

Raizada Lab Biosafety Containment Level 2 (CL2) and General Safety Training Manual (*Draft March 25, 2021*)

Manish N. Raizada, PhD (raizada@uoguelph.ca)
Professor, Department of Plant Agriculture, University of Guelph

TABLE OF CONTENTS

1. Principal Investigator
2. Designate(s)
3. Responsibilities
4. 24-Hour Emergency Contacts
5. Locations
6. Biohazard Materials Used In the Lab
7. Summary of Work
8. Biohazard Permit(s)

9. Training Program SOP
 - 9.1. New Laboratory Trainee Orientation Checklist
 - 9.2. Ongoing Training Procedures
 - 9.3. GHS Pictograms Reminder
 - 9.4. General Laboratory Safety – Quick Tips

10. Emergency Response Plan (spills, exposure, fire, etc.)
 - 10.1. Emergency Exit Procedure
 - 10.2. Emergency egress (exit) routes
 - 10.3. Emergency/Safety Equipment in Room 310
 - 10.4. RESPONDING TO A FIRE
 - 10.5. RESPONDING TO FLOODING
 - 10.6. RESPONDING TO LOSS OF POWER
 - 10.7. RESPONDING TO A MEDICAL EMERGENCY
 - 10.7.1. Quick Overview of medical emergency response
 - 10.7.2. Detailed Medical Emergency Response
 - 10.7.2.1. MEDICAL ASSESSMENT DECISION TREE
 - 10.7.2.2. MINOR INJURIES
 - 10.7.2.3. SERIOUS ("CRITICAL") INJURIES
 - 10.8. Chemical Spills
 - 10.9. QUICK OVERVIEW OF BIOHAZARD SPILL RESPONSE
 - 10.10. SPECIFIC BIOHAZARD SPILL SOPs
 - 10.10.1. If the spill occurs INSIDE the biological safety cabinet
 - 10.10.2. If the spill occurs OUTSIDE the biological safety cabinet
 - 10.10.3. If the spill occurs INSIDE a centrifuge

11. Raizada Lab Fire Prevention

12. Raizada Lab Autoclave, cylinder and cryogenic safety
 - 12.1. Autoclave Operating Procedure
 - 12.2. Gas Cylinder Operating Procedure
 - 12.3. Cryogenic Operating Procedure (liquid nitrogen, dry ice)

13. Raizada Lab Chemical Safety
 - 13.1. Chemical Safety Quick Points
 - 13.2. Chemical Exposure Response

- 13.3. Chemicals - General Lab Safety and Standard Operating Procedures
- 13.4. Chemical Spill Containment and Cleanup Procedure
- 13.5. Safe Chemical Mixing, Storage and Labeling
- 13.6. Chemical waste procedures

14. RAIZADA LAB BIOHAZARD CONTAINMENT LEVEL 2 (CL2) STANDARD OPERATING PROCEDURES

- 14.1. Definitions of biohazard risk groups and containment levels
- 14.2. Location of work: CL2 map and features
- 14.3. CL2 Entry/Exit Requirements and SOP
- 14.4. CL2 Standard Operating Procedures
 - 14.4.1. CL2 General Safety Precautions
 - 14.4.2. CL2 Personal Protection
 - 14.4.3. CL2 Surface Disinfection Procedure
 - 14.4.4. CL2 Disposal: Liquid culture and agar/phytagel disinfection and autoclave procedures
 - 14.4.5. CL2 Biosafety Cabinet (BSC) Standard Operating Procedure
 - 14.4.6. SOP for Cleaning the Front Grille and Catch Pan of the CL2 Biosafety Cabinet
 - 14.4.7. CL2 Centrifuge Operating Procedure
 - 14.4.8. CL2 Incubator/Shaker Operating Procedure
 - 14.4.9. CL2 Electroporator Operating Procedure
 - 14.4.10. CL2 Transporting Live Biohazards in the Building or on Campus
 - 14.4.11. CL2 Microscope Operating Procedure
 - 14.4.12. Plant Growth Containment Plan
 - 14.4.13. Raizada Lab Code of Conduct for Field Spraying with Microbes

15. Medical Surveillance SOP

17. Biohazard Inventory Control Plan

18. Biohazard import/transfer SOP

19. CL2 Equipment Maintenance SOP

20. Housekeeping, Monthly Inspection & Annual Lab Safety Meeting with CL2 Biosafety Drills

20.1. Lab Housekeeping

20.2. Monthly Laboratory Self-inspections

20.3. Annual Raizada Biosafety Emergency Spill Drill and General Lab Safety Meeting and Clean-up

21. Waste Disposal SOP for Biohazards, Chemicals, Regular Garbage and Recyclables

22. Working Alone Policy

23. Safety Documentation

24. Helpful Resources

25. Acknowledgements

26. Raizada Lab-Specific, On-Site Training Acknowledgment and Compliance Agreement Signature Page

Appendix I – List of Lab Signage, Posters and Log Sheets

Appendix II – Department of Plant Agriculture Training Module Checklist

1. Principal Investigator

Manish N. Raizada (raizada@uoguelph.ca), Office Room 406 Crop Science
1-519-824-4120 x53396

2. Designate(s)

Travis Goron, Post-Doctoral Fellow (gorontr@uoguelph.ca)

3. Responsibilities

Safety is a joint responsibility of all lab members, but within this framework:

3.1. Manish Raizada has ultimate responsibility for the security and management of all lab safety including biohazardous materials, including not limited to:

- Registration of projects using biohazardous materials by application to the Biosafety Committee.
- Advising all investigative staff of the medical surveillance program.
- Ensuring security of the work area.
- Ensuring that inventory records are maintained.
- Performing risk assessments for the work to be undertaken and developing a risk management plan
- Performing risk assessments for the work to be undertaken and developing a risk management plan.
- Preparation and regular review of a written workplace-specific biosafety manual including standard operating procedures.
- Reporting any incidents involving biohazardous materials including suspected illnesses.
- Training and supervision of all staff and students.

3.2. The Raizada Lab Equipment Officer (Dr. Travis Goron) is responsible for:

- training, maintenance and repair of all equipment
- organize laminar, chemical and biosafety hood safety certifications and repairs as needed
- ensure laminar/biosafety hood filters are replaced
- purchasing new equipment as needed
- keeping all equipment sign up sheets up to date in lab

3.3. The Raizada Lab Safety Officer (former technician and current PhD student Anuja Shrestha, later to be replaced by OMAFRA technician Erik Glemser) has the following responsibilities:

- complete the monthly lab safety checklist (see below)
- organize lab clean ups and keep clutter in lab to a minimum
- keep Emergency Lab Contact Info sheet up to date, posted and distributed
- update lab chemical inventory and MSDS sheets
- update lab safety signage
- disposal of broken glass
- disposal of sharpies containers
- ensure biohazard autoclave binder and labels are available
- arrange pickup of hazardous chemicals
- oversee autoclaving of plant and bacterial waste (ask users for assistance)
- proper disposal and recording of bacterial plates (bag labeling, etc.)
- ensure lab coats are washed, added and replaced
- help with other lab safety issues
- order lab safety supplies as needed
- maintain biosafety lab spill kit
- ensure flammables are placed in flammables cabinet

- ensure that safety shower area of free of any obstacles
- run the eye wash station weekly**
- advise Manish on Lab Safety issues/concerns

3.4. The Raizada Laboratory Covid Officer (technician Ben McFadyen) is responsible for:

- Keeping Covid-19 PPE supplies replenished including masks, hand gels, hand soap, paper towels and 70% ethanol disinfectant bottles
- obtaining PPE and disinfectant supplies from the UofG stockroom

3.5. The Raizada Laboratory Greenhouse and Field Trial Officer (technician Erik Glemser) will:

- help personnel with greenhouse and field trials: safety, equipment, transportation, ordering, space reservations, forms

3.6. The Department of Environmental Health and Safety, through the Biosafety Officer administers the biosafety program at the University by:

- Preparing and verifying the accuracy and completeness of license applications.
- Administering the registration/permitting process.
- Providing guidance and training on biological safety to personnel at all levels of responsibilities.
- Liaising with and reporting to government authorities as required concerning the use of biohazardous materials.
- Auditing and inspecting work areas and procedures for compliance.
- Maintaining records associated with the Biosafety Program
- Developing, reviewing and revising general biosafety related procedures.
- Investigation biohazard-related incidents.

3.7. The Crop Science Building Joint Health and Safety Committee will:

- maintain and update hiring and other training checklists and materials pertaining to safety
- undertake annual laboratory inspections and provide advice to the laboratory
- update lab and building personnel about changes to safety policy and regulations
- serve as a liaison to EHS

4. 24-Hour Emergency Contacts (updated Mar 25, 2021)

Room 310 Lab Phone: 519-824-4120 x54084

Raizada Lab What's App Group has all lab members (contact Travis at 226-337-4833 to join)

Name	Position	Phone/SMS	Email
Emergency	x2000 or 519-840-5000		
Campus Police (Emergency)	x2000 or 519-840-5000, or 911		
Environmental Health and Safety	519-824-4120 x 53282		
Physical Resources	519-824-4120 x 53282		

Additional Contacts (updated Mar 25, 2021)

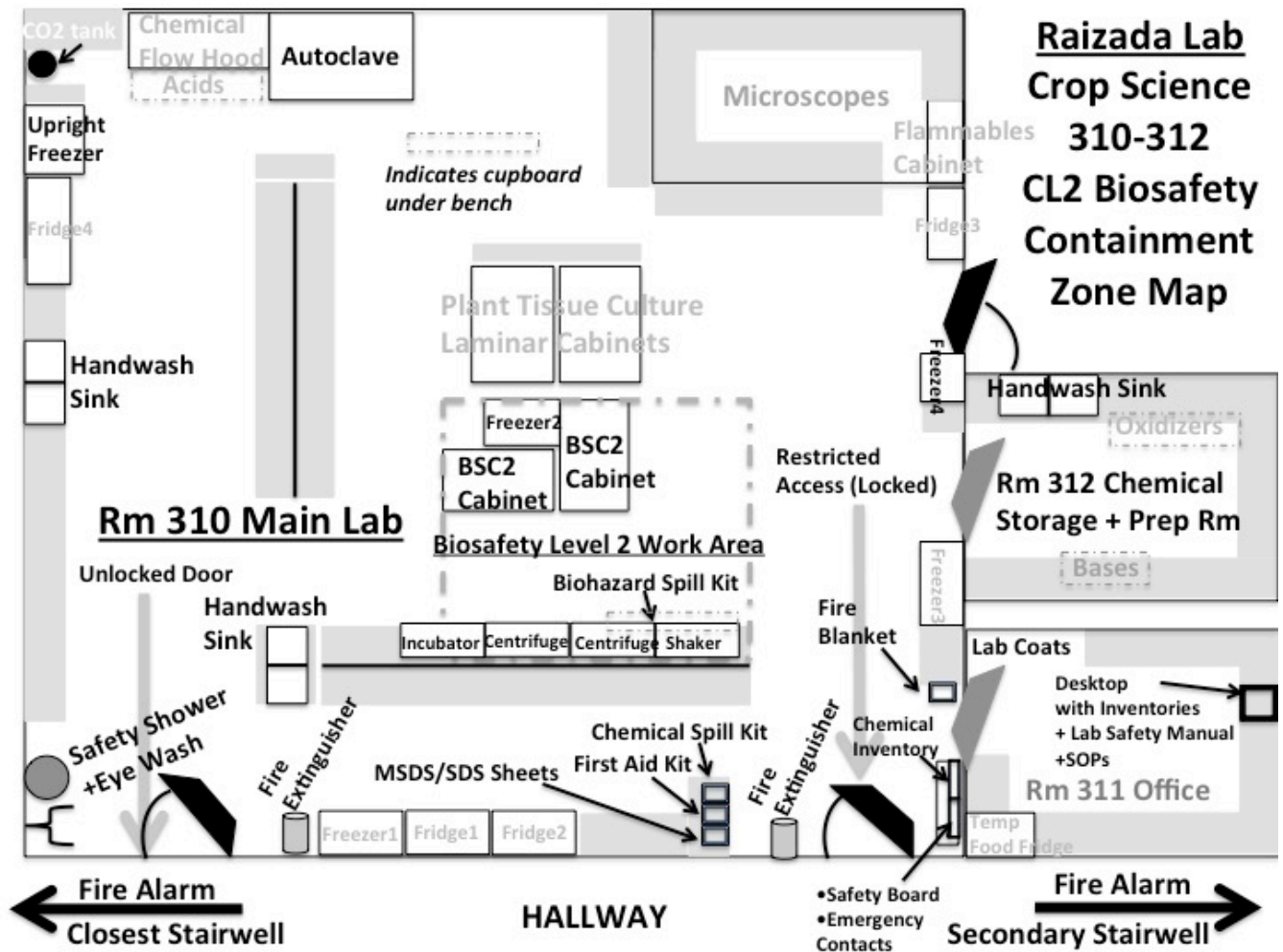
Name	Position	Phone/SMS	Email
Christi Cooper	Manager, Agricultural & Veterinary Safety	x 52049	mcooper@uoguelph.ca
Gausiya Khan	Biosafety Officer	x 53190	gkhan@uoguelph.ca
Alicja Zachertowska	Lab Safety Officer	x 54270	azachert@uoguelph.ca
Rachel Pinto	Manager, Occupational Hygiene and Safety	x 54855	rpinto@uoguelph.ca
Chris Grainger	Common Room Equipment and Autoclaves	Room 205/222 x 52509	cgrainge@uoguelph.ca
Sue Couling	Crop Science Growth Facilities Manager	Room 135 x 52476	scouling@uoguelph.ca
Crop Science First Aid Responders and Fire Wardens	Updated names posted on Room 310 Exit Doors		

PLEASE NOTE

In case of violence, harassment or threats	Use the illness or injury report form and submit it to Occupational Health and Wellness; a Violence Incident Report can be sent directly to campus police – call x2000 or 911
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5. Locations

The Raizada Lab is Located in Room 310 of the Crop Science Building within which are two additional rooms, Room 311 and Room 312. A CL2 Containment Zone is located within Room 310, with the focus being two Level 2 Biosafety Cabinets. Detailed maps are below:



6. Biohazard Materials Used In the Lab

- Bacteria Containing Recombinant Plasmid Visual Reporters Such as Green Fluorescent Proteins
- Plant Endophytic Microbes

7. Summary of Work

The Raizada Lab studies the beneficial microbiome of crop plants, focusing on corn, but also side projects in wheat, cucurbits and turfgrass. The lab isolates probiotics, in particular endophytes, which are microbes that naturally inhabit plants without causing disease – they are implicated in helping plants with promoting root growth, nutrient acquisition (e.g. nitrogen fixation) or fighting disease. For this reason, the lab refers to them as ‘crop probiotics’.

8. Biohazard Permit(s)

Pending approval of application(s) to EHS.

9. Training Program SOP

9.1. New Laboratory Trainee Orientation

1. All new lab members must complete the mandatory training modules listed in the document entitled, Department of Plant Department of Plant Agriculture Safety Training/Documentation – Standard Operating Procedures (May 13, 2020 update) (approved by Christi Cooper, EHS) (Appendix II)

- EHS Worker Health and Safety Awareness
- EHS WHMIS
- EHS Laboratory Safety
- EHS Supervisor Health and Safety Awareness and Due Diligence (Manish Raizada only)
- EHS Farm, Field and Landscape Safety – Summer Training (as appropriate)
- Plant Ag: Growth Facility Training (as appropriate)

2. ALL Raizada Lab members working in the lab must also complete the EHS Biosafety course, which includes:

- Federal, provincial, municipal, laws, regulations and standards regarding use of biohazardous materials.
- University Biosafety Policy and Program and University requirements regarding use of biohazardous materials.
- Permits.
- Responsibilities.
- Risk assessment including potential hazards, zoonoses, and lab-acquired infections.
- Medical Surveillance.
- Biosecurity.
- Principles of containment.
- Physical facilities.
- Administrative controls.
- Engineering control.
- Operational practices.
- Personal protective equipment.
- Personal practices and hygiene.
- Disinfection and sterilization.
- Waste management.
- Emergency procedures and reporting.
- Transport of biohazardous materials: shipping, receiving, importing, exporting.

2. Trainees must complete a quiz for each EHS training course and then copies of completion certificates are kept on file [by Tara Israel for graduate students, and by Kelly Scholtes for all other lab members (i.e. technicians, post-doctoral fellows, research associates, undergraduates)].

3. **Workplace specific training:** New trainees receive on-site lab specific training, prior to commencing work, during which this 60-page lab manual is discussed page by page. Personnel then must read and sign the last page of this manual in acknowledgement. A copy of the signed manual is kept with the trainee and with Manish Raizada. The on-site training includes:

- Hazards of the particular biohazardous materials in workplace.
- Laboratory standard operating procedures (SOPs) and workplace-specific safety manual.
- Laboratory-specific techniques.
- Proper use of departmental autoclaves
- Proper use of laboratory equipment such as centrifuges, biological safety cabinets, shakers, microscopes, incubators, etc.
- Laboratory-specific emergency procedures and reporting.
- Location and use of emergency equipment: fire alarm pull-station, fire extinguisher, eyewash,

safety shower, spill kit.

- Field work safety basics
- Laboratory-specific record keeping.
- Laboratory-specific waste management.
- Packaging and transporting of biohazardous material within the work area.

9.2. Ongoing Training Procedures

1. Additional ongoing training resources: Lab members will be encouraged to review this manual which is meant to be comprehensive, as well as other resources noted in Section 24, on an on-going basis. These links are also posted on the Safety Tab and Homepage QuickLinks (bottom of homepage) on the Raizada Lab website: www.RaizadaLab.weebly.com
2. Person-to-person training will be ongoing, including safety reminders and time to discuss safety concerns at daily WhatsApp Group meetings (during Covid) and during bi-monthly lab meetings.
3. The Raizada Lab Safety Officer will perform a formal monthly self-inspection check and then report back to the lab at the subsequent group meeting to discuss any safety areas of concern.
4. Signage, SOPs and posters throughout the lab, including on the Lab Safety Board will also served as reminders of good safety/biosafety practices.
5. An entire day annually (during the first 2 weeks of January) will be devoted to reviewing lab safety including CL2 Biosafety. This will include an emergency biohazard spill response drill (see Section 20.2).
6. It is the responsibility of all lab personnel as well as Manish Raizada to ensure that training that expires is repeated as necessary; lab members will not be permitted to work in the lab or field until their training status is up to date.
7. To be proactive, Manish Raizada will review the status of EHS training certificates of all lab personnel at the Annual Raizada Biosafety Emergency Spill Drill and General Lab Safety Meeting and Clean-up during the first 2 weeks of January each year (see Section 20.3).

