in beakers are good for collecting solid waste and small volumes of liquid waste. Once full, these bags need to be added to the biohazard waste containers for correct disposal.
* Used needles, disposable syringes, scalpel blades, pipettes, and other sharp items are to be placed in puncture resistant containers marked with a biohazard symbol for disposal. Used needles should not be re-capped, bent, broken or manipulated by hand. Discard these items intact to prevent accidental skin puncture.
* Exercise care in all procedures and manipulations to minimize aerosol formation. Aerosols can be generated while using pipettes, wire loops and even during the removal of a screw cap or a rubber bung from a culture tube. Rapid and forceful ejection of the contents of a blow-out pipette can also produce an aerosol. In general, slow and unhurried movements are to be preferred in microbiological work, but with minimum delays between operations.
* Biosafety cabinets must be certified annually. Check the certification sticker on the front of the unit to verify your biosafety cabinet’s condition. Contact Name of PIs in case the certification is expired. To assure sterility inside the cabinet and establish proper air flow for containment, the blower should be turned on at least five minutes before infectious materials are to be put into the biosafety cabinet. Spray the safety cabinet with 70% Ethanol before use. To collect liquid waste on the biosafety cabinet, use the provided containers. After each course day, put the closed containers out for autoclaving. Only if autoclaving is not possible (e.g. due to equipment failure), disinfect the liquid waste by adding bleach to a final concentration of ≥10% bleach, and leave at room temperature for 30 minutes to ensure sufficient contact time with disinfectant.
* Never place anything over the front or rear grill of a biosafety cabinet. Disrupting the airflow into the front grill allows contaminated air from inside the cabinet to blow into the lab or directly at the person sitting at the cabinet. It also allows non-sterile air from the room to blow into the biosafety cabinet over the experiments. Materials should be placed in the biosafety cabinet so as not to block air flow into the rear grill. Leave a few inches for air to flow around objects. Any disruption of the air flow in the cabinet decreases its effectiveness. Before starting your work in the biosafety cabinet, make sure that you have everything you need in the cabinet. The fewer times you pull your hands out of the cabinet, the less air flow disruption. Work in the biosafety cabinet should be performed in the center of the work surface of the cabinet whenever possible. After the biosafety cabinet has been emptied, wipe inner surfaces with 70% Ethanol. At the end of the day, the UV light should be turned on for 30 min (automatic timer). No one should be in the room during this time.
* When using incubators, all users should check that they have the correct settings (normally 37°C and 5% CO2). If an alarm is sounding, check the panel for the identifying blinking light. 1) If there is no obvious reason for the alarm, contact Name of PIs. 2. The “CO2 Low” (or High) message indicates a deviation from 5% CO2. Check the hose from the wall to the unit. 3) The “tank farm” must be checked for empty tanks once/week. Always keep one CO2 gas cylinder working and the other spare one is full for changing. To order a new bottle and organize pick-up of the old bottle, contact the department’s lab manager name and email. Decontaminate incubators by heat sterilization on a regular basis, as indicated in the manual. Always remove the CO2 sensor before heat sterilization as it will get damaged during this process.
* Gas bottles for incubators (most commonly CO2, but can be N2 in case of hypoxia incubators) are cylinders that contain compressed gases and should hence not be subjected to rough handling or abuse. Such misuse can seriously weaken the cylinder, rendering it unfit for further use or causing a highly dangerous fracture. To protect the valve during transportation, the cover cap should be screwed on tightly by hand and remain on until the cylinder is securely chained up in place and ready for use. Gas bottles should never be rolled or dragged. The preferred transport, even for short distances, is by cart with the cylinder strapped in place with a chain. The contents of gas bottles must be clearly labeled. Empty cylinders should be separated from full ones and clearly labeled as such. Gas bottles must be strapped into the rack and secured with the metal chain. Readily combustible substances, such as oil or grease, must not be permitted to come into contact with oxygen cylinder valves, regulators, gauges or fittings. Suspected leaks can be checked by applying a dilute soap solution. Bubbles will occur where there is a leak. It is crucial that there is always one full replacement bottle available. To order a new gas bottle, contact the department’s lab manager name and email. Reminder: You have to take the gas cylinder training before starting this course.
* Other important aspects:
	+ After manipulating biological agents, make sure all containers are tightly closed.
	+ Only sealed containers are to be used when centrifuging cell cultures.
	+ Test tube cultures should always be kept in test tube racks. Never place the test tubes horizontally on the bench top.
	+ Never pipette anything by mouth.
	+ Clean materials to be autoclaved should be placed in leak proof containers or specific autoclaving bags and properly labeled with autoclave tape.
	+ Living cultures must not be removed from the laboratory without permission.
	+ Biological material when transported between buildings must be in a double container system. If any materials is dropped or spilt whilst being carried between buildings, the accident must be reported and the spillage must be treated immediately.
	+ Laboratory refrigerators, cold rooms and other equipment must not be used to store any food for human consumption.
	+ All vessels containing viable biological materials should be properly labeled to provide information to users in case of breakage and spillage. Label information should include: organism present, special features if any, and initials of experimenter.
	+ The location of first aid kits, spill kits, emergency showers and fire extinguishers should be made known to all laboratory workers.

