

**BIOSAFETY STANDARD OPERATING PROCEDURES - DR. CAROLYN CUMMINS' LABORATORY**

**1. CONTACT INFORMATION**

|                                |                     |                                |                             |
|--------------------------------|---------------------|--------------------------------|-----------------------------|
| <b>Principal Investigator:</b> | Carolyn Cummins     | <b>Department:</b>             | Pharmacy                    |
| <b>Building and room:</b>      | Leslie Dan, Rm 1140 | <b>Lab Phone #:</b>            | 416-978-6146                |
| <b>PI Phone #:</b>             | 416-946-3466        | <b>PI Email:</b>               | carolyn.cummins@utoronto.ca |
| <b>Alternate Contact:</b>      | Ricky Tsai          | <b>Alternate Contact Phone</b> | 647-887-3883                |
| <b>Alternate Contact</b>       | r.tsai@utoronto.ca  |                                |                             |
| <b>SOP Revision Date:</b>      | July 2014           |                                |                             |

**2. RESPONSIBILITIES**

**2.1. PI Responsibilities**

2.1.1. Ensure all procedures documented in this SOP are followed by all personnel working in the lab.

**2.2. Research Personnel Responsibilities**

2.2.1. Follow all procedures as listed in the SOP.

**3. BIOLOGICAL AGENTS AND OTHER INFECTIOUS MATERIAL**

| Agent                                          | Description                                                                       | Risk Group | Potential Hazards                                                                     | Signs & Symptoms                                   | Required Immunizations |
|------------------------------------------------|-----------------------------------------------------------------------------------|------------|---------------------------------------------------------------------------------------|----------------------------------------------------|------------------------|
| E.coli bacteria                                | Agents are not associated with disease in healthy adult humans – low risk.        | 1          | Ingestion, mucous membrane exposure                                                   | Diarrhea and stomach cramps; conjunctivitis        | None                   |
| Human cell lines (HEK, HeLa, RAW264.7, MLO-Y4) | Agents are associated with human disease which is rarely serious – moderate risk. | 2          | Percutaneous injury, ingestion, mucous membrane exposure                              | N/A                                                | None                   |
| Adeno associated virus (AAV)                   | Viral vectors                                                                     | 2          | Adenovirus is a pathogen of respiratory, gastrointestinal mucosa and mucous membranes | Cold, flu/pneumonia; conjunctivitis; gastric upset | None                   |
| Lentivirus                                     | Viral vectors                                                                     | 2          | Adenovirus is a pathogen of respiratory, gastrointestinal mucosa and mucous membranes | Cold, flu/pneumonia; conjunctivitis; gastric upset | None                   |

#### **4. INTRODUCTION TO GENERAL SAFETY AND TRAINING FOR THE BSL-2 LABORATORY**

##### **4.1. Required Training**

The minimum requirements for qualification to work in the Cummins lab are:

- U of T Biosafety Training (<http://www.ehs.utoronto.ca/services/biosafety/training.htm>)
- Online WHMIS (see U of T Biosafety link provided above)
- Memorandum of Understanding (MOU) and Agreement on Biosafety
- Documentation is to be kept on file in lab.
- Additional training is required for those individuals working with research animals.
- Additional training is required for those individuals working with radioactivity.

This document will provide the basis of training in conjunction with EHS training. Refer to the EHS website (<http://www.ehs.utoronto.ca/services/biosafety.htm>) and/or the UofT [Biosafety Policies and Procedures Manual](#) for more information.

##### **4.2. Administrative Procedures**

It is the responsibility of each employee to carefully consider every action taken in the BSL-2 lab and its potential impact on possible exposure or contamination, and to follow established Standard Operating Procedures (SOPs) and protocols diligently and without variance.

- No employee will be trained to work in the lab without the express permission of Dr. Cummins
- New SOPs and protocols must be approved by the PI before initiation.
- Current SOPs and protocols will be reviewed and/or revised by Ricky and Dr Cummins annually.

##### **4.3. Description of Laboratory**

The Laboratory is located in the Pharmacy building in rooms 1140 (main lab) and 1125 (tissue culture). There is a -80C storage freezer in Room 1145. The desk/office space is in Room 1160.