9.3. GHS Pictograms Reminder

GHS pictograms are central to your awareness of hazards in the lab, and so have been pasted below as a constant refresher:

GHS Pictograms and Hazards

Flame	Exclamation Mark	Health Hazard
 <ul style="list-style-type: none"> Flammable Self-Reactive Pyrophoric Self-Heating In Contact with Water, Emits Flammable Gases Organic Peroxide 	 <ul style="list-style-type: none"> Irritation (skin or eyes) Skin Sensitization Acute Toxicity (harmful) Specific Target Organ Toxicity – Single Exposure (drowsiness or dizziness, or respiratory irritation) Hazardous to the Ozone Layer 	 <ul style="list-style-type: none"> Carcinogenicity Respiratory Sensitization Reproductive Toxicity Specific Target Organ Toxicity – Single or Repeated Exposure Germ Cell Mutagenicity Aspiration Hazard
Skull & Crossbones	Exploding Bomb	Flame Over Circle
 <ul style="list-style-type: none"> Acute Toxicity (fatal or toxic) 	 <ul style="list-style-type: none"> Explosive Self-Reactive (severe) Organic Peroxide (severe) 	 <ul style="list-style-type: none"> Oxidizer
Corrosion	Gas Cylinder	Environment
 <ul style="list-style-type: none"> Corrosive (skin, eyes, or metals) 	 <ul style="list-style-type: none"> Gas Under Pressure 	 <ul style="list-style-type: none"> Hazardous to the Aquatic Environment (acute or long-term)

9.4. General Laboratory Safety – Quick Tips

Key objectives:

1. Protect ourselves from exposure to the hazards present

2. Prevent the migration of contaminants out of the lab
3. Mitigate the impact of a serious incident (e.g., fire, spill)

Key points:

- Report all potential hazards or safety issues to lab supervisor/advisor.
- Know the emergency response procedures.
- Keeping aisle and exit routes clear.
- Know evacuation routes from the lab and the location of emergency equipment such as pull stations, fire extinguishers, spill kits, emergency showers/eyewashes, first aid kits, fire blankets
- Don't let others come in to the building behind you after hours if you don't know them.
- Never leave lab doors propped open.
- Do not bring food, drinks, tobacco products or cosmetics into the lab (food or drink permitted in the lab office during Covid).
- **Closed-toe, closed-heel shoes** constructed of a resistant material (preferably leather) **are required to be worn in all laboratory areas**. Sandals, and open-toed shoes are not permitted.
- Always wear suitable clothing, a lab coat, and required personal protective equipment (PPE) when working with potentially hazardous materials.
- Dispense hazardous chemicals inside a fume hood.
- Never allow gloved hands to touch surfaces outside of the lab, or 'clean' surfaces in the lab such as door knobs, phone receivers, or computer keyboards.
- Do not wear lab coats in common areas or stairwells (during Covid, since walking through the Pauls' Lab is not allowed, you can wear your lab coat in the hallway but only to the adjacent door leading to the Pauls lab).
- Keep personal items separated from hazardous materials.
- All materials dispensed from their original containers must be labeled.
- Place a sign on any unattended experiments and provide contact information so you can be reached if something goes awry.
- If using personal headphones when doing lab work, keep the volume low – you need to be able to hear if equipment is functioning properly, and be able to hear emergency alarms.
- be aware of other people present and their location.
- Keep incompatible chemicals separate (no acids with bases, no flammables with oxidizers) – look at the labels and/or SDS if you aren't sure.
- Always assume gloves are contaminated. You put on gloves to prevent something from touching your skin. The logical conclusion is that whatever it was you didn't want on your skin is now on the exterior of the glove. Change gloves frequently and never allow a gloved hand to touch a common surface (like a door knob, phone receiver, etc.).
- Lab coats protect you, and the clothes that you own, from becoming contaminated. It is a requirement that lab coats be worn in the lab for any work involving hazardous materials. **Lab coats cannot be worn outside of building**. If you will need a lab coat at your destination on another floor, simply place your coat in a plastic bag and carry it with you.
- A clean work area goes a long way to reducing cross-contamination.
- For any hazardous agent it is important to consider how the inevitable small spills, droplets and splashes can be controlled. Working over disposable bench paper is an easy way to control contamination. Alternatively, you can work over a low tray that has cleanable surfaces. Regardless of the method you use, be sure to clean your work area when finished, and if there are any small spills, clean them up using an appropriate method as soon as possible (refer to the specific spill SOPs below for more details).
- Another area with high potential for spreading chemical/biological contamination is the transport of materials through hallways. Make use of carts and secondary containment trays when moving materials around the building.
- Another factor to consider is the storage of your personal belongings; as much as possible, you should keep personal items out of the lab. If you do have items you need in the lab,

keep them well separated from potentially contaminated areas.

- Ensure that the access to eyewash stations, showers, and fire extinguishers is unobstructed.
- Ensure that fire extinguishers have adequate charge (needle on pressure gauge is in green zone).
- Ensure spill kits and first aid kits are readily accessible and appropriately stocked.
- Electrophoresis: The use of voltages of approximately 200 V and currents of more than 80 mA in electrophoresis procedures could create the potential for an electrical shock if the equipment is not operated properly. Turn off power before connecting leads, opening the lid or reaching into the chamber.
- Do not leave water blocks or small heating blocks on overnight (exception are PCR machines).

10. Emergency Response Plan (spills, exposure, fire, etc.)

Immediately sound the fire alarm if needed, leave the building, and then call x2000

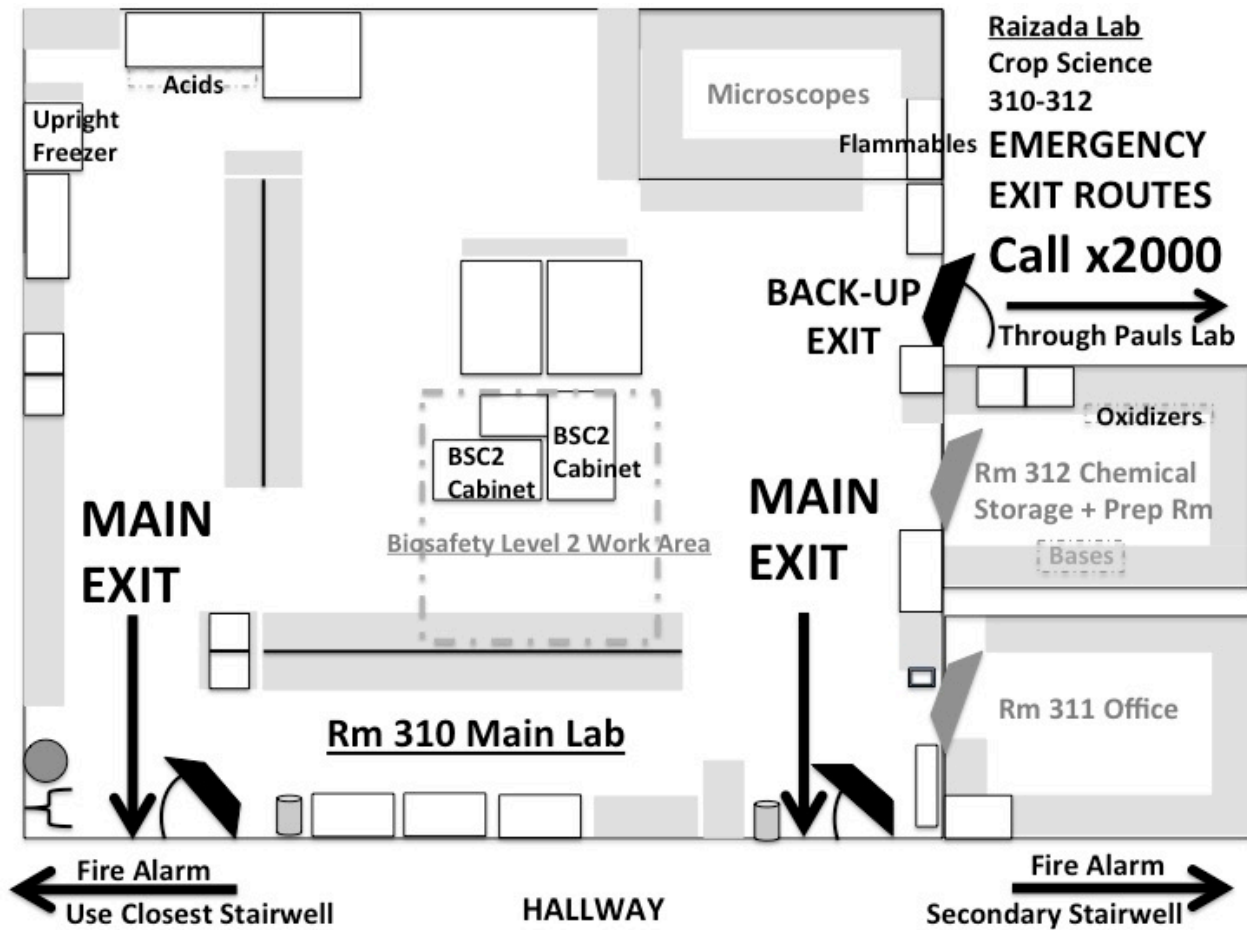
10.1. Emergency Exit Procedure:

When you hear the evacuation alarm, IMMEDIATELY:

1. Extinguish any open flames (e.g. alcohol lamp).
2. Close chemical fume hood sash and biosafety cabinets – if in use, keep the biosafety cabinets switched on however.
3. Exit the lab or office, close door behind you, but do not lock, but lock the lab office which has valuables.
4. Exit via the closest stairwell, DO NOT use the elevator.
5. Exit the building and move to a safe distance.
6. Report any informed knowledge you have of the incident to the Fire Marshall or by calling **x2000 or 519-840-5000**.
7. Do not re-enter the building until authorized to do so by a Fire Warden (identified by an armband) or an emergency official (e.g. Fire Marshall).

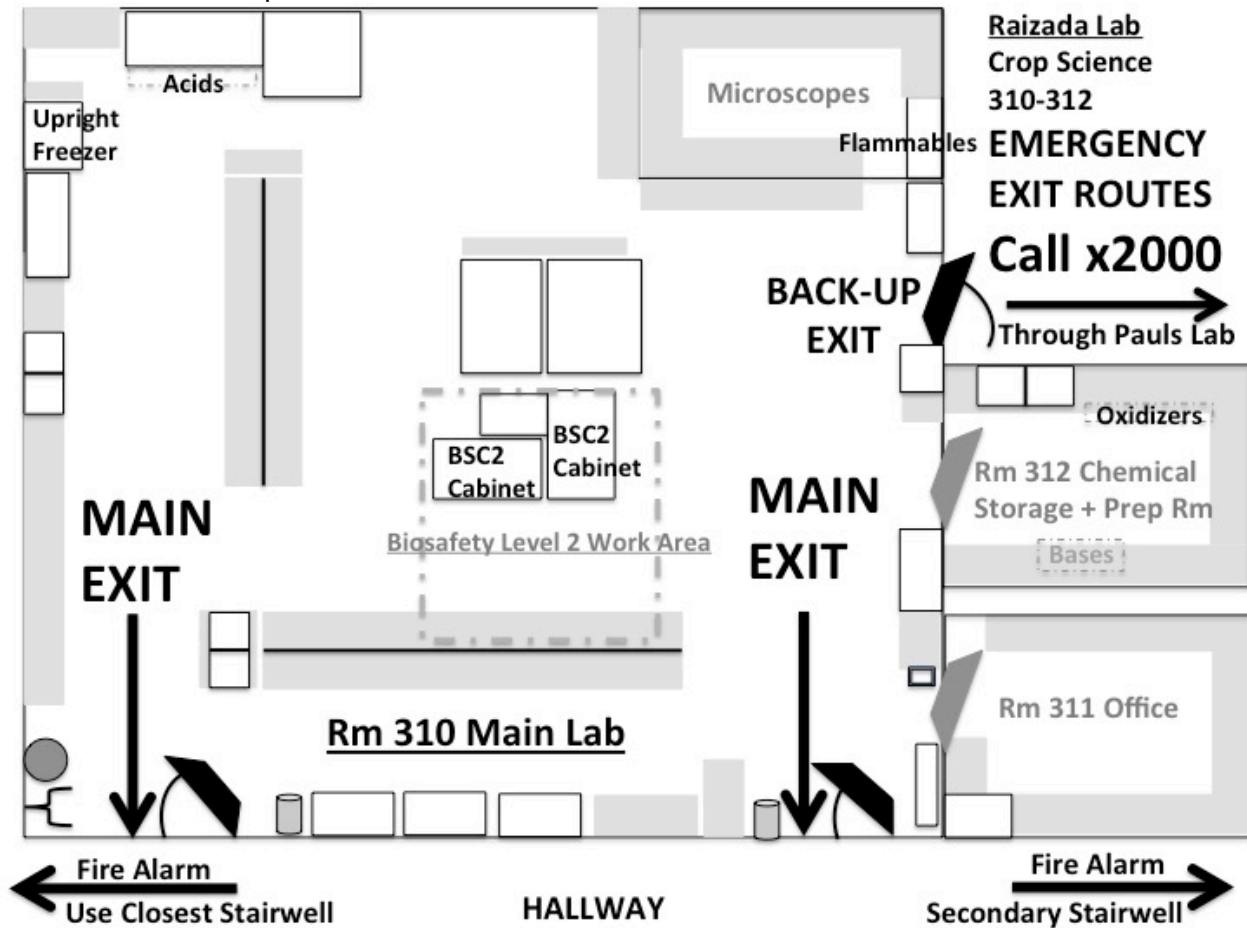
10.2. Emergency egress (exit) routes

See the map below and also posted in the lab:



10.3. Emergency/Safety Equipment in Room 310

See below and also posted in the lab:



Room 310 contains:

- Autoclave
- 2 Fire Extinguishers
- Fire Blanket
- Eye Wash Station
- Safety Shower
- First Aid Kit
- Chemical Spill Kit
- Biosafety Spill Kit
- Yellow Biohazard Sharps Containers
- Red (regular) Sharps Containers
- Broken Glass Disposal
- Biohazard Waste Container
- Material Safety Data Sheets /SDS Sheets
- Toolkit (across from autoclave)
- Flammables Cabinet (orange)
- PPE stocks

10.4 RESPONDING TO A FIRE

1. Sound the alarm at the closest pull station. Fire Alarm Pull Stations: located on the 3rd floor hallway close to both stairwells. If a fire alarm rings, immediate evacuation is mandatory. DO NOT try to get your personal belongings.
2. If a colleague in the lab is on fire, wrap them in a fire blanket located in front of the lab office OR roll on the ground rapidly to help extinguish the flames.
3. Notify others in the lab and move quickly to the nearest safe exit or stairwell.
4. If you need assistance to evacuate, move to the nearest safe stairwell and have a colleague or fire warden notify the emergency authorities of your location.
5. DO NOT use any elevators.
6. If you are last to leave an area or room, close doors behind you but do not lock to allow emergency personnel to enter easily.
7. Once outside, follow instruction of fire wardens and move well away from the building. We have people who voluntarily serve as Fire Wardens. During an evacuation they are identifiable by their bright orange hats; please follow their instructions and be respectful.
8. Re-entry to the building may proceed once the alarm bells have stopped ringing (for a minimum of 2 minutes) unless instructed otherwise by emergency response personnel.

Use of a fire extinguisher:

1. If a fire is small and controllable, you may use one portable extinguisher to put out the fire:
2. Before attempting to extinguish a fire ensure that the fire department has been notified, others in the vicinity have been alerted and the evacuation has begun.
3. Always have an exit behind you, if the fire does not go out you will be able to safely exit without having to navigate around the fire.
4. Make sure you are familiar with the operation of an extinguisher (now is not the time to figure it out) and that the extinguisher is suitable for the type of fire. Your safety is paramount, if it is unsafe or you are uncomfortable trying to put the fire out, don't.
5. To operate an extinguisher, use the P-A-S-S technique:
P - Pull the pin. Hold the extinguisher by the carrying handle and pull/twist the pin out. You do not want to place your hand on the trigger because this will make it impossible to pull the pin out.
A - Aim at the base of the fire. Aim the nozzle at the leading edge of the fire.
S - Squeeze the trigger. Fully depress the trigger with the palm of your hand and expel the entire extinguisher onto the fire.
S - Sweep back and forth. Sweep the extinguisher side to side; ensure that you extend beyond the edges of the fire to completely cover the base of the fire.

10.5. RESPONDING TO FLOODING

1. Notify campus police at Extension x2000 if severe, but if modest, then contact Physical Resources at x53282 so that the water source can be switched off.
2. The most immediate risk from water is electrical, so please ensure that there are no wires or electrical near the flood zone but DO NOT put yourself at risk of electrocution.
3. Electricity-non-conducting rubber gloves should be used when handling water to reduce the risk of electrocution. They are located on the shelf across from the autoclave in Room 310.
4. The main flooding risk in Room 310 is from the autoclave; in this case, please also contact Chris Grainger (cgrainge@uoguelph.ca) so that the autoclave service provider can be contacted to initiate a repair if needed.

10.6. RESPONDING TO LOSS OF POWER (INCLUDING PR WORK)

1. Close the sashes to the chemical fume hood and biosafety cabinets.
2. Please close the doors including the Lab Office Door, and exit the building using the stairwells.

3. If the interruption to chemical exhaust or biosafety hoods has resulted in a hazardous leak of chemicals, vapours or biohazards then treat this situation as an emergency and pull the fire alarm to ensure a prompt evacuation of the building and call x2000.
4. In non-emergency situations, contact Physical Resources (x53282).

10.7. RESPONDING TO A MEDICAL EMERGENCY

10.7.1. Quick Overview of medical emergency response

Obtain first aid assistance. The names and contact numbers of departmental first aiders are posted in our lab. Additionally, the locations of the University's first aid stations are:

- Student Health Services, JT Powell Building
- Occupational Health and Wellness (OHW), Alexander Hall
- Campus Police/Fire Prevention, mobile service

Students may obtain further medical treatment from Student Health Services. Employees can seek medical treatment or advice through Occupational Health and Wellness. For emergencies, dial x52000 and request emergency assistance.

In an emergency, do not attempt to transport the casualty to the hospital yourself. Dial x52000 and request an ambulance.

For non-critical injuries, notify Manish Raizada as soon as possible and ensure an [Incident Report](#) is submitted to OHW 24 hours. Don't wait for signatures if they are delayed:

<https://www.uoguelph.ca/hr/system/files/Incident%20Report%20Form%20-%20July%202020%20AODA.pdf>

Fax to 519-780-1796 or email to ohw@uoguelph.ca.

Specific Incidents:

- Cuts** – if someone suffers a severe cut, place pressure on the wound, and if possible elevate the wound above the heart.
- Punctures** – if the object is still lodged in the person's body, do not remove it. Call x52000 immediately and request medical assistance.
- Fainting** – if someone is about to faint, have them sit or lie down. If they have fainted in a seated position, steady them and put their head between their knees. If they have fallen to the ground, roll them to their back and elevate the legs 20-30 cm. If someone sustained an x52000 and request emergency medical assistance.
- Needle sticks** – Rinse the wound for 15 minutes and encourage bleeding. Determine whether it is a 'clean' or potentially 'dirty' needle. If the needle was potentially contaminated with an infectious substance, advise the victim to immediately contact Occupational Health and Wellness (x52647) or Student Health Services (x52131) and seek medical treatment. Outside of regular hours, advise the victim to seek immediate medical treatment (i.e. Emergency Room). Prophylaxis for hepatitis and HIV must be started as soon as possible following exposure.
- Seizures** – help the person to the floor and clear away nearby objects. Try to prevent the person from striking objects in the area and harming themselves during the seizure. Do not attempt to restrain the victim or force anything into their mouth. Placing any object in the

mouth of a seizure victim only increases the likelihood of choking. Dial x52000 immediately and request medical assistance; be sure to inform them if the victim is having trouble breathing or any other relevant details.

•**Choking** – call for help. If the airway is only partially obstructed and there is air exchange, encourage the victim to continue coughing. If the airway is fully obstructed, characterized by the inability to speak, cough, or breathe adequately, administer the Heimlich maneuver by alternating 5 back blows with 5 abdominal thrusts to dislodge an upper airway obstruction.

•**Cardiac emergency** - if someone is showing symptoms of a heart attack (sudden pain in arm, chest, neck, jaw or back; pale skin), call x52000 and request an ambulance be sent to your location. Get the person to a sitting position and remove any constrictive clothing. Monitor their pulse and provide reassurance. If the victim goes into cardiac arrest and you are trained, administer CPR until emergency authorities arrive. Campus Police and Fire Division vehicles are equipped with portable Automated External Defibrillators (AED) and provide mobile first aid services. Additionally, there are AED locations in different buildings on campus.