# Chapter 5 Waste Disposal

Always ascertain the correct procedure for disposal of waste materials in order to minimize

1. the hazardous nature of these materials
2. the risk of user exposure to still-hazardous wastes
3. the risk of contaminating the environment with these materials

**Chemicals and Liquid Waste (non-biohazardous)**

* Solvents and corrosive chemicals should not be poured down the sink, but placed into the appropriate waste bottles, which should then be disposed of in the correct manner.
* All chemical waste has to be collected in appropriate storage bottles, separated according to the following groups: Acids, Bases, Alcohols, Thiols and Sulfides, Formalin and Formaldehydes. Additional collection bottles will be created as required.
* Storage takes place in the respective safety cabinets depending on the danger of the waste (e.g. flammable, corrosive).
* Solid chemical waste will be kept in the original containers whenever possible
* All waste containers must contain a disposal sheet that shows the type of waste, the volume, the date and the experimenter. All lab members are advised to fill the sheet immediately upon disposal.
* Once full, waste containers are picked-up by Gary Zinsmeister from RIT Environment, Health & Safety. The pick-up in Institute Hall is coordinated by the department’s lab manager name and email.

**Sharps**

* Sharp objects, such as broken glassware, needles and blades, must be disposed of in a sharp container and not in the general waste.
* Biohazardous sharp containers must be autoclaved before disposal (see below).
* Sharp containers are picked-up by Gary Zinsmeister from RIT Environment, Health & Safety. The pick-up in Institute Hall is coordinated by the department’s lab manager name and email.

**Biohazardous Waste**

* Any biohazardous waste must be decontaminated prior to disposal. Hence, all biohazardous wastes should be treated chemically or by autoclaving in order to minimize their hazardous nature prior to disposal.
* Treat all liquid biohazards by autoclaving.
* All solid biohazardous wastes must be disposed of into the biohazard waste containers only, equipped with autoclavable waste bags. Do not discard into the regular waste bins.
* For the collection of smaller amounts of biohazard waste during the experiment, small biowaste bags (in bag holders) can be used. The bags must be transferred into the biohazard waste containers for disposal.
* Full biohazard waste bags are being transported to the name of PI or person who will be transporting on the 3-side-contained transportation cart, where they will be autoclaved.

**Tissue**

* Leftover tissue of bovine tissue after cell isolation must be transferred into Ziploc bags.
* Bags must be labelled with content, name and date and stored at -20°C until further disposal.
* Once sufficient tissue waste has accumulated, it gets picked-up by Gary Zinsmeister from RIT Environment, Health & Safety. The pick-up in Institute Hall is coordinated by the department’s lab manager name and email.

# Chapter 6 Accident Management

Accidents can and do occur in laboratories. As with all such incidences, the foremost concern is to ensure that safety to all users is preserved. Once all users have been brought to safety, one can then begin to deal with the effects of the accident, i.e. to prevent the threat from spreading from the site of the accident. This might necessitate the containment of spills or leaks.