##### **4.4 General Laboratory Safety**

Work with the agent will be performed in the Cummins Lab

- Laboratory employees must immediately notify the PI and/or EHS in case of an accident, injury, illness, or overt exposure associated with laboratory activities.
- No eating, drinking, smoking, handling contact lenses, or applying cosmetics in the lab at any time.
- No domestic animals or unaccompanied minors (persons under the age of 18), or immuno-compromised persons will be allowed to enter the lab at any time

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- Food, medications, or cosmetics should not be brought into the lab for storage or later use. Food is stored outside in areas designated specifically for that purpose.
- No open-toed shoes or sandals are allowed in the laboratory.
- PPE includes gloves, lab coat, and eye protection.
- All skin defects such as cuts, abrasions, ulcers, areas of dermatitis, etc. should be covered with an occlusive bandage.
- Mouth pipetting is prohibited; mechanical pipetting devices are to be used at all times.
- All procedures are to be performed carefully to minimize the creation of splashes or aerosols.
- Follow all manufacturer's instructions and SOPs when using any of the laboratory equipment.
- Wash hands:
  - After removing gloves, and
  - Before leaving the laboratory.
- Razor blades, scalpels, and hypodermic needles ("sharps") should be discarded into the "yellow sharps" container in lab. Do not recap needles. In lab when in use these should be kept in a petri dish with a lid for safety.
- Work surfaces will be decontaminated as needed with 70% ethanol and work surface covers (blue absorbant pads) should be changed as needed. Effective dilution for ethanol is 70-85%. Stocks of ethanol are available in lab. Alcohols are effective against a broad spectrum of bacteria and many viruses. Ethanol is fast acting and leaves no residue and is non-corrosive.

### 4.5. *Laboratory Waste Disposal*

- *Level 2 biological waste*: to be decontaminated in *Yellow Buckets lined with yellow plastic bags*. Please see Level 2 biohazard list to check to see if you are working with a Level 2 reagent. Yellow bags are then tied up (twist tie / knot) when full and placed in Room 1140 near the South sink for weekly garbage pick up. Please write on the top of the bag: "Cummins Lab, PB1140, Cert #10001980"
- *Clear glass waste* are to be triple rinsed and place in the large green recycling bin in the hallway. *Coloured glass waste* are to be triple rinsed and placed in another box separately labeled in the hallway.
- *Regular lab plastics* are to be triple rinsed and place in the orange recycling bin in the hallway.
- *Solid chemical waste* (EtBr and Phenol-Chloform) go in their own respective green bucket and are brought up to the Penthouse fully labeled and lid sealed shut when full. Liquid chemical waste go into the matching labeled 4L glass jars, also bought to Penthouse when full. Labels for chemical waste can be picked up from the Penthouse room or contacting EHS.
- All other *solid non-recyclable waste* go into grey trash bins as regular garbage.
- Liquid waste (non-organic) can be decontaminated with bleach, bringing the solution to 10% bleach, and discarded in the sink.
- Bleach is effective against vegetative bacteria, fungi, most viruses at 1:100 dilution. Effective against bacterial spores at 1:10 dilution. Bleach is very corrosive and is rapidly inactivated by organic matter.

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### 4.6. *General Biosafety Cabinet Safety*

- Turn on the blower in the cabinet at least 10 minutes before placing any items into the hood.
- Check the certification sticker and Magnehelic gauge to verify that the biosafety cabinet is working properly.
- Check the air flow indicator to verify that the air flow is operating properly.
- Gloves must be worn at all times.
- DO NOT disrupt the airflow through the hood by placing ANY item on the grills or by opening the door to the corridor.
- In general, the interior of the hood should be considered to be a contaminated zone, even though every effort is made to keep the surfaces clean, as is consistent with accepted good microbiological practice and sterile technique.
- Clean the inside surfaces of the BSC with 70% ethanol after completion of work.
- Allow the blower to run for at least 10 minutes following use.
- The UV light is turned on at the end of the day (all night). UV lights must be turned off before work begins in the hood. Do not look directly at UV lights as this can cause eye damage.
- If alarm sounds, do not panic and stop working immediately. Follow emergency shut down procedure (pull down sash, remove gloves and wash hands) and then notify Dr Cummins immediately.