•**Active Threat** - If you become aware of a violent situation, such as an armed person on campus:

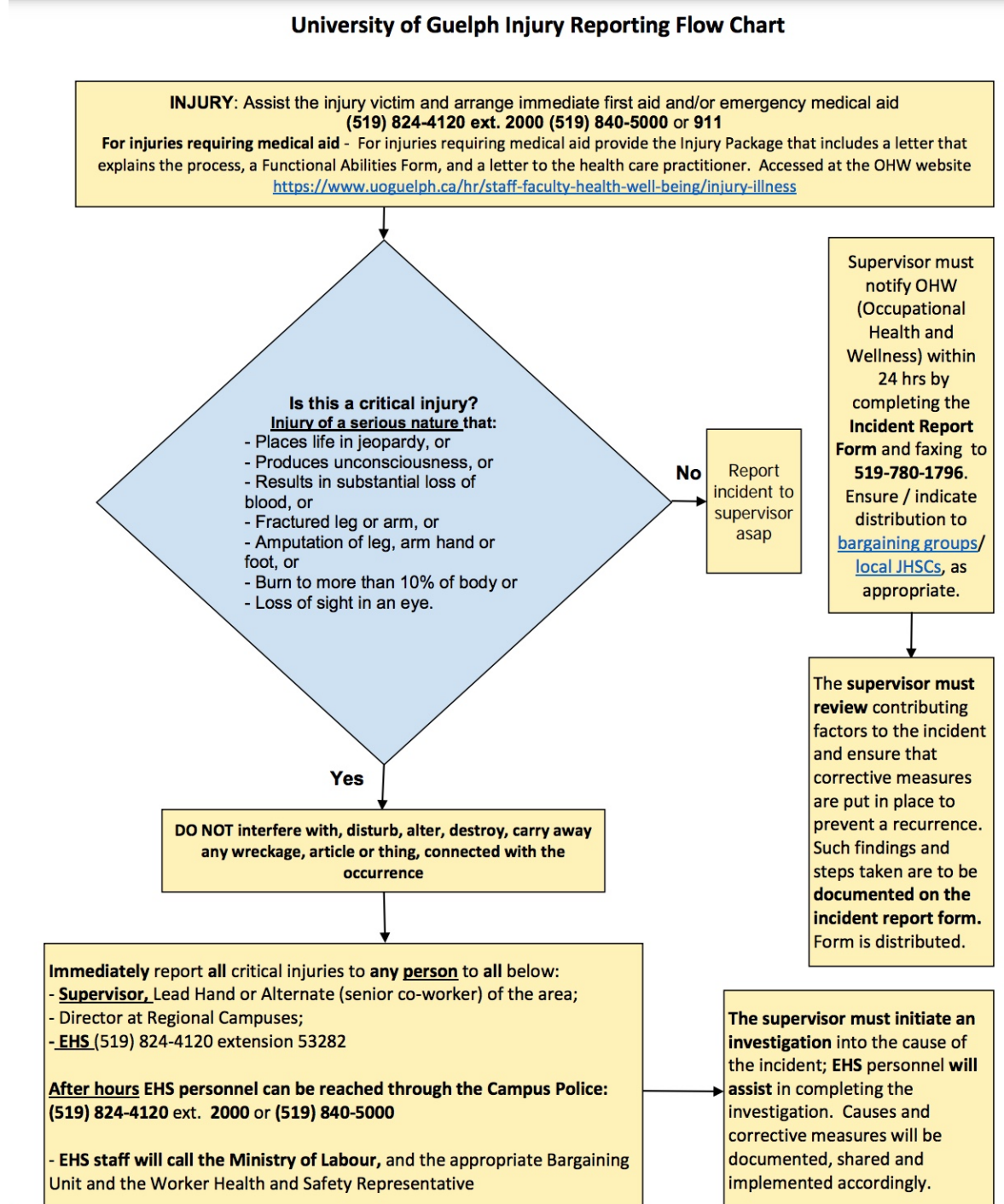
- If possible, lock yourself in the nearest safe room and stay out of sight.
- If safe to do so, dial x52000 and request help.
- If you are certain you can get to an exit safely, move quickly and evacuate the building.

10.7.2. Detailed Medical Emergency Response

10.7.2.1. MEDICAL ASSESSMENT DECISION TREE:

Assess the scene and determine the level of aid required referring to the public injury reporting flow chart – see the chart below and here is the PDF:

<https://www.uoguelph.ca/hr/sites/uoguelph.ca.hr/files/public/Injury%20Reporting%20Flow%20Chart%202016.pdf>



Complete an Injury/Illness Incident Report Form Within 24 hours:

<https://www.uoguelph.ca/hr/system/files/Incident%20Report%20Form%20-%20July%202020%20AODA.pdf>

Fax to 519-780-1796 or email to ohw@uoguelph.ca.

10.7.2.2. MINOR INJURIES:

Render first aid assistance if permission is granted.

Non-Student Researcher: If the injury requires further medical attention, the lab member may either contact Occupational Health Services on the first floor, room 179 in Alexander Hall, building 31 during regular working hours, **extension 52647**, or may follow up with the physician of his/her choice. If a hazardous material is involved, send a copy of the SDS/Material Safety Data Sheet with the casualty. Prior to receiving medical aid off campus, notify Occupational Health Services, extension 52647, during regular working hours. **After hours, phone extension 52000 for transportation and assistance.**

There is no charge to the employee for transportation to medical aid for a work related injury if warranted.

Student Researcher: If the injury requires further medical attention, the student may go for assessment during regular working hours to Student Health Services extension 52131. They will need a student ID and the UHIP card. **After hours, the student must use Guelph medical clinics**

<https://wellness.uoguelph.ca/sws/resources-category/medical-clinics>

If the student wishes to seek medical aid off campus, the student is responsible for his/her own transportation. If the student lives in Residence on campus, transportation assistance may be available. The student can contact his/her Residence Manager for information. If a hazardous material is involved, send a copy of the Safety Data Sheet with the casualty. If a student needs to leave the class, send an escort with the student if the injury warrants or if the student requests one. After hours, SafeWalk can accompany the student during the SafeWalk hours of operation (19:30 to 02:30). Phone extension 53200 to contact SafeWalk.

There are several "after hours" clinics in Guelph. These walk-in clinics are preferable to the Guelph General Hospital Emergency Room for minor injuries that occur outside regular working hours.

Reporting: Please report all incidents, including 'near misses'.

Complete an "Injury/Incident Report Form" for all incidents no matter how minor and fax within 24 hours to Environmental Health and Safety, 519 824-0364. Fax to 519-780-1796 or email to ohw@uoguelph.ca.

<https://www.uoguelph.ca/hr/system/files/Incident%20Report%20Form%20-%20July%202020%20AODA.pdf>

Report to the Department Chair and the supervisor within 24 hours.

10.7.2.3. SERIOUS ("CRITICAL") INJURIES:

CALL: Extension 52000 for assistance 24hours per day. The dispatcher will arrange for a taxi or ambulance as appropriate. The dispatcher will have campus police meet the ambulance or taxi to guide it to the scene of the accident. Do not call 911 directly. Ambulance and taxi drivers are not familiar with campus and valuable time can be lost while they try to find you.

RENDER FIRST AID: if permission is granted. Keep the casualty comfortable. Make sure their personal belongings are secure.

SDS: If a hazardous material is involved, send a copy of the Safety Data Sheet with the casualty.

PRESERVE THE SCENE: Do not allow anyone to interfere with, disturb, destroy, alter, or carry away any wreckage, article or thing at the scene or connected with the occurrence until permission to do so has been given by a Ministry of Labour inspector except for the purposes of preventing further harm relieving human suffering, or preventing unnecessary damage to equipment or other property.

REPORTING: Report the incident by telephone to Environmental Health and Safety, x53132, during regular working hours as soon as the casualty has been stabilized. If outside regular working hours, campus police will contact the appropriate agencies. Complete an "Injury/Incident Report Form" immediately. Fax to 519-780-1796 or email to ohw@uoguelph.ca.
<https://www.uoguelph.ca/hr/system/files/Incident%20Report%20Form%20-%20July%202020%20AODA.pdf>

10.8. Chemical Spills:

Our lab is equipped with Chemical Spill Kit (on the Safety Shelf near the lab entrance, see lab map above) for chemical, oil and solvent spills. Contents include:

- Small broom and dustpan
- Absorbent padding or paper
- Protective gloves
- Spill-X Solvent Adsorbent for treatment of solvent spills
- Spill-X Acid Neutralizer for treatment of Acid Spills
- Spill-X Caustic Neutralizer for treatment of caustic spills

These kits are **not** to be used for strong oxidizers.

Complex Spills:

- Considered too large (>4 Litres) or too hazardous to be cleaned up without special equipment.
- Phone extension 52000 for assistance in cleaning up these

Hydrochloric Acid Spills: Concentrated hydrochloric acid releases very corrosive vapours that necessitate the use of respiratory protection. Spills larger than a litre may require assistance, call extension 52000; otherwise use acid neutralizer located in the spill kits.

Flammable Solvent Spills: The only open flames permitted in our lab will be from small alcohol lamps to sterilize metals that will not fit into the Bactoincinerators; these are permissible only in the Biosafety Cabinets and Plant Tissue Culture Hoods on metal surfaces, not on lab benches. Use the solvent suppressant spill control material in the spill kit and place all used material in the fume hood until hazardous waste pickup can be arranged.

10.9. QUICK OVERVIEW OF BIOHAZARD SPILL RESPONSE (bacterial, fungal cultures) (also see p106 of the 2017 EHS Biosafety Manual)

The first consideration is your safety. If biohazardous material has spilled on you, remove contaminated clothing and thoroughly wash the affected areas. If material gets in your eye, flush at the eyewash for 15 minutes. If it is a spill that creates a hazard for others in the area (e.g. aerosolized pathogen), notify everyone in the lab and have them evacuate.

You want to avoid inhalation of pathogens. If the spill is in the lab and there is concern of airborne exposure, evacuate the room and allow 30 minutes for aerosols to settle. If the spill occurs in a biosafety cabinet, lower the sash as much as possible and ensure the fan is running for at least 10 minutes. If a tube of pathogenic material breaks in a centrifuge, put a sign on the equipment and leave the centrifuge closed for 30 minutes for droplets to settle before you attempt to clean the spill.

Specific directions are provided in the detailed SOPs below. However, the basic technique is to cover the spill with absorbent material, soak with a suitable chemical disinfectant, allow enough contact time for the disinfectant to work, then bag all waste and send for autoclaving. It is also important to notify your supervisor of the spill and complete an EHS Incident Report.

****Emergency biological spill response drill to be held in the first two weeks in January annually***

****See the Biological Spill Response Plan Posted in the CL2 zone in Room 310.***

****Consult the Biological Spill Kit located across from the Biosafety Cabinets***

****If the spill is outside the Biosafety Cabinet, ask other members of the lab to exit, put the Spill Hazard sign on all 3 entrances to the lab (these are in the Spill Kit) and lock the doors. The Biological Spill Kit contents are below. Then proceed below as follows:***



Room 310: The Biological Spill Kit also contains absorbent pads, a pail with lid, disposable coveralls, disposable boot covers, concentrated bleach and hydrogen peroxide (e.g. Spor-Klenz or PeridoxRTU, for decontaminating centrifuge and metal surfaces), and N95 masks and pre-made “Do not enter: Biohazard Spill” signs. 70% ethanol disinfectant is located in the flammables cabinet.

10.10. SPECIFIC BIOHAZARD SPILL SOPs:

10.10.1.If the spill occurs INSIDE the biological safety cabinet (see the Room 310 Biological Spill Kit on the map above)

1. Close the sash as much as possible and allow the cabinet to run for 15 minutes to allow the filters to remove aerosolized particles.
2. Alert persons in the immediate area and secure the area.
3. Put on appropriate clothing and personal protective equipment including eye protection, clothing, and nitrile gloves (double glove).

4. Use tongs or forceps to pick up broken glass and other sharp objects and place them in a puncture-proof yellow sharps container for decontamination.
5. Contain the spill using absorbent pads or paper towels from the Biological Spill Kit (see map). Place pads in an autoclave biohazard bag for decontamination.
6. Soak the spill with 1/10th diluted bleach or 70% ethanol or use peroxide if a spill concerns spores, and allow to stand for 15 to 20 minutes, then soak up with paper towel or absorbent pad.
7. Remove the absorbent pads or paper towels and place in a biohazard autoclave bag inside the cabinet. Clean up residual material working from the outside to the center of the spill using paper towels or absorbent wipes placing them carefully into the autoclave bag. Close the bag with tape, then wipe the outside of the bag with disinfectant before removing from the cabinet.
8. Decontaminate equipment and other non-disposables inside the cabinet before removing from the cabinet. Place in autoclave bags or trays as appropriate. Be careful to ensure that contaminated objects are not taken out of the cabinet before being treated.
9. Wipe down the cabinet with disinfectant-soaked wipes again to remove any residual material. Treat the wipes as contaminated.
10. Lift the exhaust grill and the tray and clean with disinfectant (see the grille cleaning SOP below). Do not open other internal surfaces of the cabinet; this may only be performed by knowledgeable NSF-certified cabinet maintenance personnel after a gas-decontamination.
11. Autoclave all waste and equipment for final decontamination.
12. Remove contaminated personal protective equipment. Soak in disinfectant in the sink for 20min and/or autoclave before sending to the laundry.
13. Wash hands and any other area that may have been contaminated with disinfectant soap and/or alcohol gel.
14. Seek medical attention immediately as per the medical surveillance plan if there is any possibility of exposure to the agents.
15. Complete an incident report as per University incident reporting procedures:
<https://www.uoguelph.ca/hr/system/files/Incident%20Report%20Form%20-%20July%202020%20AODA.pdf>
 Fax to 519-780-1796 or email to ohw@uoguelph.ca.
16. Provide the PI a list of all personnel present in the room at the time of the release for further follow-up.

10.10.2. If the spill occurs OUTSIDE the biological safety cabinet (see the Room 310 Biohazard Spill Kit on the map above)

Spills outside the biological safety cabinet may generate aerosols that are dispersed in the laboratory. All persons who are not involved in the spill mitigation must leave the area until declared safe for re-entry.

1. Hold your breath and move away from the spill. Alert persons in the laboratory and secure the area. Close laboratory doors and post warning signs to prevent entry.
2. Notify Manish Raizada or designate immediately so that he/she may provide assistance.
3. Remove any contaminated clothing and place in autoclave bags for decontamination.
4. Vacate the laboratory for at least 1 hour to allow aerosols to be carried away and for heavier particles to settle.
5. Put on appropriate clothing and personal protective equipment including eye protection, clothing, impermeable gloves (double glove) and fitted N95 mask. Air-purifying respirators may only be used if personnel are enrolled in the respirator program through Environmental Health and Safety (Occupational Hygienist, x54855)
6. Use tongs or forceps to pick up broken glass and other sharp objects and place them in a puncture-proof sharps container for decontamination.
7. Contain the spill using absorbent pads. Place pads in an autoclave bag for decontamination.
8. Soak the spill with 1/10th bleach or 70% ethanol and allow to stand for 15 to 20 minutes, then soak up with paper towel or absorbent pad.

9. Remove the absorbent pads/paper towels and place in a biohazard autoclave bag. Clean up residual material working from the outside to the center of the spill using paper towels or absorbent wipes placing them carefully into the autoclave bag. Close the bag with tape and then wipe the outside of the bag with disinfectant.
10. Decontaminate equipment and other non-disposables by wiping down with disinfectant and/or autoclaving. Treat all wipes as contaminated waste.
11. Decontaminate surfaces and other items such as bottles, equipment, bench tops, and furniture in the vicinity of the spill if there is any likelihood of contamination.
12. Autoclave all waste and equipment for final decontamination.
13. Remove contaminated personal protective equipment. Soak in disinfectant in the sink for 20min and/or autoclave before sending to the laundry.
14. Wash hands and any other area that may have been contaminated with disinfectant soap and/or alcohol gel. If showers are available, wash all exposed areas of the body with germicidal surgical scrub or soap and water.
15. Contaminated Shoes: Step on disinfectant soaked paper towels or soak spill with disinfectant. If necessary, soak shoe soles in bath containing disinfectant for 20 minutes. Dry all surfaces and 'footprints' before leaving the lab. Do not walk out of the lab until shoes are thoroughly decontaminated.
16. Seek medical attention immediately as per the medical plan if there is any possibility of exposure to the agents.
17. Complete an Injury/Illness Incident Report Form within 24 hours:
<https://www.uoguelph.ca/hr/system/files/Incident%20Report%20Form%20-%20July%202020%20AODA.pdf>
Fax to 519-780-1796 or email to ohw@uoguelph.ca.
18. Provide the PI a list of all personnel present in the room at the time of the release for further follow-up.

10.10.3. If the spill occurs INSIDE a centrifuge (see the *Room 310 Biological Spill Kit on the map above*):

1. Close the lid of the centrifuge immediately.
2. Alert persons in the laboratory and secure the area. Close laboratory doors and post warning signs to prevent entry.
3. Vacate the room for at least 30 minutes to allow aerosols to settle.
4. Notify Manish Raizada or designate immediately so that he/she may provide assistance.
5. Wear personal protective equipment including lab coat, nitrile gloves (double glove), eye protection and fitted N95 mask.
6. Use tongs or forceps to pick up broken glass and other sharp objects and place them in a puncture-proof sharps container for decontamination.
7. Contain the spill using absorbent pads or paper towels. Place pads in an autoclave bag for decontamination.
8. Decontaminate the inside of the centrifuge by wiping rotors and buckets with disinfectant. **DO NOT USE BLEACH ON METAL. You can use 70% ethanol or hydrogen peroxide (located in the Biohazard Spill Kit).** Remove the rotors and buckets to the biological safety cabinet for a more thorough cleaning. Clean the inside of the centrifuge with the disinfectant. Use a minimum contact time of 20 minutes. Do not use bleach unless the equipment is designed to withstand the corrosive properties. Never use bleach on ultra-centrifuge rotors or buckets.
9. Autoclave all waste and equipment for final decontamination or place in burn boxes for incineration as per departmental procedure.
10. Remove contaminated personal protective equipment. Soak in disinfectant in the sink for 20min and/or autoclave before sending to the laundry.
11. Wash hands and any other area that may have been contaminated with disinfectant soap and/or alcohol gel.

12. Seek medical attention immediately as per the medical surveillance plan if there is any possibility of exposure to the agents.

13. Complete an Injury/Illness Incident Report Form within 24 hours:

<https://www.uoguelph.ca/hr/system/files/Incident%20Report%20Form%20-%20July%202020%20AODA.pdf>

Fax to 519-780-1796 or email to ohw@uoguelph.ca.

14. Provide the PI a list of all personnel present in the room at the time of the release for further follow-up.

11. RAIZADA LAB FIRE PREVENTION

1. Review the Room 310 maps for emergency fire exits, fire extinguisher, fire blanket and fire alarm locations.

2. Extension cords should never be connected in series and only for temporary use, and the connection point should be away from water or potential future spills/floods (so never on the ground).

3. There are no gas Bunsen burners in the lab.

4. Small open alcohol flames are permitted in the plant tissue culture laminar flow hoods and biosafety cabinets (to sterilize larger metal tools) but should be minimized, and kept distant from any flammables.

5. All flammable liquids should be stored in the orange Flammables cabinet in the back of the lab, and any overflow stocks near the Loading Stock (speak to Sue Couling for space).

6. If you are walking by the flammables cabinet, please always double check that it is locked.

7. The maximum volume of 70% ethanol allowable outside the flammables cabinet when not in use is 100ml and it must be clearly labelled.

8. Alert lab personnel and Manish Raizada if you observe larger volumes of flammables on someone's bench, and immediately move it to the Flammables Cabinet. Fire prevention is a shared responsibility.

9. Please ensure that oxidizers and flammables are never in close proximity in case there is a spark. Consult the lab map for their storage locations.

10. Do not leave water baths on overnight.

11. Reduce cardboard clutter in the lab as much as possible.

12. RAIZADA LAB AUTOCLAVE, CYLINDER & CRYOGENIC SAFETY

12.1. Autoclave Operating Procedure

1. Physical resources is responsible for steam/water pipes (call x53854), while the Autoclave Service Provider for problems with the machine itself (contact Chris Grainger to schedule a technician visit: cgrainge@uoguelph.ca)

2. There are 4 autoclaves in the building. Do not use any autoclaves without an experienced lab member providing training, read the instructions posted and complete the login sheet posted.