# Basic Information

* The laboratory is equipped with emergency safety equipment such as eye wash, safety shower and first aid kit. All workers must be familiar with emergency and first aid procedures.
* The laboratory contains fire extinguishers. Workers who have received training may use them if necessary.
* Access to emergency exits and equipment should never be blocked
* Report all incidents of contamination and/or injury to Name of PIs and department’s lab manager name and email. It should be noted that the reasons for reporting these incidences is solely for the purpose of ensuring the safety of the users, and not for the purpose of punishment.

## Common Accidents: What to do

## *Acid and alkali in eyes*

## Irrigate thoroughly with solution from eyewash bottle or, if this is not available, cold water from the tap. Obtain medical attention immediately.

## *Extensive splashing with acid or alkali*

Douse with water (use a shower) and remove affected clothing. Obtain immediate medical attention.

## *Thermal burns*

Apply cold water to affected area, dry carefully, cover with dry dressing and seek medical assistance. Severely burnt persons should be kept warm until medical assistance arrives.

***Fires***

Any unintentional fires, regardless of size, extinguishment or amount of damage, must be report to Public Safety immediately. **Call (585) 475 3333 or Text (585) 205 8333**.

## *Cuts and wounds without exposure to biohazards*

Cuts are dangerous because they tear the skin and make the interior of the body vulnerable to toxins and other dangerous agents. If a cut is bleeding, apply pressure to it with a bandage, or with gloved hands, to staunch the flow of blood. Then, the wound should be cleaned very thoroughly by rinsing with running water. Dress simple wounds with iodine and gauze/plaster. Deep lacerated wounds need further medical attention after application of pressure dressing to stop bleeding. If there is detachment of body parts like parts of fingers, place them in a container packed with ice and bring them along with the patient to the Emergency Department at University of Rochester (Change to “designated medical treatment location”).

## *Cuts and wounds with exposure to biohazards*

In the event that the cut was caused by an object known to contain or to be contaminated by a biohazardous material, wash the wound with running water and apply wound disinfectant. Thereafter, seek immediate medical attention and notify Name of PIs and department’s lab manager name and email immediately. Needles are commonly used the laboratory and it is possible that a user might be accidentally stabbed with one. A needle-stick injury like this is similar to a cut and poses the same dangers even if it doesn’t bleed profusely. It should hence be treated as a cut.

***Bruises***

A blunt blow to parts of the body might result in mild internal bleeding leading to a bruise, just beneath the skin. In the event of a bruise apply ice cubes wrapped in a piece of cloth to the injured area. If possible, apply pressure and raise the injured part of the body to reduce swelling and bruising.

# Spills: What to do

* A spill kit is available in the laboratory
* Make sure to put up clear warning signs to others to keep clear of the contaminated area until decontamination is completed. Always double-glove your hands
* ***Solid sample spill***
	+ Dispose of the contaminant, along with the outer-most pair of gloves into the biohazard bin.
	+ Disinfect the contaminated surface with 10% bleach for 30 min. If the surface is sensitive to bleach (e.g. safety cabinet), ensure that the time does not exceed 30 min to avoid surface damage.
	+ Wipe away the bleach, then disinfect with 70% ethanol for 30 min.
	+ Dispose of all wiping towels, along with the second pair of gloves, in the biohazard bin.
* ***Liquid spill***
	+ Most important: Prevent spread of the liquid
	+ Use either a spill kit sponge pad or wad of paper towels to surround the liquid.
	+ Proceed to soak up the main body of the spill with absorbent mats or sprinkle with crystalising agent Super-sorb
	+ Once the spread of the spilled biohazardous material is stopped or retarded, remove soaked material into the biohazard bin, together with the outermost pair of gloves
	+ Disinfect the contaminated surface with 10% bleach for 30 min. If the surface is sensitive to bleach (e.g. safety cabinet), ensure that the time does not exceed 30 min to avoid surface damage.
	+ Wipe away the bleach, then disinfect with 70% ethanol for 30 min.
	+ Dispose of all wiping towels, along with the second pair of gloves, in the biohazard bin.
* In case of extensive bodily contact with a contaminant, remove all contaminated clothing and rinse yourself thoroughly under the emergency shower. Use soap if possible.
* In case of a minor contamination, wash the area thoroughly with soap and rinse in the sink.