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### 4.7. *General Accident Procedures (further info see Section 9)*

Spills - Apply paper towels to absorb the spill, and then soak paper towels with *10% bleach before disposal*. For spills outside the biosafety hood, alert others in the area. Use N95 mask if there is a possibility of harmful aerosols.

Biological spills in the lab – please refer to pertaining section in the EHS Protocol attached at the end of this booklet. The Spill Kit is labeled and located in a black back underneath Victor Machine in Room 1140.

Follow all aspects of all other emergency SOPs without exception.

## 5. CONTAINMENT REQUIREMENTS

### 5.1. *Laboratory Entry & Exit*

Entering the lab to begin work:

- Put on appropriate PPE
- Gather all materials for the experiment.

Exiting Laboratory:

- Before exiting the lab, be sure that all required documentation has been completed, the hood and work area are clean, all contaminated waste materials are disposed of properly, and stocks have been returned to the proper storage area.
- Wash your hands.

5.2. *Specimen Transport:*

Transport of biological materials to another building or lab within the same building should be done in a spill proof sealed container, and in a secondary container. Please use a cart if transporting large amounts of agents.

5.3. *Biosecurity:*

Please keep doors to the lab and office area locked at all times. No unauthorized people are allowed in the lab and visitors are to be accompanied at all times. All Level 2 biologicals must be kept in a spill proof container, with proper signage (door signs/storage signs) and secured (locked). If you see any suspicious person, please report to campus police (416-978-8222).

5.4. *Work within the laboratory:*

Cell Culture Work:

- During cell culture work persons must be wearing *gloves and a lab coat*.
- Preparation of primary containers of agent stock solutions and manipulations of primary containers of agents should be conducted in a biological safety cabinet.
- The user should verify inward airflow of the biological safety cabinet before initiating work
- All work should be done within the operationally effective zone of the biological safety cabinet.
- Care should be taken to avoid contaminating medium or other cell culture reagents.
- Collect regular pipet tips in a beaker in the hood and then place into trash when work is completed. Discard pipets and tips appropriately by placing them in the Yellow Bin beside the BSC. Regular plastics are tripled washed and recycled (green bin in hallway). Level 2 reagents go into Yellow Bin lined with yellow bags and tied up with twist tie/knot when full and bring into Room 1140 for weekly garbage pick up.
- When vacuum lines are used with systems containing agents, they will be protected by a liquid trap containing bleach. The aspiration tubing should then be flushed with 10% bleach after use. **NOTE: No biological agent-containing material should be allowed into the drain of a sink unless the material has been decontaminated with bleach!**
- Working outside the Biosafety Cabinet: Working outside the hood includes such actions as transporting samples from the hood to a centrifuge, incubator, or water bath.
- Vials or tubes being transported to the centrifuge, water bath, etc. should be in a stable rack. No liquid (biological waste) should be allowed into the sink drain unless it contains a final concentration of 10% bleach.
- Decontaminate items entering or exiting the BSC with 70% ethanol.
- The interior of the hood should be cleaned after each use.

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### Adeno-Associated Virus (AAV) – injections in mice

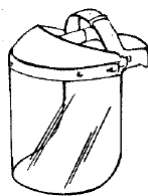
- AAV aliquots are kept in -80C freezer (Room 1145, locked) until use.
- Injections are performed in BSL Level 2 cabinets in DCM Brown Suite: PPE is worn (mask, double shoe covers, double gloves, double gown); surfaces are cleaned before/after procedure.
- After injections are completed, outer layer of gloves, shoe cover and yellow outer gown is disposed in red biohazard bag in the Brown Suite as you exit the room.
- Animals in static cages (no openings) are quarantined in DCM Brown Suite for 3 days.
- Cage is changed to transfer animals to the house room in DCM Red Suite; dirty cages are bagged and autoclaved by DCM staff prior to disposal.
- After quarantine, animals are treated as regular mice and can be brought back into Red non-sterile Suite. Bagged carcass (proper "body bags") and cage are disposed of in DCM Necropsy fridge and dirty rack respectively. Proper PPE (lab coat, gloves, mask) is used during the procedure and discarded in bin in Necropsy Room.
- All surfaces are wiped with either 70% ethanol or 1:16 Virox.