3. When opening an autoclave, first put on heat insulating (orange) gloves, located directly across from the autoclave in Room 310, stand back, and then open slowly to release any hot steam to prevent burns. A face shield is also available.

4. Any materials should be located in a secondary autoclave tray to capture overflow liquids or broken glass: we have previously paid thousands of dollars in repair costs when this practice was not respected.

5. Prior to autoclaving bottles, loosen the lids to 2/3rd of their capacity to prevent a seal from developing. Remember to seal after autoclaving.

6. Place autoclave indicator tape on the tops of materials.

7. Contaminated waste containing RG2 microbes should be immediately autoclaved in Room 310 if possible to minimize transport outside of the lab. They should be placed in a biohazard bag with a tracking sticker and autoclave tape, then finally delivered downstairs to the autoclave area, where it will be disposed by Sue Couling. Delivery should be in secondary containment.
8. Biohazardous waste especially RG1 microbes can be placed in a biohazard bag with a tracking sticker and delivered to the downstairs autoclave area, where it will be autoclaved and disposed by Sue Couling. Delivery should be in secondary containment.

12.2. Gas Cylinder Operating and Transport Procedure

1. Room 310 typically has one compressed CO₂ cylinder for making dry ice.
2. Gas cylinder regulators are single stage or dual stage: one indicators pressure that is being delivered and the other the pressure in the cylinder – dual stage allows for a step-down in pressure that is delivered.
3. Do not empty cylinder completely.
4. Do not expose to high temperature.
5. Remove the regulator when not in use or when being transported
6. Transport using the appropriate cylinder-specific cart and chain both cylinders;
7. In the lab, ensure that the cylinder is secured to the wall.
8. Spare cylinders are located on the 1st floor and empty cylinders should be returned there; contact Sue Couling (scouling@uoguelph.ca).

12.3. Cryogenic Operating Procedure (liquid nitrogen, dry ice)

>Dry Ice Safe Operating Procedure

1. Touching dry ice can result in frost bite. Please use insulated gloves when handling.
2. The dry ice maker can spew small pieces of ice, so always wear Safety Glasses.
3. Never store dry ice in a refrigerator/freezer (especially deep chest freezers). Dry ice will sublime at -78°C and could asphyxiate the person opening the equipment.

>Liquid Nitrogen Safe Operating Procedure

1. When dispensing liquid nitrogen from the large dewar, always LEAVE THE DOOR OPEN
2. Touching liquid nitrogen result in frost bite or nerve damage. Please use insulated gloves when handling.
3. Liquid nitrogen can expand to 800x leading to cracking/explosion if put into an improper container, so need the proper dewar flasks. Please also use Safety Glasses.
4. Cylinders and dewars should not be filled to more than 80% of capacity with liquid nitrogen, since expansion of gases during warming may cause excessive pressure build-up.
5. Boiling and splashing always occur when charging or filling a warm container with liquid nitrogen or when inserting objects into liquid N. Perform these tasks slowly to minimize boiling and splashing.
6. Use tongs to withdraw objects immersed in liquid nitrogen.

13. RAIZADA LAB CHEMICAL SAFETY

13.1. Chemical Safety Quick Tips

- Know the hazards of the materials with which you are working. Review the SDS prior to handling a new chemical.
- Don't accumulate unnecessary inventory - check to see if the lab already has the chemical, and order only as much as you need.
- Never store incompatible materials together. Acids CANNOT be stored with bases; flammables CANNOT be stored with oxidizers.
- Never put flammable solvents in a fridge unless the fridge is specifically designed to accommodate flammables which Room 310 does not. There many recorded cases of fridges

and freezers exploding.

- ADD acids to water, NEVER water to acids.
- Chloroform is acutely toxic if breathed for long periods, and carcinogenic if ingested. It can cause you to be dizzy/faint when breathed in. Use in the Chemical Hood.
- Formalin/Formaldehyde is a carcinogen. Use in the Chemical Hood.
- Acrylamide powder is a neurotoxin though safe once polymerized.
- Never return unused chemicals to the reagent bottles.
- Always use the fume hood for dangerous experiments, in particular, those which give off toxic, flammable or nauseating vapours (chloroform, beta-mercapthoethanol, osmium tetroxide, phenol).
- Corrosive liquids can be particularly hazardous as they act rapidly upon contact. Examples of common corrosive liquids are:
 - Strong acids (hydrochloric acid, nitric acid, etc.)
 - Strong bases (aqueous sodium hydroxide, potassium hydroxide, ammonia, etc.)
 - Strong oxidizing agents (peroxides, etc.)
- Ammonia is a corrosive gas if inhaled.
- Explosives (ammonium nitrate, urea) should be stored in a secondary container in a secure, dry location away from other groups.
- Never “sniff-test” a chemical.










13.2. Chemical Exposure Response (also see Emergency Response Section above)

1. In case of minor or major skin exposure, run under water in the sink or safety shower as appropriate continuously for 15min.
2. In case of eye exposure, use the eye wash continuously for 15 min.
4. First Aid is permitted, seek medical health as appropriate and report the incident to Manish Raizada (see Emergency Response Section above).
5. Complete an Injury/Illness Incident Report Form within 24 hours:
<https://www.uoguelph.ca/hr/system/files/Incident%20Report%20Form%20-%20July%202020%20AODA.pdf>
Fax to 519-780-1796 or email to ohw@uoguelph.ca.

13.3. Chemicals - General Lab Safety and Standard Operating Procedures

1. In case of power failure or water shutdown, all lab work must stop since there may be failures with the eyewash station and safety shower. It may also cause a ventilation malfunction, so close the sash to the fume hood.
2. Be aware of the chemical and safety properties of chemicals, before working with them, aided by the GHS pictograms below and SDS/MSDS sheets including when they are combustible, flammable, toxic, carcinogenic or corrosive. **Here they are again as a reminder:**

GHS Pictograms and Hazards

Flame	Exclamation Mark	Health Hazard
 <ul style="list-style-type: none"> Flammable Self-Reactive Pyrophoric Self-Heating In Contact with Water, Emits Flammable Gases Organic Peroxide 	 <ul style="list-style-type: none"> Irritation (skin or eyes) Skin Sensitization Acute Toxicity (harmful) Specific Target Organ Toxicity – Single Exposure (drowsiness or dizziness, or respiratory irritation) Hazardous to the Ozone Layer 	 <ul style="list-style-type: none"> Carcinogenicity Respiratory Sensitization Reproductive Toxicity Specific Target Organ Toxicity – Single or Repeated Exposure Germ Cell Mutagenicity Aspiration Hazard
Skull & Crossbones	Exploding Bomb	Flame Over Circle
 <ul style="list-style-type: none"> Acute Toxicity (fatal or toxic) 	 <ul style="list-style-type: none"> Explosive Self-Reactive (severe) Organic Peroxide (severe) 	 <ul style="list-style-type: none"> Oxidizer
Corrosion	Gas Cylinder	Environment
 <ul style="list-style-type: none"> Corrosive (skin, eyes, or metals) 	 <ul style="list-style-type: none"> Gas Under Pressure 	 <ul style="list-style-type: none"> Hazardous to the Aquatic Environment (acute or long-term)

3. MSDS sheets have been replaced by SDS sheets which are located via Chemwatch here (you must sign in via the EHS website):

<https://www.uoguelph.ca/hr/hr-services-environmental-health-safety-portal/safety-data-sheets>

See the lab poster entitled, “(“Looking for an MSDS/Safety Data Sheet?”)

4. Hard copies of the MSDS/SDS sheets for commonly used chemicals in Room 310 are located on the safety shelves near the entrance to the lab (see map)

5. Currently the chemicals present in the lab are listed on our chemical inventory sheets posted on the Lab Safety Board (see map) and also kept in the Room 310 desktop. In the future, we will migrate our chemicals in the UofG Online Chemical Inventory System.
6. Wear long pants, socks, closed shoes, secure hair back, no loose jewelry and no contact lenses.
7. There should be no open flames in the lab, except small alcohol lamps placed in the plant tissue culture flow hoods. Use the Bacto-incinerator for all other work.
8. Working alone is prohibited for acute toxins, highly reactive or explosive, highly corrosive, or open flames with flammable materials.
9. When working with volatile or toxic/carcinogenic substances, work deep inside the chemical fume hood. The chemical flow hood should be left ON at all times. The sash needs to be mostly closed for it to function. Prior to use, ensure that there is a Kimwipe attached to the inside wall of the hood and that it is floating, as an indicator of exhaust air flow. Work 15 cm back from the front edge. Completely close the sash when the fire alarm goes off. Fume hoods on campus are equipped with a warning device that will alarm when the airflow is inadequate for the hood to function as designed; notify Physical Resources x53854.
10. Chloroform (if ingested), acrylamide and formaldehyde are suspected carcinogens, so use in the chemical flow hood.
11. Ethidium bromide is no longer permitted in our lab and has been replaced with Red Safe.

13.4. Chemical Spill Containment and Cleanup Procedure

Also see Emergency Response Procedures (above)

Room 310 is equipped with Chemical Spill Kit (on the Safety Shelf near the lab entrance, see lab map above) for chemical, oil and solvent spills. Contents include:

- Small broom and dustpan
- Absorbent padding or paper
- Spill-X Multi-Purpose Spill Treatment Kit (Acid Neutralizer, Alkali Neutralize, Vapour Suppressor, Solvent suppressor)

These kits are **not** to be used for strong oxidizers.

Complex Spills:

- Considered too large (>4 Litres) or too hazardous to be cleaned up without special equipment.
- Phone extension 52000 for assistance in cleaning up these

Hydrochloric Acid Spills: Concentrated hydrochloric acid releases very corrosive vapours that necessitate the use of respiratory protection. Spills larger than a litre may require assistance, call extension 52000; otherwise use acid neutralizer located in the spill kits.

Flammable Solvent Spills: We should have no open flames in our lab. Use the vapour suppressant spill control material in the spill kit and place all used material in the fume hood until hazardous waste pickup can be arranged.

13.5. Safe Chemical Mixing, Storage and Labelling

1. Only 100 ml bottles of 70% ethanol are permitted outside of the flammables cabinet.
2. Flammable liquids have a flashpoint of less than 37.8°C Please ensure that all flammables including alcohols (ethanol, methanol, iso-propanol, etc) are stored in the flammables cabinet. This also includes acetone, benzene, acetonitrile, formamide, toluene, xylene, hexane, acetic acid, and diethyl ether. **Organic acids** such as acetic acid, butyric, and formic acids are

combustible materials and should be stored in a flammable storage cabinet. In fact any organic acid can be stored with the flammable solvents. **Non-flammable solvents** include chloroform and methylene chloride which do not need to be kept in a flammable storage cabinet.

3. Flashpoints of common chemicals are: ethanol (13°C), methanol (11°C), acetone (-18°C), glacial acetic acid (39°C), diethyl ether (-45°C), ethylacetate (-45°C).

4. Explosives (ammonium nitrate, urea, silver nitrate) should be stored in a secondary container in a secure, dry location away from other groups.

5. Bases are incompatible with acids, flammables and oxidizers and should be stored on their own away from water sources (e.g. under sink). Examples: sodium hydroxide, potassium hydroxide, ammonium hydroxide, and organic amines.

6. Cyanide containing materials release hydrogen cyanide gas when mixed with acids.

Examples: sodium cyanide, potassium ferricyanide, potassium ferrocyanide, and sodium thiocyanate.

7. ADD acids to water, NEVER water to acids.

8. There are special locations for acids, bases, and oxidizers in the lab (see Room 310 map).

9. Lab chemicals must be segregated according to the chart below (the EHS Lab Manual 2018 has a list of incompatible chemicals on Table 14 on p80):

Table 1. Required segregation of lab chemicals:

	Water reactive / Pyrophoric	Explosives	Flammables (solids & liquids)	Oxidizers (solids & liquids)	Corrosive Acids (solids & liquids)	Corrosive Bases (solids and liquids)	Non-flammable solvents & regulated chemicals	Low-hazard solids & liquids
Water reactive / Pyrophoric	✓	x	x	x	x	x	x	x
Explosives	x	✓	x	x	x	x	x	x
Flammables (solids & liquids)	x	x	✓	x	x	✓	✓	x
Oxidizers (solids & liquids)	x	x	x	✓	x	x	x	Secondary containment required
Corrosive Acids (solids & liquids)	x	x	x	x	✓	x	x	< 2 M acidic solutions
Corrosive Bases (solids and liquids)	x	x	✓	x	x	✓	Secondary containment required	< 2 M caustic solutions
Non-flammable solvents & regulated chemicals	x	x	✓	x	x	Secondary containment required	✓	✓
Low-hazard solids & liquids	x	x	x	Secondary containment required	< 2 M acidic solutions	< 2 M caustic solutions	✓	✓

Refer to the [Lab Safety Manual for further information](#) 

10. In particular, all oxidizers must be kept away from flammable/combustibles (e.g. nitrate) since they can be explosive if needed, or with friction or a spark. Oxidizers include bleach, chlorine, potassium permanganate, perchloric/chromic/nitric acids. Remember to wear protective clothing when using an oxidizer as it can irritate skin/eyes.

11. All oxidizers should be in secondary containment like stainless steel trays separating each type of oxidizer. Oxidizers (liquid and solid) can be stored in secondary containment on a lab shelf or dedicated oxidizer cabinet.
12. Corrosives in our lab include sulphuric acid, acetic acid, NaOH, ammonia, KOH, and phenol. Wear gloves and safety glasses at all times with these chemicals.
13. Mineral acids (e.g. HCl) should be stored in a dedicated acid cabinet in secondary containment, and are incompatible with bases such as NaOH, NH₄OH, flammables and oxidisers. They must be stored separately away from water sources (e.g. under sink) and in some cases from each on plastic trays. Examples – hydrochloric acid, nitric acid, sulphuric acid, phosphoric acid, perchloric acid.
14. If storing or transporting any chemical larger than 3 ml, please note all contents (e.g. NaCl, TrisHCL, 30% glycerol).
15. If a commercial chemical is not in the original container, a workplace label is required that contains the product name, precautionary measures, reference to MSDS.
- 16 Non-flammable solvents such as dichloromethane and dimethylformamide can be stored with secondary containment on lab shelves.

13.6. Chemical waste procedures (additional details below)

1. Waste cannot be put down the drain that has a pH of <5.5 or >9.5, has a strong odour, contains dyes/pigments, exceeds a set concentration that is considered dangerous to humans, animals or the environment, or is hazardous as governed by the Guelph Sewer Use Bylaw. If not sure, ask Manish Raizada or EHS.
2. Hazardous waste is defined by the 9 SDS pictograms.
3. Hazardous lab waste is picked up by EHS. Please package the waste appropriately, label with a yellow tag from EHS, store safely (e.g. chemical safety hood), and submit a disposal form to EHS (all forms/tags available from the EHS website): <https://www.uoguelph.ca/hr/hr-services-environmental-health-safety-programs-laboratory-safety/hazardous-waste>
4. Sharps containers are autoclaved and submitted via chemical waste by EHS.
5. Broken glass should be placed in the broken glass container and is removed by custodial services.
6. Biohazard waste should be autoclaved then can go into non-hazardous waste but should have clear autoclave tape on it.

14. RAIZADA LAB BIOHAZARD CONTAINMENT LEVEL 2 (CL2) STANDARD OPERATING PROCEDURES

****Consult the University of Guelph Biosafety Manual for Additional Details and Procedures:**

<https://www.uoguelph.ca/hr/hr-services-environmental-health-safety-programs/biosafety-forms>

DETAILED CL2 SOPs

14.1. Definitions of biohazard risk groups and containment levels

•Excerpts from Public Health Agency Canada Laboratory Biosafety Standard, 2nd Edition 2015: <https://www.canada.ca/en/public-health/services/canadian-biosafety-standards-guidelines/secondedition.html>

•Risk Group 1 (RG1 low individual and community risk)

Microorganisms that are a) not capable of causing disease or b) unlikely to cause disease in healthy workers or animals. These agents can be handled on an open bench top in a well-designed and functional laboratory using the practices normally employed in a basic microbiology laboratory. Also referred to as Containment Level 1 (CL1). RG1 pathogens are not regulated by CFIA or the Public Health Agency of Canada.

•Risk Group 2 (moderate individual risk, limited community risk)

A pathogen that can cause human or animal disease but, under normal circumstances, is unlikely to be a serious hazard to laboratory workers, the community, livestock, or the environment. Laboratory exposures rarely cause infection leading to serious disease; effective treatment and preventative measures are available and the risk of spread is low. Work with these agents should be done in a Containment Level 2 (CL2) laboratory, with a biological safety cabinet available for manipulations that may create an aerosol. Risk Group 2 bacteria are not uncommon isolates from natural sources such as soil, water, food, and your body. When working in a lab that uses Risk Group 2 bacteria you must be aware of the particular bacteria involved, and the particular risks posed by these bacteria. The primary exposure hazards associated with organisms requiring CL2 are through the ingestion, inoculation and mucous membrane route. Agents requiring CL2 facilities are not generally transmitted by airborne routes, but care must be taken to avoid the generation of aerosols (aerosols can settle on bench tops and become an ingestion hazard through contamination of the hands) or splashes. Primary containment devices such as Biological Safety Cabinets (BSCs) and centrifuges with sealed rotors or safety cups are to be used as well as appropriate personal protective equipment (i.e., gloves, laboratory coats, protective eyewear). As well, environmental contamination must be minimized by the use of handwashing sinks and decontamination facilities (autoclaves).

•Risk Group 3 (high individual risk, low community risk)

A pathogen that usually causes serious human or animal disease. There is no CL3 facility on the University of Guelph campus.

•Risk Group 4 (high individual risk, high community risk)

Viruses that usually produces very serious human or animal disease, often untreatable. There is no CL4 Containment Facility on campus and only one in all of Canada in Winnipeg.

•For which RG2 microbes may truly be pathogenic to humans, please consult:

Database of Pathogen Safety Data Sheets (Government of Canada):

<https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/pathogen-safety-data-sheets-risk-assessment.html>

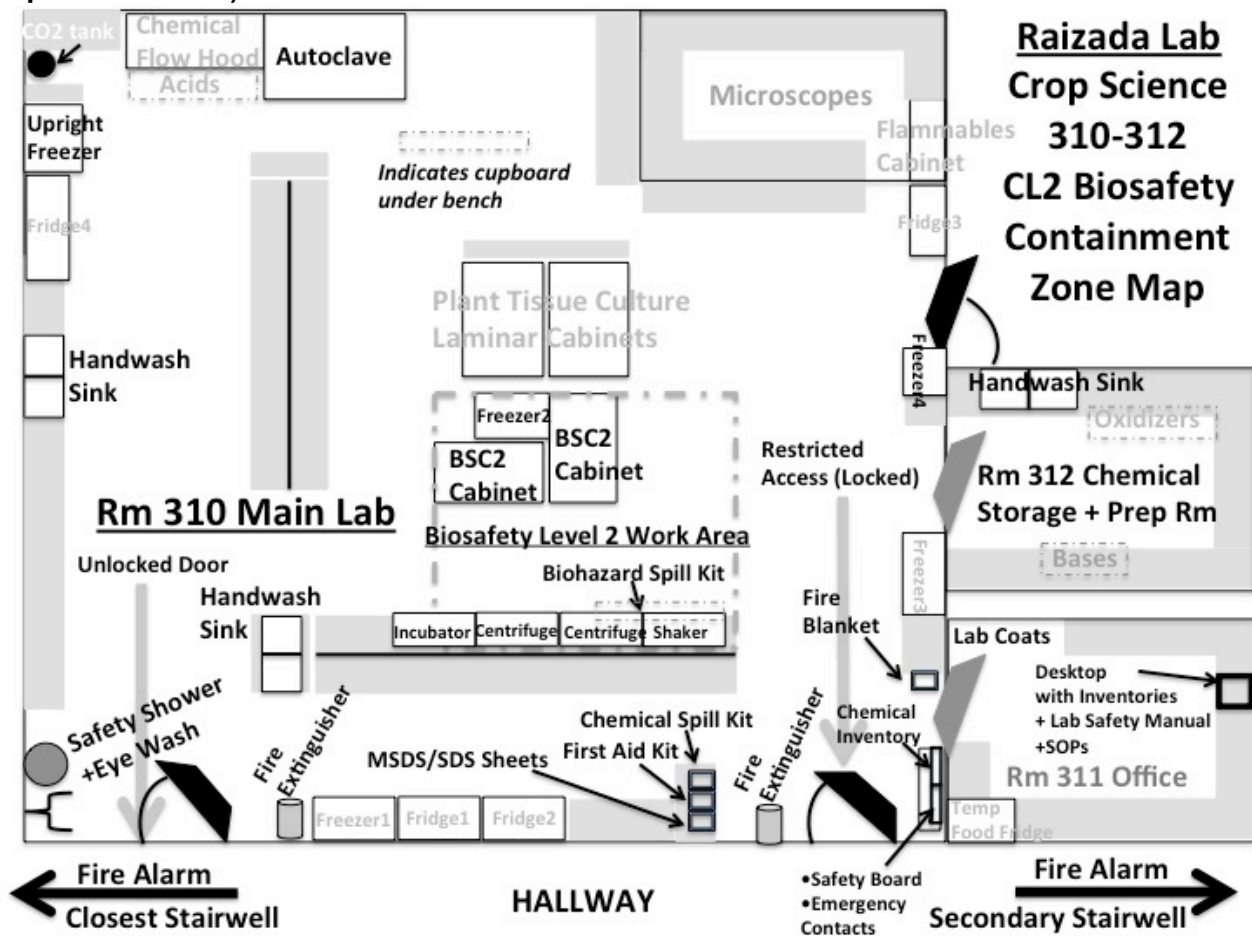
• Microbes on this database that are potential human pathogens and common endophytes include:

--**Aspergillus fungi**
--*Bacillus anthracis*
--*Bacillus cereus*
--*Bacteroides spp.*
--*Burkholderia/Pseudomonas mallei*
--*Burkholderia (Pseudomonas) pseudomallei*
--**Citrobacter spp.**
--*Clostridium spp.*
--**Enterobacter spp.**
--*Fusobacterium spp.*
--**Klebsiella spp.**
--**Lactobacillus spp.**
--*Micrococcus spp.*
--*Mycobacterium spp.*
--**Pseudomonas spp.** (excluding *B. mallei*, *B. pseudomallei*)
--**Serratia spp.**
--*Staphylococcus aureus*
--*Streptococcus spp.*
--*Yersinia spp.*

14.2. Location of work: CL2 map and features

1. Work with RG2 microbes is to take place in Room 310, at the active CL2 work zone which is in the center of the lab and marked by tape on the floor (see map below). Opening Petri dishes or tubes or any activity that can cause exposure to potential aerosols of RG2 microbes, is strictly limited to the Biosafety Cabinets.
2. As indicated in the map below, Room 310 contains no windows and has: appropriate PPEs for CL2, an overhead shower, eye wash, autoclave, Biosafety signage on all entrances and in the lab, two Class II Type A Biosafety cabinets (Canadian Cabinets BM4-2A-49) with active certification and benchtop keepers for biohazard bags, biohazard waste bin that is segregated from the general waste stream, handwashing sinks with soap, locked/closed doors, storage spaces for PPE, coat hooks for lab coats near an exit, decontamination technologies, a Biosafety Spill Kit, and locked -80°C freezers. The active CL2 work zone within the lab is marked by tape and has all the equipment required for routine work including a bacterial shaker, centrifuge with a sealed rotor, incubators, electroporator, biosafety cabinets and a biohazard waste bin.
3. The CL2 maps are below:

Room 310 CL2 map (the active CL2 work zone is in the center of the lab and marked by tape on the floor):



14.3. CL2 Entry/Exit Requirements and SOP

There are no windows in Room 310-312 or in Room 422 or any of the labs in the Crop Science Building.

1. The hallway doors into Room 310 must be kept closed and locked at all times, even when the lab is hot.
2. Only Raizada Lab personnel who have passed EHS Biosafety Certification (which must include anyone authorized to work in Room 310) are permitted to enter into the Lab using the entrance closest to the CL2 Biosafety 2 working area.
3. All other personnel including building personnel interested in using the Room 310 autoclave or microscope facility must enter using the distal entrance (near the Lab Safety Shower) or back entrance to Room 310 and must complete the log in/out sheets located at these entrances.
4. Gloves should be removed when exiting Room 310 and placed into the Biohazard Waste.
5. Before exiting the lab, especially if the Biohazard Waste has just been opened, wash hands thoroughly for 30 seconds with soap (preferred) or alcohol gel.
6. Upon entering the lab, full PPE must be worn (see CL2 SOP below).

14.4. CL2 Standard Operating Procedures

14.4.1. CL2 General Biosafety Precautions

1. Minimize your work and time in the CL2 work zone (see lab map) unless you are working with live microbial cultures or other biohazards (e.g. we have a second centrifuge).
2. Personal items or belongings are restricted in Room 310 (e.g. coats, bags, electronic equipment, except during the Covid pandemic). Outside coats and belongings should be stored in Room 311 or your personal office.
3. Eating or drinking, storing of either food, personal belongings, or utensils, applying cosmetics, and inserting or removing contact lenses are no longer permitted in Rooms 310-312, except during Covid where eating/drinking/food storage is permitted in Room 311 (lab office) to minimize community spread of the virus. After Covid, only drinking water from closed water bottles will be permitted in Room 311 (lab office) to reduce the need to exit/re-enter the lab.
4. You must remove your gloves, wash or disinfect your hands for 30 seconds and remove any contaminated PPE prior to entering Room 311 to minimize spread of microbes onto desktops and paper, and because drinking water is permitted in this room (and eating during Covid).
5. Long hair should be tied back or restrained so that it cannot come into contact with hands, specimens, containers, or equipment.
6. Children less than 16 years of age are not permitted in the laboratory.
7. Doors to Room 310 must NOT be propped open (this does not apply to internal within the laboratory).
8. Open wounds, cuts, scratches, and grazes should be covered with waterproof dressings.
9. The Lab is to be kept clean and tidy.
10. Storage of materials that are not pertinent to the work and cannot be easily decontaminated should be minimized; paperwork and report writing should be kept separate and conducted in Room 311 (lab office)
11. Remove gloves when answering telephone, opening doors, entering corridors.
12. Disinfectants effective against the agents in use must be available at all times along with a complete biological spill kit (see below).
13. Use of needles, syringes, and other sharp objects should be strictly limited to those occasions when other alternatives are not suitable. Caution should be used when handling needles and syringes to avoid autoinoculation and the generation of aerosols during use and disposal. All procedures should be performed in the biological safety cabinet; needles should not be bent, sheared, or recapped; and they should be promptly placed in a University Sharps container. The sharps container must not be filled above the marked line. Once full, the container is sealed, marked with Manish Raizada's name and laboratory room number.
14. Leakproof, impact resistant and disinfectant resistant containers with clip on lids are to be used for the transport of infectious materials within facilities (e.g., between laboratories within the building).
15. If temporarily storing liquid cultures or Petri dishes with microbes in fridges, they should be sealed with Parafilm tape or placed inside a Petri dish plastic sleeve which should be sealed completely with tape. A clear "RG2 microbe" label should be written on the Petri dish or sleeve if known or suspected. Your name and the date should be clearly labeled. All microbes in Petri dishes, tubes, etc. including RG1 should be placed in secondary containers in the fridge (e.g. greenhouse trays with no holes) to avoid spillage into the fridge, and if liquid is detected in the secondary container, it should be disinfected with 1/10 diluted bleach or 70% ethanol spray for 30 min.
16. All spills, accidents, or exposures to infectious materials and losses of containment must be reported immediately to Manish Raizada.

17. Do not touch any desktops with gloves on, and always be aware of touching any surfaces – i.e. within the lab but outside of the CL2 work zone (e.g. fridge handles) -- with gloves that might have been exposed to microbes or other biohazards. From now, all desktop keyboards in the lab must be covered with a washable, protective thin cover. Double glove as necessary, removing the outer glove in the Biosafety Cabinet.
18. Lab coats are not to be worn in stairwells or common areas of the building. Contaminated lab coats should be autoclaved or chemically disinfected prior to laundering.
19. Hands should be washed thoroughly (at least 15-20 seconds of rubbing with soap) whenever gloves are removed, and prior to leaving the laboratory.
20. To prevent indirect or cross contamination, gloves should be preferably changed or removed before handling items likely to be touched by others not wearing gloves, such as doorknobs, light switches, equipment/computer controls, lab notebooks, written instructions or writing instruments.
21. The use of equipment controls such as flexible keyboard covers that can easily be disinfected is encouraged.
22. Laminated copies of SOP's or instructions lend themselves to decontamination and pens and markers can be cleaned with ethanol after use.
23. Routine chemical disinfection of work surfaces is necessary to prevent the spread of potentially pathogenic material.
24. Benchkote should be changed on a regular basis and following any minor spills.
25. Non-essential items and personal belongings must be kept away from areas where biohazards are handled.
26. Sharps are to be discarded in containers that are leakproof, puncture-resistant, and fitted with lids, or specially constructed for the disposal of sharps waste. Sharps are never to be disposed in the regular waste.
27. All biohazardous waste materials must be decontaminated. Liquid biohazardous materials are to be chemically disinfected or autoclaved prior to release to sanitary sewers. Dry biohazardous materials are to be autoclaved prior to disposal in the regular waste stream or collected by a disposal company specializing in the handling of biohazardous waste.
28. Autoclave runs must use indicator tape to ensure efficacy of the sterilization cycle.
29. Never hold your pipet aid or pipettor upside down when a used pipet/tip is in place, to prevent a microbe from dripping into the pipettor.
30. Never remove a contaminated pipettor, or pipet aid from the biosafety cabinet.
31. Eject/remove pipet tips directly into a biohazard waste container inside the biosafety cabinet, never on to the BSC surface.
32. Avoid touching your eyes.
33. Keep things away from your mouth such as pencils, hands, scoopulas, pens, etc.

14.4.2. CL2 Personal Protection:

1. **Class II B2 Biosafety cabinets** - The key issue is whether or not a tube or Petri dish is open or a potential microbial aerosol can be released such as via active pipetting: in this situation, the task MUST be performed in the Biosafety Cabinets. If DNA is being isolated from microbes, you can remove the tubes from the biosafety cabinet after the cell lysis step, but you must remove gloves used for earlier steps (and replace with fresh gloves, or double glove and remove the outer glove) and you must place the tubes into new racks, leaving the original rack in the biosafety hood until it can be decontaminated with 70% ethanol
2. **Eyes and Face** – Wear Goggles or Face Shields. Contact lenses are not permitted in CL2 laboratories.
3. **Feet** – Must wear closed, water-proof shoes (no sandals or canvas-topped shoes). In case of a spill, wear disposable foot covers (available in the Biohazard Spill Kit)

4. **Hands** – Wear gloves when handling hazardous reagents. Nitrile gloves generally provide superior protection to vinyl. Latex gloves are not purchased by the Raizada Lab. Double glove if you leaving the biosafety cabinet to continue working with the RG2 microbes outside the cabinet (e.g. to centrifuge): first remove the outer glove that might be exposed to microbes inside the cabinet (into the cabinet biohazard waste bag). While wearing gloves, you should not handle telephones, doorknobs, or other objects that others might handle without using gloves. Gloves are not to be worn in the hallways. Take extra clean gloves with you when exiting the lab with a biohazardous agents. They should be discarded afterwards in a biohazard bag within the Biosafety Cabinet (not outside), which should then be closed and placed into the biohazard waste.

5. **Arms** – If it is expected that your arms in the biosafety cabinet will become exposed to RG2 liquids, then disposable arm sleeves should be worn and discarded afterwards in a biohazard bag within the Biosafety Cabinet (not outside), which should then be closed and placed into the biohazard waste.

6. **Clothes/Lab Coats** – You are required to wear long pants and a buttoned-up lab coat when working in Room 310. Lab coats not only protect the wearer from chemical and biohazards, but they are also part of the containment barrier that protects other people from these hazards. When used properly, biological agents should be contained on lab coats rather than your clothes. By taking off your lab coat before leaving the lab, these contaminants are then contained within the lab. Please do not to wear lab coats in the hallways/elevators to minimize the spread, except if you are transporting biohazardous materials. If you need a lab coat at your intended destination within the building, but not during transport, place in a biohazard bag or Tupperware container.

7. **Lungs** – Fitted N95 respirators should be worn in any situation where there is a chance of a microbial aerosol (e.g. open tubes, spill).

8. **Hygiene** – Wash your hands before leaving for lunch or home. This is a surprisingly effective way of minimizing the spread of biologicals out of the laboratory

9. **Jewelry** – Not permitted unless there is no chance of it becoming contaminated during a spill.

14.4.3. CL2 Surface Disinfection Procedure (also see p79 of 2017 UofG biosafety handbook)

1. Use benchcoat on counter tops and remember to dispose in the Biohazard waste, immediately if there is any suspicion of droplets.

2. For the Biosafety Cabinet and any equipment or Pipetman surfaces, use disinfectant as described in this section: disinfectant kills when applied to non-living objects, while a sanitizer reduces number of vegetative bacteria to a safe level. Decontamination means sterilization or disinfection; sterilization (e.g. autoclaving) kills everything including spores.

3. For routine cleaning of surfaces including the biosafety cabinet, noting that spores can be resistant to some disinfectants, the options are as follows:

- bleach: must be 5-6% active ingredient (so dilute 1/10th in water); but only a high concentration and has some activity against endospores. DO NOT use bleach on metal parts of centrifuges.

- ethanol; 70% is most effective, with variable activity against spores; a longer contact time is needed against fungi.

- hydrogen peroxide (e.g. Accel TB, Spor-Klenz, PeridoxRTU): effective against spores and fungi, and compatible with aluminum so can be used on metal parts of centrifuges

- iodine (e.g. Wescodyne, Ioprep): effective against bacterial spores

4. For spills (see Emergency Procedure above for details), after being protected by PPE and evacuating other, then use an absorbent pad and paper towels, then pre-clean with soap/water then use 50% concentrated bleach to disinfect.

14.4.4. CL2 Disposal: Liquid culture and agar/phytagel

1. Small and large biohazard bags (e.g. Fisher 01-826B) and the biohazard log book are located in the CL2 work zone cabinet. Please re-order as needed so we always have an ample supply. No biohazard bag, autoclaved within the lab or in the general facility on the First Floor, can leave the lab without the Lab MNR# identifier and recording in the log book, to allow for tracking.
2. When working in the Biosafety Cabinet, always have a biohazard bag on a holder available.
3. For liquid microbial cultures, especially in small-medium volumes, the researcher may add 1/10th (final) v/v concentrated bleach INSIDE the biosafety cabinet, cover, gently mix to coat the sides of the tube with disinfectant, and incubate for 30 minutes and then discard in the sink with running water.
4. For liquid microbial cultures in small volumes (e.g. Eppendorf tube), it is recommended that you pour the cultures into a larger disposable plastic tube for bleach sterilization (above) INSIDE the biosafety cabinet or sealed glass container for autoclaving (seal the top prior to removing from the biosafety cabinet). Except for trace volumes, DO NOT directly place small tubes containing substantial liquid cultures directly into the biohazard bags for autoclaving, as the tubes might leak and spill prior to autoclaving.
5. Liquid cultures cannot be autoclaved in plastic as it will melt, but must be poured into glass beakers and covered prior to removal from the Biosafety Cabinet, for immediate autoclaving in Room 310.
6. Petri dishes containing contaminated agar should be autoclaved but can melt and/or leak inside a biohazard bag before autoclaving and hence should first be placed into a small biohazard bag or sleeve and closed, prior to being placed into the Biohazard waste (secondary containment).
- 7. We do not want aerosols to be dispersed from the Biohazard waste container itself, so ensure that all tubes are completely closed, and if there any chance of aerosol release from contaminated items, place first in a secondary bag (tied up), prior to being placed into the Biohazard waste.**
8. Autoclaving: We have an autoclave in Room 310: use the liquid cycle to disinfect liquid cultures or contaminated solid items. Use autoclave tape and only biohazard bags placed in secondary containment autoclave trays. See the Autoclave SOP above. After autoclaving, solid item should be tagged with an MNR# biohazard label and deposited to the first floor. Alternatively, for solid items in biohazard bags, they can be tagged with an MNR# biohazard label and deposited downstairs on the first floor potting area where they will be autoclaved. Please record any disposed biohazard bags (with an MNR# label) in the biohazard waste log book located in Room 312 in a drawer near the door.
9. Sharps, needles and capillary pipettes, are collected in the YELLOW sharps containers. Pasteur pipettes are collected in plastic trays to be autoclaved. Broken glass is collected in yellow waste bins in each lab for disposal by housekeeping services; however if the glass was contaminated with microbes, please autoclave first.
10. Do not overfill sharps containers or autoclave bags

14.4.5. CL2 Biosafety Cabinet (BSC) Standard Operating Procedure

1. Room 310 Class II Type A Biosafety cabinets (Canadian Cabinets BM4-2A-49): The biosafety cabinets are equipped with HEPA filters to filter out >0.3 um particles including bacteria. These cabinets recirculate and keep airflow away from the user, whereas the plant tissue culture cabinets blow air towards you which you do not want. Use the plant tissue culture cabinets only to pour plates and similar tasks where living microbes are not present.
2. If you are working with a tube or Petri dish that is open or a potential microbial aerosol can be released such as via active pipetting, then the task MUST be performed in the Biosafety Cabinets. If DNA is being isolated from microbes, you can remove the tubes from the BSC after the cell lysis step, but you must remove gloves used for earlier steps (and replace with fresh gloves, or double glove and remove the outer glove) and you must place the tubes into new racks, leaving the original rack in the BSC until it can be decontaminated with 70% ethanol.
3. Do not to store equipment or supplies inside the BSC.

4. Before working in the BSC, ensure that the BSC certification has not expired (Call CON-Test at 1-800-321-3816 to re-certify or BTO company 289.232.4823 <http://btoservices.com/Contact.htm>), check that the sash is in the appropriate location, confirm inward airflow with a tissue, prevent grille obstructions that can disrupt the air flow, ensure the alarm is at the “on” position, disinfect interior surfaces with 70% ethanol, assemble and load all materials needed including a benchtop keeper with small biohazard bag, liquid waste container, gloves and possibly disposable sleeves, then wait 5min before starting.
5. If your lab coat sleeves are loose, they might touch contaminated surfaces or liquids; in this case, use tape around your wrist area OR use disposable biohazard sleeves.
6. Only one person should work in the cabinet at a time.
7. Remove broken or visibly contaminated gloves (e.g. microbe droplets on gloves) immediately inside the BSC.
8. BSC disinfection should include, while the BSC has been running for >5 min: the bottom surface, interior walls and glass pane with 70% ethanol. Use hydrogen peroxide disinfectant if you are concerned about the presence of spores. Never put your head inside the cabinet to clean.
9. Never use the UV light and never leave it on afterwards.
10. Never completely close the window sash with the motor running as it could cause motor burnout.
11. However, in case of **a power outage only**: close or cover all open containers, surface sterilize all interior surfaces and objects, and discard all materials into the biohazard bag in the cabinet and close it. The sash on the cabinet must be closed as much as possible to prevent outflow of microbial aerosols into the room; turn off the blower motor. Remove contaminated gloves and wash your hands.
12. There are no Bunsen burners in the Raizada Lab. To sterilize use the Bacto-incinerator (desirable); a less desirable option are small alcohol lamps.
12. Keep all materials at least 10 cm inside the sash opening.
13. Keep discarded or contaminated material near rear of cabinet on one side until the end of your experiment but without disrupting air vents.
14. Avoid moving your hands, pipettes, or other items over open containers or plates to avoid cross-contamination.
15. Minimize enter and exiting of arms but otherwise do it straight and slowly to prevent disrupting air flow. Double glove if you leaving the biosafety cabinet to continue working with the RG2 microbes outside the cabinet (e.g. to the centrifuge in the CL2 work area): first remove the outer glove that might be exposed to microbes inside the cabinet (into the cabinet biohazard waste bag).
16. At the end of use, do not initially switch off the cabinet in order to allow the HEPA filter to keep filtering out bacteria from aerosols. Close open containers before turning off the BSC, place contaminated gloves and materials in the biohazard bag within the cabinet then close the bag before removing from the cabinet into the large Biohazard waste, disinfect then remove any killed liquid cultures (see above), then put on clean gloves and disinfect all containers, equipment (e.g. test tube racks) and surfaces with 70% ethanol, discard all waste in the autoclave or biohazard bag (see above), then allow the BSC to run for >5 minutes before switching off. Remove your inner gloves into the external Biohazard Waste and wash your hands thoroughly for 30 seconds with soap.
17. In case of a spill, consult the Emergency Procedure Spill SOP above in the lab manual.
18. Check/complete the Front Grille and Catch Pan Cleaning Sheet Log located on each BSC unit. Every month, remove the Catch Basin grille and disinfect as noted in the SOP below.