### Lentivirus (AAV) – injections in mice

- Lentivirus aliquots are kept in -80C freezer (Room 1145, locked) until use.
- Experiments are performed in BSL Level 2 cabinet in Room 1125. PPE is worn (mask, double shoe covers, double gloves, double gown); surfaces are cleaned before/after procedure.
- PPE includes but not limited to: double glove, lab coat, goggles
- When the experiment is completed, gloves are disposed in the yellow biohazard bin lined with yellow bag.
- A bottle of bleach is also used to double rinse all disposable plastics inside the BSC cabinet prior to disposal (tips, plates etc) in the yellow biohazard bin lined with yellow bag.
- All surfaces are wiped with 1:16 Virox.

### Working with Liquid Nitrogen

- Make sure face shield (one in Room 1125, one in room 1140) is secure on your head along with a pair of eye goggles prior to handling any liquid nitrogen.
- Ensure PPE is worn (lab coat and gloves), and double glove with the blue pair of Cryo-glove for further protection when handling frozen samples. **Never** submerge your hand in liquid nitrogen.
- Make every effort to prevent liquid nitrogen spillage, which results in cracking of the floor. Large Styrofoam containers can be used to help contain liquid nitrogen during sample transfer.



## 6. PROPER USE OF EQUIPMENT

### 6.1. *Biological Safety Cabinets*

Class II cabinets (Type A, B1, B2 and B3) provide personnel, environment and product protection. Air is drawn around the operator into the front grille of the cabinet, which provides personnel protection. In addition the downward laminar flow of HEPA filtered air within the cabinet provides protection by minimizing the chance of cross contamination along the work surface of the cabinet. Because cabinet air passes through the exhaust HEPA filter, it is contaminant-free (environmental protection) and may be re-circulated back into the laboratory (Type A) or ducted out of the building (Type B).

- To assure sterility inside the cabinet and establish proper air flow for containment, the blower should be turned on at least ten minutes before infectious materials are to be put into the biosafety cabinet.
- Biosafety cabinets must be certified prior to use. A HEPA Filter technician certifies our BSC hood annually.
- The biosafety cabinet air flow (“Magnehelic”) gauge should be checked (reading is equal to approximately 0.5 inches) to assure proper operation of the cabinet before placing any materials into it. Readings indicate relative pressure drop across the HEPA filter. Higher readings may, therefore, indicate filter clogging. Zero readings may indicate loss of filter integrity. In either of these cases, notify Ricky and/or Dr Cummins.
- 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 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, make sure that you have everything you need in the cabinet. The fewer times you pull your hands out of the cabinet, the less disruption of the air flow.
- Work should be performed in the center of the work surface of the cabinet whenever possible. Work outward progressing from clean to dirty (contaminated).
- Waste disposal: solid waste. Dispose of waste properly in the appropriate garbage bin underneath the BSC. Liquid waste is collected in the trap (containing bleach) underneath the BSC. Liquid waste is decontaminated by adding additional bleach and soaking for approximately 20 minutes before disposal.
- After the cabinet has been emptied, wipe inner surfaces with 70% ethanol. Do not shut down the blower. Check with other lab users before shutting the BSC down and putting the UV light on overnight.
- The bleach in the vacuum traps must be changed when the flask is about  $\frac{3}{4}$  full. To discard trap liquid, first treat with fresh bleach for 20 minutes, and then empty it into the sink.
- The vacuum filters must be replaced if clogged or if liquid makes contact with the filter. Used filters should be placed in the waste to be autoclaved.