14.4.6. SOP for Cleaning the Front Grille and Catch Pan of the CL2 Biosafety Cabinet

1. Check/complete the Front Grille and Catch Pan Cleaning Sheet Log located on each BSC unit. Every month, remove the Catch Basin grille and disinfect as noted in the SOP below.

2. Ensure BSC is running and remains running for the entire cleaning procedure.
3. Wear proper PPE to work at a BSC (see above).

Clean and Remove Front Grille

1. Wet wipe the top surface of the outside edge of the BSC and front grille a total of 3 times with pre-soaked (10% or 1/10th diluted bleach) paper towels. Place used paper towels into waste bag.
2. Remove the grille, turn upside down and place onto the work surface.
3. Wet-wipe the underside of the grille for a total of 3 times with pre-soaked paper towels. Place used paper towels into waste bag.
4. Scrub any gross contamination remaining on the grille with appropriate scrubbing tool. Remove all loosened debris by wet-wiping another three times.
5. Spray top of grille and underside of grille thoroughly with 70% ethanol. Let sit for 5 minutes.
6. Remove front grille from BSC.
7. Rinse front grille in sink.

Clean Catch Basin

1. Take care when handling paper towel when the BSC is on. They can get sucked up into the HEPA filter and we will have to have it removed (\$\$\$).
2. First examine the basin: Note any sharps or broken glass. Note: You want to remove as much loose solid material as possible before flooding.
3. Saturate the entire surface of the catch basin by misting with decontamination solution to prevent fly away debris. You want to capture as much solid material and not let it get sucked up into the HEPA filters.
4. Let sit for 5 minutes, keep spraying, do not let it dry out.
5. Using an appropriate tool (such as: tongs, plastic scraper, mini dustpan, toilet brush or long handled scrub brush) remove as much loose solid material, sharps or broken glass. Place sharps or broken glass into a sharps container.
6. Collect debris into a plastic bag inside the BSC if you are positive no sharps are present. If the debris contains sharps that you cannot pick out, place the wet, sharps-containing muck into a wide mouth sharps container.
7. Flood catch basin with decontamination solution.
8. Let sit for 15 minutes or the recommended contact time for the decontamination solution.
9. Absorb liquid into paper towels or absorbent pads and deposit into biohazard waste bag inside BSC.
10. Flood the catchbasin a second time with tap water to remove chemicals from the decontamination solution is required.
11. Dry the catchbasin with paper towels ensuring that you HOLD ON to the dry paper towels as they may get sucked up into the fans.
12. Replace front grille.

14.4.7. CL2 Centrifuge Operating Procedure

1. The biosafety danger of this equipment is that a tube can break, causing spills and release of aerosols. Wear full PPE especially with RG2 or suspected RG2 microbes. Do not operate a centrifuge inside the Biosafety Cabinet as the large size of the equipment will disrupt the internal air flow, and the vibration may damage the biosafety cabinet.
2. Any RG2 samples must be prepared in the Biosafety Cabinet and then transported to the centrifuge in secondary containment.
3. There are multiple centrifuges in Room 310 (see lab map). For live cultures, please use the centrifuge(s) located in the CL2 work area.
4. Two primary ways to prevent a tube breaking are: (1) to use plastic that is rated to withstand high speed centrifugation; and (2) to symmetrically load and balance all tubes using the digital scale as needed, and use of balance standards which should be located next to the centrifuge (in the case of the microcentrifuge).

5. Never operate the rotor without the cover closed and locked in place and/or an O-ring sealed safety bucket/cups in place to prevent aerosol loss.
6. Do not leave the centrifuge until it has reached its full operational speed; if vibration occurs, stop the run immediately. Ensure that you have the proper PPE including a fitted N95 respirator and then proceed with *Cleaning a Biological Spill Inside a Centrifuge SOP* (see above in Emergency Response section). In case of a spill, do not use bleach which is corrosive to the metal of a centrifuge, but instead use hydrogen peroxide which is in the Biohazard spill kit.
7. After spinning RG2 or suspected RG2 microbes, only open the buckets/cups in the Biosafety Cabinet to prevent aerosol escape into the lab.
8. At the end of centrifugation with RG2 or suspected RG2 microbes (live cultures), clean and disinfect the buckets (interior and exterior) and lids prior to removing them from the Biosafety Cabinet with 70% ethanol, let stand 5 minutes and then dry.
9. For bacterial DNA isolations involving RG2 or suspected RG2 microbes, after the cell lysis step, other centrifuges in the lab may be used and aerosol/disinfection procedures are no longer necessary.

14.4.8. CL2 Incubator/Shaker Operating Procedure

1. If a microbe is RG2 or suspected RG2 or transgenic (RG1 or RG2), then please use the dedicated incubator or shaker-incubator in the CL2 Work Area.
2. Ensure your 96-well plates, tubes or flasks are not overfilled (recommended ¼-1/2 of the max volume).
3. Ensure your tubes or flasks are closed to prevent splashes if RG1; ensure flasks are covered with a filter to prevent loss of RG2 aerosols.
4. Ensure your tubes or flasks are labelled with your name.
5. Load your flasks or tubes into the shaker.
6. Make sure the shaker platform is balanced as much as possible.
7. For the shaker, increase the RPM's slowly to research the desired speed.
8. Watch the shaker to ensure flasks are secure and the platform is balanced: we do not want shaking that could lead to a spill.
9. At the end, wait until the platform comes to a complete stop before removing tubes to prevent damage or a spill.
10. Inspect the area to determine if a spill has occurred. If a spill has occurred, follow the Emergency Procedure Biohazard Spill SOP above, but briefly:
 - a. Turn the shaker off, add a sign to the equipment noting a spill. Notify all owners of tubes/flasks within the shaker. Leave closed for 30 minutes.
 - b. Ensure you have full PPE (fitted N95 mask, complete lab coat, sleeves, etc).
 - c. After 30 minutes, open the shaker to retrieve any broken glass with tongs and place into the yellow biohazard sharps container.
 - d. Wipe down all intact samples with 70% ethanol and remove. The owner of those samples is responsible for their disposition.
 - e. Lay down paper towels to absorb puddles of liquids.
 - f. Add 70% ethanol or hydrogen peroxide (see Biohazard Spill Kit) to the paper towels and let sit for 30 min.
 - g. Please do not use bleach as it is corrosive to the metal components of the equipment.
 - h. Mist the remainder of the surfaces inside the shaker with 70% ethanol.
 - i. Retrieve all paper towels into the solid biohazardous waste.
 - j. Saturate paper towels in 70% ethanol or hydrogen peroxide and wet-wipe the inside surfaces of the shaker twice.
 - k. Retrieve all paper towels into the solid biohazardous waste.

14.4.9. CL2 Electroporator Operating Procedure

1. This is stored in the CL2 work area, but for active use, please use it in the Biosafety Cabinet and follow the BSC SOP.
2. The critical time of exposure to potential microbial aerosols is when the liquid culture tube and cuvettes are open.
3. As ice is used in this procedure, be careful to wipe the outside of all tubes prior to submerging in ice, but if there is any concern about microbial contamination of the ice water, then the ice water should be bleach-disinfected for 30min or autoclaved (after pouring in a flask) following the appropriate SOP, prior to being discarded in the sink.
4. Please remember to leave the Biosafety Cabinet on for at least 5 minutes at the end of your experiment, following the BSC SOP.

14.4.10. CL2 Transporting Live Biohazards in the Building or on Campus

It is understood that you will try to perform all your experiments in Room 310, but there may be situations where you need to move living cultures. However, this represents an opportunity to cause spread of microbes outside of the Lab, so great care must be taken.

1. Please place microbes, etc. especially RG2 or suspected RG2, or any transgenic microbes (RG1 or RG2) in a shatter-proof, leak-proof container (e.g. Tupperware) in the Biosafety Cabinet and close the lid.
2. Place on a cart if needed. Imagine a scenario where you might trip with a small box of microbes causing a spill, so a cart would prevent this.
3. Before leaving Room 310, remove your lab coat and gloves and wash your hands for 30 seconds with soap. Your lab coat may be contaminated with microbes and you do not want to brush up against elevator walls with it.
4. Remember to keep alcohol gel, spare gloves and your lab coat with you, to use at your destination.
5. At your destination, put on new gloves and the appropriate PPE; if you are leaving living microbes there, tape a sign on the equipment noting: your name, Raizada Lab, your cell phone number, and microbe Risk Group. If RG2, suspected, unknown or a mixture of RG1 and RG2, note it as "Risk Group 2" microbes. Do not write the abbreviation (i.e. "RG2"). Imagine if there is a spill and someone else finds it: they must be warned of the potential danger.
6. Before exiting your new location, remove your gloves to take back to the Biohazard Waste in Room 310, then wash your hands again for 30 seconds with soap or alcohol gel before touching any door handles.

14.4.11. CL2 Microscope Operating Procedure

1. When imaging microbes or plants inoculated with microbes, please try to limit microscopy to the Room 310 Microscope Facility. If the Summerlee Building Confocal Microscope Facility must be used, ensure that all microscope slides are prepared in the Biosafety Cabinet in Room 310 prior to leaving the building, with cover slips, then placed in sealed Petri dishes, which are then placed in closed tertiary containment (e.g. closed Tupperware, see Transporting Live Biohazards SOP above). Do not take tubes containing live cultures with you, and there should be no need to do so.
2. Take multiple pairs of gloves, alcohol gel, a Kimwipe box, extra strips of Parafilm, and 70% ethanol with you to the microscope session.
3. Before touching the microscope or keyboard, wash your hands with soap and water or alcohol gel for 30 seconds.
4. During the microscope procedure, beware of any liquid touching your hands or the microscope stage, AND note that earlier users may have spilled microbes onto those surfaces, so assume that surface contamination including on your hands.
5. As needed, dampen Kimwipes with 70% ethanol and scrub down surfaces without damaging equipment. Repeat this at the end of your experiment.
6. At the end of the experiment, place microscope slides back into Petri dishes, seal with Parafilm tape and put back into tertiary containment (e.g. closed Tupperware).

7. Remove your gloves to take back to Room 310 (also place in the Tupperware), then wash your hands with soap and water or alcohol gel for 30 seconds before touching any door knobs (see Transporting Live Biohazards SOP above).
8. After you have returned to Room 310, put on new gloves and dispose of contaminated glass (microscope slides, cover slips) in the Yellow Tips Container, and dispose of any other contaminated waste (e.g. the Petri dishes, gloves) in the Biohazard Waste.

14.4.12. CL2 Plant Growth Containment Plan (based on NIH Guidelines)

https://osp.od.nih.gov/wp-content/uploads/2019_NIH_Guidelines.htm

Following Biosafety Permit approval:

1. OVERVIEW: Only non-transgenic RG1 microbes will be permitted in open pots in the Crop Science Greenhouse Facility. Transgenic RG1 microbes in open pots will only be allowed in closed plant growth cabinets/chambers, not growth rooms. RG2 microbes (transgenic or not) will only be permitted in closed plant tissue culture bottles/jars (e.g. Magenta jar) with a 0.2 micron filter to prevent bacterial escape.
2. No RG2 microbe or transgenic microbe (RG1 or RG2) can be seed coated or painted onto a plant outside of the CL2 Biosafety Cabinets except without an amended Biosafety Permit, but this request is unlikely to be approved by Manish Raizada.
3. After painting a microbe onto an open plant (i.e. not in a sealed test tube or Magenta jar) in the CL2 Biosafety Cabinet, treated plants must remain in the Cabinet for a minimum of 1 hour (or until the coating agent is dry), to minimize any liquid droplets or aerosols outside of the cabinet. Microbe-treated tissue culture plants in sealed containers are not subject to this requirement.
4. For a RG2 microbe or transgenic microbe (RG1 or RG2), the maximum foliar application permitted per plant is 1 ml ($<10^6$ cfu/ml or OD600=1.0), applied using a disposable, autoclaved cotton swab (e.g. leaf painting), or seeds can coated within a tube in a bacterial culture. Microbe-treated tissue culture plants in sealed containers are not subject to this limitation, but the microbe must be pipetted directly into the tissue culture tube if applied as a foliar. The plant must be placed onto secondary containment such as a large 150 mm Petri dish or disposal bench coat paper, to reduce spillage onto the Cabinet surface, both during treatment (all plants) and for the 1-hour drying period (open plants). If the lab coat sleeves may touch plants containing transgenic microbes, then long disposable sleeves should be worn and placed in a biohazard bag upon exiting the chamber for autoclaving, along with the gloves.
5. Once treated, tissue culture plants must be sealed immediately with their lids and/or a breathable membrane containing a 0.2 micron filter to prevent escape of microbes, PRIOR to removal from the Biosafety Cabinet.
6. After drying, open-treated plants (i.e. not in sealed containers) must be placed in a sealed, non-shattering secondary container such as a Tupperware box for transport (e.g. to a growth cabinet).
7. Microbially pre-treated open plants (e.g. approved transgenic RG1 bacteria) placed in a plant growth cabinet (e.g. on soil, sand or Turface) in pots must be placed into disposable, leak-free secondary containment (i.e. plastic flat trays) so that no effluent from the pots is discharged onto the floor of the plant growth cabinet. The secondary containment must be larger than the anticipated diameter of the foliar radius at the end of the experiment. It would be best to cover the entire floor of the cabinet with secondary containment trays. At the end of the experiment, these must be autoclaved in biohazard bags or disinfected and disposed of.

8. Location: For transgenic bacteria, only a plant growth chamber/cabinet can be used that is listed on the Biosafety Permit. The Crop Science Plant Growth Chamber Facility does not contain any windows or doors to the outside. For transgenic microbes, no plant growth chamber can be used that is contained within a room that has a window or door to the outside environment. No transgenic bacteria are permitted inside greenhouses, to prevent their aerosols from being released into the environment from the roof vent. Transgenic bacteria are also not permitted inside the indoor growth rooms, as aerosols would be difficult to contain, and the entire room cannot be sterilized.

9. For watering or fertilization in a plant growth cabinet, use gentle hand watering only directly into the pots within secondary containment: do not use a hose or apply water/nutrients directly onto leaves to minimize splashing, and pour slowly to prevent soil splashing of microbes.

10. Remember to disinfectant/autoclave any items that have come into contact with leaves or soil such as scissors: systemic microbes can be anywhere.

11. No concurrent experiments can occur within the plant growth cabinet.

12. The Plant Growth Cabinet must be clearly marked with a Biohazard Poster noting the hazard on all Cabinet doors, and the plant growth cabinet must be locked at all times, with a log maintained of all accesses to the cabinet by authorized personnel noted on the Biosafety Permit.

13. If plants are to be transported back to the lab for experimentation, they must be placed into sealed Tupperware or other boxes and placed into the Room 310 Biosafety Cabinet (e.g. for plant tissue grinding).

14. Personal Protection:

--Normal CL2 precautions must be used when working at a growth chamber with potted plants containing transgenic bacteria, even if RG1 transgenic bacteria, including use of: a fitted N95 respirator, fully buttoned lab coat, gloves, lab goggles, long pants, no open-toed shoes, and tied back hair.

--- Furthermore, the lab coats used for growth chamber visits must be laundered frequently due to potential aerosol exposure and hence authorized personnel should have multiple lab coats available.

---No eating/drinking or application of cosmetics are permitted in the vicinity.

---It is important to ensure that there is a sink with soap in the vicinity to permit thorough handwashing.

---If the lab coat sleeves may touch plants containing transgenic microbes, then long disposable sleeves should be worn and placed in a biohazard bag upon exiting the chamber for autoclaving, along with the gloves.

---Please remember to bring a biohazard bag for each growth chamber visit and tie it before exiting the chamber area.

15. Termination:

--At the termination of the experiment, all plants, growth media and secondary containment must be autoclaved.

---Do not remove the secondary containment trays if there is liquid on the surface: allow the liquid to evaporate first prior to removal.

--Do not autoclave pots as they will melt; all pots including any residual soil or sand must be soaked in 10-fold diluted bleach for a minimum of 30 minutes.

---All surfaces of the biosafety cabinet must be thoroughly treated with an appropriate disinfectant (e.g. 10-fold dilution of bleach) for a minimum of 30 minutes, while the secondary containment remains on the floor.

---Do not wash the floor panels inside the plant growth chamber if the chamber contains a drain that might connect to the general sewer system. Instead, if there is a drain, then after the walls have dried, the secondary containment can be removed, and then the floor panels removed and placed onto plastic sheets, then sprayed with disinfectant for 30 minutes and then allowed to dry.

---All paper towels, gloves and contaminated materials must be autoclaved. Near the plant growth chambers is a large autoclave in the potting area, and the biohazard bag should be taken

directly there, not to Room 310, so please bring a biohazard Raizada tracking label with you but remember to log it into the book in Room 310.

---Plant tissue culture glass tube or jars should be autoclaved on the liquid cycle in secondary containment; any agar/phytigel should be removed first if possible, and disposed of in biohazard bags placed within secondary bags to prevent leakage, prior to autoclaving.

16. If plants have been treated with a transgenic microbe, they will not be permitted to go to reproductive maturity where they might produce pollen or seed.

17. Only plants can be treated with a transgenic microbe that cannot regenerate asexually in the wild.

18. The only transgenic vectors (i.e. plasmid) that can be introduced on potted plants in growth chambers are those listed in the Biosafety Permit, limited to reporters such as GFP and an herbicide/antibiotic selection marker such as Kanamycin.

19. No vectors can be used that might contain toxins, can cause harm to humans, or increase the virulence of the microbe on humans/animals or cause danger to the environment.

20. Only vectors can be used that are entirely from prokaryotic sequences.

21. No vectors can be used that might cause integration into bacterial host chromosomes unless explicitly noted in the Biosafety Permit.

22. The total volume of transgenic microbes coated onto plants must be limited and recorded, and at no point exceed more than 4L of microbial culture per week.

23. For non-transgenic RG1 greenhouse experiments that are approved, the same protocols apply as in transgenic RG1 experiments in plant growth cabinets, including no concurrent experiments, except that:

-- secondary containment is not required for pots;

-- disposable sleeve covers are not required, but frequent laundering of lab coats should occur;

-- plants can be sprayed directly in the greenhouse, but tissue painting is preferred; fitted N95 respirators is required during spraying/painting, along with PPE as noted above, and disposable shoe covers during the spraying. Full body Tyvex-type suit may be preferable for some microbes.

-- individual greenhouses do not need to be locked; however the entire Crop Science Greenhouse Facility is always locked and card activated which provides a log of visitors.