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- Spills in the BSC: leave the cabinet turned on. Wearing gloves and lab coat, spray or wipe cabinet walls, work surfaces and equipment with disinfectant (70% ethanol, do NOT use bleach). Flood surfaces if necessary and collect excess in drain pans below the work surface. Allow 30 minutes contact time. Soak up excess with paper towels. Ensure that no solid debris is blown into the area below the grille. Surface disinfect all items that may have been splattered before removing them from the cabinet. Discard all clean up materials into biohazard waste container. Wash hands and exposed skin areas with soap and water. Notify EHS if the spill overflows into the interior of the cabinet. It may be necessary to do a more extensive decontamination of the cabinet. Similar instruction are found in the EHS Biological Spills attachment at the end of this booklet.
- Personal Protective Equipment (PPE): When using a biological safety cabinet, protective clothing, including gloves and a long-sleeved body covering (gown, laboratory coat, smock, coverall, or similar garment) should be worn so that hands and arms are completely covered to prevent contamination of cultures, skin and street clothing.
- Eye protection should be worn when handling infectious organisms or chemicals.
- These requirements also apply to anyone working in the area while someone else is working at the biosafety cabinet.
- Decontaminate BSC annually using the DCM procedure attached to the back of this booklet and adopt it to our lab. This requires the unit to be out of commission for approximately 24 hours so check with other lab users first.

### 6.2. *Incubators*

#### Upright CO<sub>2</sub> Incubators

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- Located in Room 1125. Set at 37°C with 5% CO<sub>2</sub>.
- Temperature should be checked each day by all users.
- Operation manuals are located in the office (Room 1160), inside the cabinet under the microwave.
- Be sure that there is sufficient water at the bottom of the incubator. Autoclaved H<sub>2</sub>O is kept under the sink in the Tissue Culture room for this purpose. Pour water directly into the base of the incubator.
- If an alarm is sounding, check the panel for the identifying blinking light. Do NOT panic.
  1. If there is no obvious reason for the alarm, contact Ricky and/or Dr Cummins.
  2. The “CO<sub>2</sub> Low” (or High) message indicates a deviation from 5% CO<sub>2</sub>. Ensure the door to the incubator is properly closed and check the hose from the wall to the unit.
  3. The “tank farm” must be checked for empty tanks once/week.
- Decontaminate incubators annually following manufacture’s protocol (found in the office). This requires the unit to be out of commission for approximately 24 hours so check with other lab users first.



### Bacterial Shaker/Incubator

- Turn off when not in use.
- Usually set to 37°C.
- Make sure containers are securely closed. There is a rack to hold centrifuge tubes in the incubator.
- Flasks should be covered with an aluminum foil cap and secured to the shaking platform using the various clamps provided.
- Clean up spills straight away using 70% ethanol and paper towels.

### 6.3. *Water Bath*

The water baths around the lab are monitored weekly by a lab personnel (lab duty) for water level, and filled with tap water only. Ensure the lids are properly secured to prevent evaporation. Put a label on the lid to warn other lab users of temperature setting/heat. To prevent growth of any organisms, water should be treated with algicide at the appropriate dilution (see vendor instructions on the bottle). Water baths are cleaned approximately every 2-3 months.

### 6.4. *Centrifuges*

- For work requiring a centrifuge spin at 4°C turn the centrifuges on half an hour before you need them to allow the temperature to drop properly. Turn them off at the end of the day.
- Always ensure the centrifuge is properly balanced. If not an ugly noise will ensue (and an error message).
- For the large centrifuge: ensure all 4 adaptors are in at all times for proper balance.
- Make sure your centrifuge caps are closed tightly. Any spills should be cleaned up straight away using 70% ethanol, paper towels and wear gloves. Dispose of as biohazardous trash.
- In case of a spill inside the centrifuge, please refer to the appropriate section in the EHS Spill Protocol attached at the end of this booklet

### 6.5. *Autoclave*

- Autoclaves use pressurized steam to destroy microorganisms, and are the most dependable system available for the decontamination of laboratory waste and the sterilization of laboratory glassware, media and reagents. For efficient heat transfer, steam must flush the air out of the autoclave chamber. Before using the autoclave, check the machine is on and available for use.
- Polypropylene containers (we have 2 in the lab) – polypropylene is a plastic capable of withstanding autoclaving but resistant to heat transfer. Therefore, materials contained in a polypropylene pan will take longer to autoclave than the same materials in a stainless steel pan. To decrease the time required to sterilize material in these containers select a container with the lowest sides and widest diameter possible for the autoclave.
- Stainless steel containers and pans – stainless steel is a good conductor of heat and is less likely to increase sterilizing time, though is more expensive than polypropylene.
- Take into account the size of the articles to be autoclaved. A 2 liter flask containing 1 liter liquid takes longer to sterilize than four 500mL flasks each containing 250mL of liquid.

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- Material with a high insulating capacity (eg. high sided polyethylene containers) increases the time needed for the load to reach sterilizing temperatures. Therefore when autoclaving racks of tips and tubes of microcentrifuge tubes try not to stack them more than two in height.
- Do NOT autoclave biohazard items. They will be collected weekly by EHS.
- Record your name, date, lab, cycle and contact info in the binder (by the sink) so we can keep track of Autoclave users.

### Preparation and loading of materials:

- Using the cart, bring all the dirty dishes to the dishwasher in Room 1149. Fill and start dish washer cycle (about 1 hour).
- When cycle is completed, remove dishes and immediately cover all exposed openings with tin foil to prevent gathering of dust.
- Loosen caps or use vented closures (ie. Tin foil)
- Place a small piece of autoclave tape on every item you wish to autoclave.
- Leave space between items to allow steam circulation.
- Household dishpans melt in the autoclave. Don't use them.
- For autoclaving liquids, fill liquid in pre-autoclaved containers only half full. Place all liquid-filled bottles in a polypropylene container and surround the bottles with a layer of water (about 5cm)

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### Cycle selection:

- Use liquid cycle (slow exhaust) when autoclaving liquids to prevent contents from boiling over. Choose the cycle based on the volume of your largest bottle. Aim to autoclave bottles filled with similar amount of liquids to ensure even sterilization.
- Select fast exhaust cycle (VacDry 20-20) for glassware.

### Removing the load:

- Check the chamber pressure is zero
- Wear lab coat, eye protection, heat insulating gloves and closed toe shoes.
- Stand beside the sliding door when opening it.
- Press "Open" and door will slowly slide down. Beware of the rush of steam.
- After the slow exhaust cycle, open autoclave door and allow liquids to cool for 20 minutes before removing. Handle with oven gloves when removing items from the autoclave.

### Monitoring and breakdown:

- In case the machine is broken, please let Ricky know. He will get in touch with Zarko who will then arrange for a service call.

### 6.6. *Chemical Fume Hood*

Laboratory fume hoods serve to control exposure to toxic, offensive or flammable vapors, gases and aerosols. Fume hoods are the primary method of exposure control in the laboratory.

- Place apparatus and equipment as far back as possible in hood for safety and optimal performance. Equipment should be placed a minimum of 8 inches inside the hood. Keep electrical connections outside of hood.
- Ensure that equipment or materials do not block the baffle vents in the back of the hood.
- When using a large apparatus inside the hood, place the equipment on blocks, when safe and practical, to allow air flow beneath it.
- Do not place electrical apparatus or other ignition sources inside the hood when flammable liquids or gases are present. Keep in mind that liquids with low flash points may ignite if they are near heat sources such as hot plates or steam lines.
- When using the fume hood, keep your face outside the plane of the hood sash and remain alert to changes in air flow.
- Work at least 6 inches back from the face of the hood.
- Always use splash goggles and wear a full face shield if there is possibility of an explosion or eruption.
- Do not make quick motions into or out of the hood, use fans, or walk quickly by the hood opening. All of these will cause airflow disturbances which reduce the effectiveness of the hood.
- Substitute less hazardous or less volatile chemicals where possible.
- Look for process changes that improve safety and reduce losses to the environment (e.g. more accurate chemical delivery systems vs. pouring volatile chemicals from bottles).
- Develop a process to evaluate research proposals ahead of time for potential emissions and look for opportunities to reduce them
- Limit chemical storage in fume hoods. Keep the smallest amount of chemicals in the hood needed to conduct the procedure at hand.
- Store hazardous chemicals such as flammable liquids in an approved safety cabinet.
- Do not allow hazardous waste to accumulate in the fume hood. A lab personnel (lab duty) will monitor the liquid waste weekly and bring full bottles up to the Penthouse for chemical waste pick up.
- Keep caps on chemical reagent bottles tight and check fitting on laboratory glassware to minimize vapor loss.
- Always use good housekeeping techniques to maintain the hood at optimal performance levels. Excessive storage of materials or equipment can cause eddy currents or reverse flow resulting in contaminants escaping from the hood.