14.4.13. Raizada Lab Code of Conduct for Field Spraying with Microbes

1. A CFIA permit or PMRA Research Notification is required. CFIA does not have a research notification process, while PMRA does. For CFIA, plan to submit 1 year in advance of the experiment; for PMRA, plan for 30 days in advance. PMRA is only for endemic microbes (i.e. where the host is endemic to Canada).
2. The concern is that field spraying with endophytic microbes may affect human or human health, create an unnatural reservoir in soil, cause long-distance spread through air, and ultimately vertical transmission into seeds intended for the food chain, or that could affect a researcher's phenotype at a research station. It is unlikely that a soil inoculant sprayed at our levels will have a significant, stable effect on the soil microbiome however. We also recognize that all of our microbes come from plants, primarily crop plants, and that these species are likely already in fields and the environment and even in seeds, though not the particular strain of study. We also recognize that even RG2 microbes are likely to be safe for humans but perhaps not in concentrated form. Despite these caveats, upon official approvals, the Raizada Lab will follow a voluntary Code of Conduct and protocol that is more stringent than the Pest Management Regulatory Agency (PMRA) of Canada.
3. We will spray only RG1 microbes from endemic host plants in field trials in Canada. There may be an opportunity to spray non-endemic RG1 microbes on AAFC Crown Land.
4. We will not spray any microbes outdoors where we do not have a whole genome sequence that verifies its taxonomy for risk group status classification. The whole genome sequence prediction must be convincing and/or placed on a phylogenetic tree to

confirm that the nearest neighbours on both sides are RG1. During Covid, due to shortages in reagents at genome facilities, if needed, a 16S sequence can be used as long as the identity across the entire 16S length (full-length) is >99% match, and if it is anchored onto a phylogenetic tree to confirm that the nearest neighbours on both sides are RG1 IN COMBINATION with antibiotic disc testing (see below).

5. RG1 status will be defined as all 3 major databases (ATCC, Canada e-Pathogen, and German DSMZ) agreeing as to the risk group status for both humans and animals (e-Pathogen). In some cases, no ruling has been made by 1-2 databases, which is OK, as long as that microbe passes our other safety criteria; if the species remains ambiguous, then the default predominant genus level classification will be used (Canada e-Pathogen database).
6. Where possible, we will undertake genome mining to look for toxicity, virulence and antibiotic resistance genes.
7. We will perform antibiotic disc assays with 10-20 antibiotics and exclude microbes that display broad antibiotic resistance. Endophytes are not under antibiotic resistance pressure, though endophytic fungi-producing antibiotics may create natural selection pressure for resistance.
8. We will try to spray only on fields that are permanent to the Raizada Lab.
9. We will establish isolation zones around sprayed plots.
10. We will not spray more than 1ml per plant.
11. We will attempt to paint plants or seed coat them, not spray.
12. For corn, we will remove tassels to prevent pollen spread. So for crosses, only pollen from non-treated plants can be used.
13. All treated plants must be chopped and ploughed under. Seeds must be tracked and care taken to drop into the field. Need to remove any volunteer plants that germinate later.
14. Full PPE must be worn during spraying.
15. No one who has any suspicion of being immunocompromised or having a chronic illness, will be allowed to spray microbes despite PPE precautions. Any researcher who does not feel comfortable spraying microbes will never be forced to do so, with no need to provide a specific reason to Manish Raizada or other lab members.
16. We will have 6-8 border rows that are untreated.
17. We will only spray plants in the center of plots so that flanking rows can act as a barrier
18. No spraying on windy days so check the weather in advance.
19. No transgenic microbes will ever be allowed.

15. Medical Surveillance SOP

1. All personnel should be aware of the Medical Surveillance program at Guelph:
<https://www.uoguelph.ca/hr/occupational-health-surveillance-0>
2. Prior to starting work with biohazards, all personnel must sign the Agreement on Biosafety form:
<https://www.uoguelph.ca/hr/occupational-health-surveillance-0>
3. All Lab workers should be aware that underlying and/or pre-existing personal health conditions might put them at increased risk of infection when working with Level 2 Pathogens. These clinical conditions may include: open skin lesions, steroid therapy, psoriasis, splenectomy, chemotherapy, immunosuppression therapy, immunodeficiency disorders, cystic fibrosis and chronic respiratory diseases. If you have a medical condition, and are not sure if it puts you at greater risk when working with biohazards, please consult Occupational Health and Safety.
4. It is the responsibility of all lab members to self-declare any medical condition that may put him/her at risk and seek medical counseling. Please inform Manish Raizada and Occupational Health and Safety.

5. Report any incidents (e.g. exposure to a biohazard) immediately to EHS; signatures can come later (see procedure above under Emergency). Complete an Injury/Illness Incident Report Form within 24 hours:

<https://www.uoguelph.ca/hr/sites/uoguelph.ca.hr/files/public/Incident%20Report%20Form%202016.pdf>

Fax to 519-780-1796 or email to ohw@uoguelph.ca.

6. Medical surveillance is an ongoing process and if your health status changes, please report to Manish Raizada and Occupational Health and Safety at 519-824-4120 ext. 52647.

16. CL2 Biosecurity Plan

The purpose of this plan is to prevent the theft, misuse or intentional release of pathogens or the data associated with them. The other elements of the Lab Manual also comprise part of the Biosecurity Plan.

1. All individuals must have biosafety training appropriate for the biosafety level of the work they undertake. Biosecurity training is mandatory for all students and staff working in labs that contain biohazardous materials.
2. The greatest threat is posed during evenings and weekends. Do not prop open doors or ever leave unlocked.
3. Room 310 doors into the main hallway should now be locked at all times. During normal working hours, only authorized personnel are to be permitted in the lab.
4. All non-lab personnel must sign in and out using the posted log sheet closest to the lab safety shower. Entering Room 310 near the lab office is no longer permitted.
5. If you are suspicious of anyone entering the lab or anyone in the vicinity of the lab then contact Campus Security immediately, extension 52000. Do not challenge the person directly.
6. The -80°C freezers must be locked at all times.
7. Never leave microbes unattended during transport.
8. An official chain of custody is needed if any microbes are shipped (i.e. a courier such as Fedex).
9. Loss, theft, or misuse of a biohazardous material must be reported to Manish Raizada and the U. Guelph Biosafety Officer.
10. The Inventory of Raizada Lab microbes should be updated annually or as it changes in real time once the taxonomy is available. The inventory should be available on the Raizada Room 311 Desktop which is normally locked (separate key from the lab), secure OneDrive Server, with a backup copy with Manish Raizada. The inventory contains the following information: (1) identification of the biohazards (species, names etc) stored and used within the laboratory; (2) location of the biohazards; (3) Risk Group status based on 16S or ITS or whole genome sequencing (note method and risk classification database(s)); (4) number of tubes and quantity. More details are in the Inventory Control Plan.
11. The lab collectively will continuously screen the -80°C glycerol stock collections for any missing tubes (lost or stolen) and report this immediately to Manish Raizada.
12. All acquisitions of biohazardous materials including purchase orders must be approved by the University Biosafety Officer and added to the Lab Inventory.
13. No Raizada Lab microbes are permitted for spraying outdoors without a permit from CFIA or Research Authorization/Notification from PMRA and will be voluntarily limited to (non-transgenic) RG1 microbes, ideally with a full genome sequence to confirm taxonomy, restricted antibiotic disease resistance and no evidence of human/livestock virulence factors.

17. Biohazard Inventory Control Plan

1. The Raizada Lab maintains an inventory of microbes (primarily bacterial and fungal endophytes isolated from crop seeds, roots and shoots) which should be updated as it changes in real time once taxonomies are available. The Lab does not possess viruses.

2. The inventory should be available on the Raizada Room 311 Desktop which is locked (separate key from the main lab key) when lab members are not present, on the secure OneDrive Server, with a backup copy with Manish Raizada.
3. The Inventory Records must be available for inspection by the University Biosafety Officer and authorized government officials.
4. The inventory contains the following information:
 - (1) identification of the biohazards (species, names etc) stored and used within the laboratory;
 - (2) location of each biohazard;
 - (3) predicted Risk Group status based on 16S or ITS or whole genome sequencing (note method and risk classification database(s)).
 - (4) number of tubes and quantity
 - (5) freezer state
 - (6) responsible contact info: Manish Raizada (raizada@uoguelph.ca)
 - (7) how generated or receipt data including import permit/transfer letter

18. Biohazard import/transfer SOP (ordering, shipping, receiving biohazards)

1. Importation, exportation and transfer of human and animal pathogens requires special paperwork. The regulations, forms and instructions on how to complete paperwork for these pathogens are on the Environmental Health and Safety website:
<https://www.uoguelph.ca/hr/sites/uoguelph.ca.hr/files/public/Quick%20Reference%20-%20Biohazard%20Import%20%2CTransfer%20%26%20Purchase.pdf>
2. At the University of Guelph, Gausiya Khan (Biosafety Officer) of EHS will provide the necessary certification to accompany your import permit request.
3. All biohazard purchases must be via iProcurement as a high value purchase order and approved by the Biosafety Officer.
4. A requisition form is required for RG2 microbes, GMOs, and recombinant DNA products.
5. To import RG1 microbes, including plasmids, we need to complete the Statement of HPTA license.
6. To import plant pests including bacterial/fungal pathogens, check to determine whether a CFIA permit is required.
7. Remember to add new microbes to the Raizada Lab Microbe Inventory.
8. To export biohazardous materials, in addition to the required approvals and permits, there are incredibly precise requirements for packaging material, paperwork and wording on parcels and paperwork for sending Dangerous Goods. Any deviation from the prescribed packaging, paperwork or wording on paperwork or packaging will result in your parcel being returned. Kevin Ecott (mailroom, extension 52274, kecott@uoguelph.ca) is the University's certified Dangerous Goods shipper. He will complete all of the necessary paperwork for your shipment, is up to date on all of the necessary shipping requirements (they change frequently) and will advise you on the necessary packaging requirements for your shipment. He will save you a tremendous amount of time. Use him.

19. CL2 Equipment Maintenance SOP

1. Biosafety Cabinets: The 2 Biosafety Cabinets in Room 310 must be certified as required which includes changing the HEPA filter. The log sheets of the certification dates of the Biosafety Cabinets are posted on the sides of the cabinets. The Biosafety Cabinets must be routinely cleaned including the front grille and trap, following the Biosafety Cabinet SOP above. All BSC users should immediately report any concerns about the inward airflow rate or other issues immediately to Manish Raizada.
2. Eyewash station and safety shower logs should be present and up to date.
3. The Room 310 autoclave shall be maintained in working order and certified, which is the responsibility of Chris Grainger.

4. The following equipment in the CL2 work area and throughout the lab, that may be in contact with microbials, should be routinely cleaned and disinfected (if appropriate, as noted in the above SOPs for specific pieces of equipment), and checked to be in proper working order by the Raizada Lab Equipment Officer, including but not limited to:

- biosafety cabinets
- centrifuges
- incubators
- shaker incubators
- electroporator
- biobeads/homogenizer
- microscopes
- pipetman including 96-well pipettman
- bacto-incinerators

5. Centrifuges should have a secure o-ring and available safety buckets/cups.

20. Housekeeping, Monthly Inspection and Annual Lab Safety Meeting Including CL2 Biosafety Drills

20.1. Lab Housekeeping

1. The Biohazard Spill kit, Chemical Spill Kit, and First Aid Kit including waterproof bandage, must be replenished and up to date. This will be the responsibility of the Raizada Lab Safety Officer.
2. The following must be screened constantly for availability by the Raizada Lab Safety Officer and other lab members: handwash soap, alcohol gel, surface disinfectants such as 70% ethanol, paper towels, benchcoat, and all PPE (including gloves in all sizes, N95 masks, disposable sleeves, foot covers, Tyvek full body covers, lab goggles), biohazard bags, biohazard bag stickers and log book, and non-overflowing Biohazard Waste, Chemical Hood clutter/waste, yellow and red sharps containers, and broken glass container.
3. The Raizada Lab Safety Officer and other lab members will be vigilant for proper chemical segregation and waste segregation.
4. The Lab will have a formal thorough cleaning and de-cluttering on an annual basis in the first two weeks of January.
5. It is important to remove clutter in/near safety cabinets, the chemical fume hood, eye wash station, safety shower, fire extinguishers, chemical spill kit and biohazard spill kit. These pieces of equipment should never be obstructed.
6. Cardboard boxes should be off the floor and properly stored.
7. Emergency showers and eyewash stations must be free of dirt and clutter and tested weekly. Physical Resources is tasked with confirming the correct function quarterly and sign in the tags attached to the equipment. A log of the testing must be available.
8. Fire extinguishers are tested regularly by the Fire Prevention Officers. Access to them must be kept clear of obstructions. Laboratory occupants, led by the Raizada Lab Safety Officer, must regularly confirm that the extinguisher regulators show their charge level in the green zone.
9. Laboratory benches should be well organized with surfaces intact, and covered with disposable benchcoat that should be replaced on a regular basis and placed into Biohazardous Waste for autoclaving if appropriate.
10. The Chemical Fume Hood should be checked. The pilot light should be working and the door should move easily. Velocity of airflow through fume hood should read at least 100 feet per minute. Physical Resources tests this annually and places a label on the hood. Never move the sash above the marked sash level and do not leave the access doors open while working. The doors are only temporarily opened to place tall items, such as cylinders, inside the hood.
11. Floors and aisles are not to be used for general storage. Hallways that lead to exit doors

must not be cluttered with items that reduce their width.

12. Sinks and drains are to be kept free of clutter.

13. Electrical apparatus are to be checked for frayed wires, missing ground prongs and exposed high voltage shock points. Check that there are enough power outlets in the room and that extension cords are not used on a permanent basis. Extension cords should never be in series.

14. The entire lab and in particular the CL2 work zone will be inspected weekly by the Raizada Lab Safety Officer for the above maintenance issues, alongside all members of the lab on a routine (i.e. daily) basis.

20.2. Monthly Laboratory Self-inspections by the Raizada Lab Safety Officer

1. The Raizada Lab Safety Officer will complete a monthly self-inspection as noted below. It must be scanned and emailed to Manish Raizada. A hard copy should also be posted on the Safety Board when completed. If there is a “No” to any question, please alert Manish and lab members but try to fix immediately also.

2. Raizada Lab Containment Level 2 and General Lab Safety -- Monthly Self-Inspection Checklist

Completed by: _____ (Lab Safety Officer) Date: _____

#	Question (If you answer no, add details in the Notes section below)	Yes	No	Fixed
1	Did you run the <u>eyewash station</u> weekly to flush out contaminants, discourage microbial growth, ensure adequate water flow?			
2	Is there unobstructed access to the emergency <u>eyewash</u> and shower?			
3	Did you check the <u>fire extinguisher</u> ? It should be easy to access (i.e. unobstructed), and the pressure indicator should be in the green area of the gauge.			
4	Are the two Biosafety Cabinets properly certified and distant from the certification end date?			
5	Are the two Biosafety Cabinets clutter free?			
6	Is the Chemical Flow Hood properly certified and the pilot light working with good airflow?			
7	Is the chemical flow hood clutter free?			
8	Is the <u>Chemical Spill Kit</u> complete? It should contain: <input type="checkbox"/> Small broom and dustpan <input type="checkbox"/> Absorbent padding or paper <input type="checkbox"/> Protective gloves <input type="checkbox"/> Acid Neutralizer <input type="checkbox"/> Alkali Neutralizer <input type="checkbox"/> Solvent Vapour Suppressant			
9	Did you find any flammables outside of the flammables cabinet such as large volumes of ethanol?			
10	Does the Lab Chemical Inventory need updating?			
11	Is the <u>Biohazard Spill Kit</u> complete? It should contain: <input type="checkbox"/> absorbent pads/paper towels <input type="checkbox"/> a pail with lid <input type="checkbox"/> disposable coveralls <input type="checkbox"/> disposable boot cover <input type="checkbox"/> gloves of different sizes <input type="checkbox"/> N95 masks			

	<input type="checkbox"/> concentrated bleach and hydrogen peroxide <input type="checkbox"/> tweezers <input type="checkbox"/> dustpan and brush <input type="checkbox"/> goggles/safety glasses <input type="checkbox"/> small and large biohazard bags <input type="checkbox"/> pre-made "Biohazard Spill: Do Not enter" signs <input type="checkbox"/> 70% ethanol should be available in the flammables cabinet.			
12	Is there an adequate supply of large and small biohazard bags, autoclave waste stickers and is the Biohazard autoclave log book is in the correct place?			
13	Was the Biohazard waste overflowing?			
14	Were the sharps containers overflowing?			
15	Is the <u>First Aid Kit</u> missing any items? The kit should contain: <input type="checkbox"/> gloves <input type="checkbox"/> tweezers <input type="checkbox"/> scissors <input type="checkbox"/> adhesive bandages <input type="checkbox"/> tape <input type="checkbox"/> gauz <input type="checkbox"/> pads or compress bandages			
16	Are any <u>PPE supplies</u> low and need replenishing? <input type="checkbox"/> handwash soap <input type="checkbox"/> alcohol gel <input type="checkbox"/> 70% ethanol <input type="checkbox"/> at least 2 full 100% ethanol jugs <input type="checkbox"/> paper towels <input type="checkbox"/> benchcoat <input type="checkbox"/> gloves in all sizes <input type="checkbox"/> N95 masks <input type="checkbox"/> disposable sleeves <input type="checkbox"/> foot covers <input type="checkbox"/> full body coveralls <input type="checkbox"/> lab goggles			
17	Is there an adequate number of clean lab coats (1 per person in the lab)?			
18	Were all 3 lab sinks and drains free of clutter?			
	Did you see any extension cords in permanent use or in series? Do we need more electrical outlets because of new equipment?			
19	Is the posted Emergency Lab Contact Info sheet up to date?			
20	Are the minus 80°C freezers locked?			
21	Are the following pieces of equipment clean or do they need disinfection? <input type="checkbox"/> biosafety cabinets <input type="checkbox"/> centrifuges <input type="checkbox"/> incubators <input type="checkbox"/> shaker incubators <input type="checkbox"/> electroporator <input type="checkbox"/> biobead/homogenizer <input type="checkbox"/> microscopes			
22	Were some lab areas cluttered and potentially represents a biohazard or fire hazard?			
23	Were cardboard boxes off the floor and properly stored? Are boxes piled too high representing a safety risk?			

NOTES/OTHER ISSUES/RECOMMENDATIONS (please include # if relevant):

Scan and email Manish Raizada. Also post hard copy on Safety Board when completed. If you answered "No" to any question, please alert Manish+ lab immediately but try to fix also.

20.3. Annual Raizada Biosafety Emergency Spill Drill and General Lab Safety Meeting and Clean-up (1/2 Day to Full Day)

Checklist. Please note this will be used to assist with the Annual Refresher Emergency Response Certification

To be completed by all lab members during the first 2 weeks of January annually (all lab members as a group)

Organized by: Manish Raizada

Date: _____

Signature: _____

#	Procedure	Completed
1	Drill: If a biohazard spill occurs INSIDE the biological safety cabinet	
2	Drill: If a biohazard spill occurs OUTSIDE the biological safety cabinet	
3	Drill: If a biohazard spill occurs INSIDE a centrifuge	
4	Drill: Biosafety spill -- lab evacuation and signage procedure	
5	Drill: Biohazard - personal exposure procedure	
6	Biohazard spill notification procedure	
7	Drill: Chemical hazard – personal exposure procedure	
8	Drill: First aid procedures	
9	General review of CL2 SOPs	
10	Review of general lab safety SOPs including the “Raizada Lab Biosafety Containment Level 2 (CL2) and General Safety Training Manual”	
11	General question and answer period of lab safety procedures including any specific concerns by lab members or PI	
12	Discussion about adequateness and locations of PPE, Chemical Spill Kit, Biohazard spill kit	
13	Discussion on status/updates to Raizada Lab Chemical Inventory	
14	Discussion on status/updates to Raizada Lab Microbe Inventory	
15	Inspection and fixing of problems related to chemical segregation, and removing old chemicals	
16	General annual lab clean up and de-cluttering completed	
17	Review of duties of Lab Members including Lab Safety Officer	
18	Review of the EHS certification status of all lab personnel	

Participating Lab Members Present:

Name (print)

Signature:

(names continued)

21. Waste Disposal SOP for Biohazards, Chemicals, Regular Garbage and Recyclables

1. There are three different types of waste streams: Recyclables, Regular Garbage, and Special Waste (chemical, biohazards, broken glass, sharps). Hazardous waste is defined by the 9 SDS pictograms. Waste cannot be put down the drain that has a pH of <5.5 or >9.5 , has a strong odour, contains dyes/pigments, exceeds a set concentration that is considered dangerous to humans, animals or the environment, or is hazardous as governed by the Guelph Sewer Use Bylaw. If not sure, ask Manish Raizada or EHS.