Proper Sash Use

- The sash should be kept closed, except when working within the hood is necessary, to contain and protect from chemical vapors, splashes, or explosions.
- Use horizontal sliding sash for partial protection during hazardous work.
- Keep the slots of the hood baffles free of obstruction by apparatus or containers.
- Keep the hood sash closed as much as possible to maximize the hood's performance. Keep the sash closed when not in use to maximize energy conservation.
- Hoods should be evaluated by the user before each use to ensure adequate face velocities and the absence of excessive turbulence.
- In case of exhaust system failure while using a hood, shut off all services and accessories and lower the sash completely. Leave the area immediately and contact Dr Cummins and/or EHS.
- **The required face velocity is 100 feet per minute (0.5 m/sec).** This velocity is capable of controlling most low-velocity cross drafts and turbulence created by normal work practices at the face of the hood. All hoods should have a sticker designating this maximum safe sash height. Keep the sash at the identified appropriate level to ensure optimal face velocity. When working with open chemicals, reduce the sash as much as possible to maximize hood performance.
- Regular testing of the fume hood should be carried out every 12 months. A sticker on the front of the fume hood indicated when an inspection was last performed.

6.7. *Agilent triple quad 6410 LC/MS/MS instrument*

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- Training and supervision to be provided by PI and/or Laboratory Personnel.

6.8. *VICTOR plate reader*

- Training and supervision to be provided by PI and/or Laboratory Personnel.

6.9. *Misonix 3000 Sonicator machine*

- Training and supervision to be provided by PI and/or Laboratory Personnel.

6.10. *QPCR 7900HT machine*

- Training and supervision to be provided by PI and/or Laboratory Personnel.

## 7. EMERGENCY EQUIPMENT

### 7.1. *Fire Extinguishers are located in each room beside the entry door. (2 in Room 1140, one by each door)*

- Fire extinguishers should be used only if the fire is small and confined to one small area! USE JUDGEMENT IN THIS! DO NOT CREATE A LIFE-THREATENING SITUATION WHILE TRYING TO EXTINGUISH A FIRE
- To operate, pull the pin to release the handle.
- Stand at a safe distance from the fire (as directed on the fire extinguisher).
- Aim the nozzle at the base of the fire, squeeze the handle to discharge the agent, and sweep completely left and right until a few seconds after seeing no fire.
- Familiarize yourself with the instructions written on the fire extinguisher. This details how to operate a fire extinguisher.
- Maintenance: Fire extinguishers are inspected annually by U of T Fire Prevention Officer.

### 7.2. *Emergency Eyewash Station:*

There are 3 eyewash stations: Room 1140, Room 1125, and Room 1133. It is a rotating duty for a lab personnel to check its operational status once a week. (Let it flush for 3 minutes to encourage fresh water flow).

### 7.3. *Repairs and Service:*

Speak to Ricky and/or Dr Cummins in the event that a piece of equipment requires service or repair.

## 8. OPERATIONAL PROCEDURES

### 8.1. *Inventory Control System:*

- Ricky keeps track of stocks and reagents in lab.
- Spreadsheets and databases are kept to ensure tracking of purchased items.
- Inventories: electronic copy (Order Book) on the lab computer in Room 1160 for reference.
- Inventories are maintained for the following: (all password protected)

Order Book:

1. Chemicals (MSDS available online through manufacture website)
2. Non-chemicals (cells, lab consumables, supplies)

Catalogue Book (lab instruments)

Mouse Database (our colony in DCM)

Primer Order Database

## 9. SAFETY CHECKS AND EMERGENCY PROCEDURES

### 9.1. *Fire Alarm / Emergency Evacuation*

- Turn off any direct sources of heat (ie. Burners, hot plates).
- Close any open containers of biological or chemical reagents\*\*\*
- Take important personal belongings (and a coat in the winter).
- Ensure the door is closed behind you and is locked.
- Exit the building through emergency stairwells located closest to you: the North (3N) or South (4S) stairwell.

\*\*\*People should also wash their hands before leaving but this depends on the severity of the emergency and what reagents they are working with.

### 9.2. *Bomb Threat*

- Do not panic and stay calm.
- Any person receiving a bomb threat over the phone should ask the caller:
  - When is the bomb going to explode?
  - Where is the bomb located?
  - What kind of bomb is it?
  - What does it look like?
  - Why did you place the bomb?
- Keep talking to the caller as long as you can and record the following:
  - Time of call
  - Age and gender of caller
  - Speech pattern, accent, possible nationality
  - Emotional state of caller
  - Background noise
- Immediately notify Dr Cummins and Campus Police (416 978 2222)

### 9.3. *Incident and Accident Reporting*

- All major and minor incidents are to be reported to EHS within 24 hours of the event.
- For U of T employees, Dr Cummins will need to fill out this form below:  
<http://www.ehs.utoronto.ca/resources/wcbproc/employee.htm>
- For students, contractors or visitors, please complete this form below:  
<http://www.ehs.utoronto.ca/resources/wcbproc.htm>
- EHS will follow up on any reported incident in an attempt to improve laboratory safety and its reporting system.
- In an event of a major medical emergency, please call 911 immediately.
- For other emergencies, follow U of T EHS Emergency and First Aid protocol  
<http://www.ehs.utoronto.ca/resources/manindex/eps/emrg1aid.htm>
- There is a first aid certified personnel on every other floor (Ricky on our floor). A first aid kit can be found on every floor across from the main elevators.

**9.4. Management of Spills**

- Apply paper towels to absorb the spill, and then soak paper towels with 10% bleach or 70% ethanol. For spills outside the biosafety hood, alert others in the area. Use N95 mask if there is a possibility of harmful aerosols. Please refer to the appropriate section in the EHS Spill Protocol attached at the end of this booklet.
- Please notify Dr Cummins. The PI will contact EHS if there was an exposure.

**9.5. Management of Accidental Exposures**

In the event of an exposure to an infectious agent or material:

Intact skin

- Remove contaminated clothing.
- Vigorously wash contaminated skin for 15 minutes with soap and water; there is a safety shower in the corridor outside lab (between Room 1138 and 1140).

Broken, cut, or damaged skin or puncture wound

- Remove contaminated clothing.
- Vigorously wash contaminated skin for 15 minutes with soap and water; there is a safety shower in the corridor outside of lab (between Room 1138 and 1140).

Eye

- Immediately flush eyes for at least 15 minutes with water at the closest eye wash station by the sink.
- Hold eyelids away from your eyeballs
- Inform Dr Cummins and seek medical attention.
- Call Campus Police at 416-978-8222 or 911 in case of emergency

Ingestion or Inhalation

- Inform Dr Cummins and seek medical attention as above.
- Do not induce vomiting unless advised to do so by a health care provider.

U of T EHS guidelines and people to notify after the incident:  
<http://www.ehs.utoronto.ca/resources/manindex/eps/emrg1aid.htm>

**10. SIGN OFF DOCUMENTATION**

*10.1. Principal Investigator Certification:*

I hereby certify that I have reviewed these practices and procedures and they represent the current operating practices in my laboratory.

| <b>Principal Investigator Name</b> | <b>Principal Investigator Signature</b> | <b>Date</b> |
|------------------------------------|-----------------------------------------|-------------|
|                                    |                                         |             |

*10.2. Personnel Certification:*

We, the undersigned, have reviewed these practices and procedures, have been trained in the appropriate methods and practices for handling potentially infectious material and agree to follow the stated practices and procedures. We understand that we must review and document compliance with these practices and procedures on an annual basis.

| <b>Personnel Name</b> | <b>Personnel Signature</b> | <b>Date</b> |
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