2. Hazardous Chemical Waste tags, like the one shown below (front and back), are available from the EHS Department. You may contact EHS at x53282 to request a supply of tags through inter-office mail. Affix a tag to the chemical waste container when you begin collecting waste. Store safely (e.g. chemical safety hood, flammables cabinet), and submit a disposal form to EHS for collection: <https://www.uoguelph.ca/hr/hr-services-environmental-health-safety-programs-laboratory-safety/hazardous-waste>

3. Biohazardous waste is dealt with by the Lab or with the help of Sue Couling on the first floor but must be tagged and tracked. Biohazard Waste needs “MNR # Labels” affixed which should be recorded in the Biohazard Autoclave Log book.

4. The following table itemizes the types of waste we may have in our lab, and how to dispose of each type:

Table 2. Disposal of different types of waste in the Lab

Waste	Examples	Disposal Procedure
Regular, non-hazardous garbage	Paper towel, non-microbe gloves, paper	Place in regular garbage containers (black bag).
Recyclable materials (not contaminated with hazardous materials)	Fine paper, newspaper	<ul style="list-style-type: none"> •Blue Labeled containers for fine paper, metal, and recyclable plastic are located in offices and labs. •Clear bags are located in select locations •Boxboard, corrugated cardboard, brown paper bags placed are to be left in the lab to be picked up by housekeeping staff. Boxes must be broken down and placed in a neat pile. Please do not obstruct exit routes.

		<ul style="list-style-type: none"> • Glass in designated yellow pails labeled noninfectious including laboratory glass waste for broken glass, empty reagent bottles, autoclaved plates and slides, empty glass vials. Will be picked up by Housekeeping staff.
Aqueous solutions (no microbes)	Phosphate buffer or Tris buffer containing hazardous chemicals (e.g. heavy metals)	<ul style="list-style-type: none"> •Aqueous solutions that contain any hazardous materials (metals, dyes, stains) must be collected and disposed of as hazardous chemical waste. •It is very important to segregate incompatible wastes; serious accidents have occurred because of the mixing of incompatible wastes, even in small quantities. When in doubt, do not mix chemicals and preferably keep the waste chemical in its original container. •Components of solutions must be identified on the hazardous waste tag and corresponding form. •NOTE – if solutions are strongly acidic or basic, label and separate. Do not mix acids and bases in waste jugs •Affix a waste tag and when full submit Surplus Chemical and Sharps Disposal requisition to EHS.
Halogenated organic solvent waste	Chloroform, methylene chloride	<ul style="list-style-type: none"> •It is very important to segregate incompatible wastes; serious accidents have occurred because of the mixing of incompatible wastes, even in small quantities. When in doubt, do not mix chemicals and preferably keep the waste chemical in its original container. •Collect in either a safety canister <25L or an empty 4L bottle. Safety canisters will be returned within a week of pick-up. •If using a safety canister, affix a waste tag to indicate the type of waste and components. Keep the lid closed except when filling, and store in flammable storage cabinet. •If reusing a 4L bottle, ensure it either contained a compatible chemical or has been cleaned of any residue. •The original label must be defaced or removed, and the bottle must be labeled with a waste tag indicating the type of waste it contains. Store in the flammable storage cabinet. •When canister or bottle is full submit Surplus Chemical and Sharps Disposal requisition to EHS.
Non-halogenated organic solvent waste	Acetone, ethanol, isopropanol, vacuum pump oil, ether, etc.	<ul style="list-style-type: none"> •Collect in either a safety canister or an empty 4L bottle. •If using a safety canister, affix a waste tag to indicate the type of waste. Keep lid closed except when filling, and store in flammable storage cabinet. •If reusing a 4L bottle, ensure it either contained a compatible chemical or has been cleaned of any residue. •The original label must be defaced or removed, and the bottle must be labeled with a waste tag indicating the type of waste it contains.

		<p>Store in the flammable storage cabinet.</p> <ul style="list-style-type: none"> •When canister or bottle is full submit Surplus Chemical & "Sharps" Disposal Request Form to EHS.
Other liquid chemical waste	<ul style="list-style-type: none"> <input type="checkbox"/> Sulfuric acid waste <input type="checkbox"/> Hydrochloric acid waste <input type="checkbox"/> Nitric acid waste <input type="checkbox"/> Caustic wastes (e.g., sodium hydroxide solutions) <input type="checkbox"/> Ammonia wastes (e.g., solutions from blueprint machines) <input type="checkbox"/> Liquid pesticides <input type="checkbox"/> Certain liquid wastes that cannot safely comingle. <input type="checkbox"/> Liquids that you are not sure whether they are dangerous or not to humans or the environment 	<p>It is very important to segregate incompatible wastes; serious accidents have occurred because of the mixing of incompatible wastes, even in small quantities. When in doubt, do not mix chemicals and preferably keep the waste chemical in its original container.</p> <ul style="list-style-type: none"> •Collect in either a safety canister <25L or an empty 4L bottle. Safety canisters will be returned within a week of pick-up. •If using a safety canister, affix a waste tag to indicate the type of waste and components. Keep the lid closed except when filling, and store in flammable storage cabinet. •If reusing a 4L bottle, ensure it either contained a compatible chemical or has been cleaned of any residue. •The original label must be defaced or removed, and the bottle must be labeled with a waste tag indicating the type of waste it contains. Store in the flammable storage cabinet. •When canister or bottle is full submit Surplus Chemical and Sharps Disposal requisition to EHS.
Clean Glass Waste	Clean glassware	<ul style="list-style-type: none"> •Clean/sterilized glass, including broken glassware can be placed in the containers marked as 'Glass' (white pail, yellow bag). •Ensure any glass going into this waste stream is clean.
Broken glass contaminated with microbes	Biohazardous glassware	<ul style="list-style-type: none"> •Place in secondary containment and autoclave for 60 min at 120°C •Then dispose of in the containers marked as 'Glass' (white pail, yellow bag).
Microbial Biohazardous Solid Waste	Used plastics (culture plates, tubes, pipette tips) disposable masks, disposable gloves, contaminated paper towels or benchcoat, etc.	<ul style="list-style-type: none"> •See CL2 Biohazardous Disposal SOP below •Collect in a commercial autoclavable bag (typically clear with red markings) placed in a closed garbage can •can autoclave in Room 310 send to the First Floor; autoclave for 60 min at 120°C) •Regularly (e.g. daily) close the top of the bag, place in a secondary container, and use a cart to take it to the autoclave designated for waste. •Do not pack waste tightly; penetration of the steam is imperative for effective decontamination.
Microbial Biohazardous Liquid Waste	Overnight culture tubes, flasks with media, etc.	<ul style="list-style-type: none"> •See CL2 Biohazardous Disposal SOP below •Add 1/10th diluted bleach for 30min then pour down lab sink with running water OR autoclave in Room 310 for 60 min at 120°C
Chemical Waste Containing Microbes	Chemical solvent + microbe	<ul style="list-style-type: none"> •Treat this as chemical waste •Aqueous solutions that contain any hazardous materials (metals, dyes, stains) must be collected and disposed of as hazardous chemical waste.

		<ul style="list-style-type: none"> •Components of solutions must be identified on the hazardous waste tag and corresponding form. •NOTE – if solutions are strongly acidic or basic, label and separate. Do not mix acids and bases in waste jugs •Affix a waste tag and when full submit Surplus Chemical and Sharps Disposal requisition to EHS. Note the microbe(s) and risk group
Microbe-contaminated Biohazardous sharps	Syringes, blades, contaminated broken glass (including Pasteur pipettes) or other sharp objects potentially contaminated with a biohazardous material	<ul style="list-style-type: none"> •Sharps must be collected in a puncture-resistant Yellow Sharps container available from Fisher. •When the bin has reached the fill line, the lid must be secured shut. •label with Pi;s Name and Lab Room number + Building •The sharps are classified as biohazardous waste hence forward the Surplus Chemical & "Sharps" Disposal Request Form to Lab Safety Officer for "Biohazard" pick-up.
Plants contaminated with microbes	Plants, soil, pots, tissue culture tubes/jars, flats	See CL2 Disposal of Plants and Associated Waste SOP below
Expired/Unneeded chemicals	Chemicals that are no longer used, Expired chemicals	Do not remove from original container. Place in a safe area of the lab, affix a yellow waste tag and submit Surplus Chemical & "Sharps" Disposal Request Form to EHS.
Compressed gas tanks	Empty CO2 or helium cylinder	<ul style="list-style-type: none"> •See Compressed Cylinder SOP •Return to first floor using the cylinder dolly (email Sue Couling if you need assistance)
Formaldehyde	Formalin preservative	Formaldehyde is an irritant and a human carcinogen and must be treated as a hazardous waste. Collect in a suitable container, affix yellow tag and when full submit Surplus Chemical & "Sharps" Disposal Request Form to EHS.
Acrylamide	Stock solutions, polymerized gels	<ul style="list-style-type: none"> •Acrylamide monomer (i.e. powder or in solution) must be treated as a hazardous waste. •Uncontaminated polymerized gels can be discarded in the regular garbage.

5. CL2 Biohazard Disposal SOP

1. Small and large biohazard bags (e.g. Fisher 01-826B) and the biohazard log book are located in Room 312 near the door. Please re-order as needed so we always have an ample supply. No biohazard bag, autoclaved within the lab or in the general facility on the First Floor, can leave the lab without the Lab MNR# identifier and recording in the log book, to allow for tracking.
2. When working in the Biosafety Cabinet, always have a biohazard bag on a holder available.
3. For liquid microbial cultures, especially in small-medium volumes, the researcher may add 1/10th v/v concentrated bleach INSIDE the biosafety cabinet, cover, gently mix to coat the sides of the tube with disinfectant, and incubate for 30 minutes and then discard in the sink with running water.

4. For liquid microbial cultures in small volumes (e.g. Eppendorf tube), it is recommended that you pour the cultures into a larger disposable plastic tube for bleach sterilization (above) **INSIDE** the biosafety cabinet or glass container for autoclaving (seal the top prior to removing from the biosafety cabinet). Except for trace volumes, DO NOT directly place small tubes containing substantial liquid cultures directly into the biohazard bags for autoclaving, as the tubes might leak and spill prior to autoclaving.
5. Liquid cultures cannot be autoclaved in plastic as it will melt, but must be poured into glass beakers and covered prior to removal from the Biosafety Cabinet, for immediate autoclaving in Room 310.
6. Petri dishes containing contaminated agar should be autoclaved but can melt and/or leak inside a biohazard bag and hence should first be placed into a small biohazard bag or sleeve and closed, prior to being placed into the Biohazard waste (secondary containment).
7. We do not want aerosols to be dispersed from the Biohazard waste container itself, so ensure that all tubes are completely closed, and if there any chance of aerosol release from contaminated items, place in a secondary bag (tied up), prior to being placed into the Biohazard waste.
8. Autoclaving: We have an autoclave in Room 310: use the liquid cycle to disinfect liquid cultures or contaminated solid items. Use autoclave tape and only biohazard bags placed in secondary containment autoclave trays. See the Autoclave SOP above. After autoclaving, solid item should be tagged with an MNR# biohazard label and deposited to the first floor. Alternatively, for solid items in biohazard bags, they can be tagged with an MNR# biohazard label and deposited downstairs on the first floor potting area where they will be autoclaved. Please record any disposed biohazard bags (with an MNR# label) in the biohazard waste log book located in Room 312 in a drawer near the door.
9. Sharps, needles and capillary pipettes contaminated with microbes, are collected in the YELLOW sharps containers. Pasteur pipettes are collected in plastic trays to be autoclaved. Broken glass is collected in yellow waste bins in each lab for disposal by housekeeping services; however if the glass was contaminated with microbes, please autoclave first.
10. Do not overfill sharps containers or autoclave bags

6. CL2 Disposal of Plants and Associated Waste Containing Microbes SOP

1. At the termination of the experiment, all plants, growth media and secondary containment must be autoclaved.
2. Do not remove the secondary containment trays if there is liquid on the surface: allow the liquid to evaporate first prior to removal.
3. Do not autoclave pots as they will melt; all pots including any residual soil or sand must be soaked in 10-fold diluted bleach for a minimum of 30 minutes.
4. All surfaces of a biosafety cabinet must be thoroughly treated with an appropriate disinfectant (e.g. 10-fold dilution of bleach) for a minimum of 30 minutes, while the secondary containment remains on the floor. Do not wash the floor panels inside the plant growth chamber if the chamber contains a drain that might connect to the general sewer system. Instead, if there is a drain, then after the walls have dried, the secondary containment can be removed, and then the floor panels removed and placed onto plastic sheets, then sprayed with disinfectant for 30 minutes and then allowed to dry.
5. All paper towels, gloves and contaminated materials must be autoclaved. Near the plant growth chambers and greenhouses is a large autoclave in the potting area, and the biohazard bag should be taken directly there, not to Room 310, so please bring a biohazard Raizada tracking label with you but remember to log it into the book in Room 310.
6. Plant tissue culture glass tube or jars should be autoclaved on the liquid cycle in secondary containment; any agar/phytigel should be removed first if possible, and disposed of in biohazard bags placed within secondary bags to prevent leakage, prior to autoclaving.

22. Working Alone Policy

1. Due to the nature of scientific experimentation, it is sometimes necessary to work during off hours (i.e. beyond 8am to 5pm on weekdays) or alone. Undergraduate students working in the laboratory will never have permission to do this. Undergrads may receive permission, however, to work in greenhouses alone after hours.
2. If required to work alone, you must inform Manish Raizada of your plans using the Raizada Lab Whats App Group so that all lab members are also aware. Please note when you have arrived and when you have left. This personnel inventory is in case there is a fire or other emergency in the building, so that rescuers know to look for you, and conversely, not to risk their lives searching empty labs.
3. If working alone, you must be fully trained on any equipment you are planning to use. If the equipment requires more than one person to operate, you may not use it.
4. If you wish to work alone but have a chronic medical problem (e.g. epilepsy), another individual must be available to check on your status at regular intervals, such as every 2 hours, or else permission to work alone will not be granted.
5. The WorkAlone feature in SAFEGryphon smartphone app is a way for anyone to virtually check in with a family member, friend, co-worker or anyone they choose if the user is working late at night or is working alone or would feel safer if they were being checked up on during this time. The SAFEGryphon app provides access to a real time security feed, emergency contact and support resources.
6. SafeWalk – is a student-run organization dedicated to providing people on campus with a safe and reliable escort after dark, 7:30pm until 2:30am every night.
7. The following activities are prohibited when working alone:
 - Working with hazardous materials - dispensing highly flammable liquids; dangerously reactive substances (e.g. peroxides, pyrophorics or water reactives); highly corrosive substances.
 - Handling of acutely toxic materials (to be identified / discussed with PI)
 - Changing compressed gas cylinders.
8. The above applies to all labs and plant growth facilities, anywhere on campus.

23. Safety Documentation

The principle of due diligence requires programs that mitigate or eliminate hazards be documented. We will document our due diligence using the following:

1. Department of Plant Agriculture Safety Training/Documentation Checklist– Standard Operating Procedures (SOP) (before starting work, updated May 16, 2020)
2. Completed EHS and Other Training Certificates of Lab Members (in their files), including:
 - EHS Worker Health and Safety Awareness
 - EHS WHMIS
 - EHS Biosafety
 - EHS Laboratory Safety
 - EHS Supervisor Health and Safety Awareness and Due Diligence (Manish Raizada only)
 - EHS Farm, Field and Landscape Safety – Summer Training (as appropriate)
 - Plant Ag: Growth Facility Training (as appropriate)
3. Agreement on Biosafety form, signed by all lab members.
4. Raizada Lab Biosafety Containment Level 2 (CL2) and General Safety Training Manual Containing SOPs (this manual)
5. UofG Completed Spill Response Plan
6. Raizada Lab Containment Level 2 and General Lab Safety – Monthly Self Inspection Checklist Records
7. Annual Raizada Lab Biosafety Emergency Spill Drill and General Safety Meeting –Checklist
8. Annual Refresher Emergency Response Certification – Completed Form
9. Raizada Lab autoclave log records
10. Biohazard autoclave log book in Room 311

11. Biological safety cabinet certification records.
12. Updated Raizada Lab Chemical Inventory
13. Updated Raizada Lab Microbe Inventory
14. Updated Raizada Lab Pathogen/Biohazard Inventory
15. Raizada Lab Room 310 CL2 entry/exit log book
16. Annual Inspection Checklist by the local Joint Health and Safety Committee
17. Incident report forms:
<https://www.uoguelph.ca/hr/sites/uoguelph.ca.hr/files/public/Incident%20Report%20Form%202016.pdf>
18. Orientation of Lab Personnel to safety resources (see Section 24 below).

24. Helpful Resources

1. Guelph Physical Resources Stockroom for PPE
<https://www.pr.uoguelph.ca/customer-services/stockroom>
2. EHS Laboratory Safety Manual (5th Edition, 2018)
https://www.uoguelph.ca/hr/system/files/LS%20manual%202018%205th%20edition%20%20final%20with%20LS%20program%20included_.pdf
3. Environmental Health and Safety (EHS) Website (UGuelph)
<https://www.uoguelph.ca/hr/hr-services/welcome-environmental-health-safety>
4. Canadian Biosafety Standard (CBS) Second Edition, Public Health Agency of Canada, Biosafety Division, Centre for Emergency Preparedness and Response.
<https://www.canada.ca/en/public-health/services/canadian-biosafety-standards-guidelines/second-edition.html>
5. Chemwatch SDS Sheets
<https://www.uoguelph.ca/hr/hr-services-environmental-health-safety-portal/safety-data-sheets>
6. Guelph Safety Databases
<https://www.uoguelph.ca/hr/hr-services-environmental-health-safety/portal>
7. NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules
 Department of Health and Human Services, National Institutes of Health (April 2019)
https://osp.od.nih.gov/wp-content/uploads/2019_NIH_Guidelines.htm
8. Canadian Food Inspection Agency, Biosafety Services Program Biohazard Containment
<https://www.inspection.gc.ca/animal-health/biohazard-containment-and-safety/eng/1300121579431/1315776600051>
9. Canada ePathogen Database for Microbial Risk Groups by Species
<https://health.canada.ca/en/epathogen>
10. United States ATCC Sheets for Microbial Risk Groups by Species
https://www.atcc.org/en/Products/Cells_and_Microorganisms/Bacteria.aspx
11. German DSMZ Microbial Risk Groups by Species
<https://www.dsmz.de/collection/catalogue/microorganisms/catalogue>
12. Database of Pathogen Safety Data Sheets (Government of Canada):
<https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/pathogen-safety-data-sheets-risk-assessment.html>

25. Acknowledgements

Manish Raizada acknowledges that parts of this manual were directly adapted from safety/biosafety manuals from EHS, the Department of Pathobiology Health and Safety Handbook (University of Guelph), College of Biological Sciences Health and Safety Handbook (University of Guelph), MCB Handbook (University of Guelph) and safety handbooks from McMaster University, Algoma University, and Western University, along with resources from the Canadian Biosafety Standard and NIH Guidelines for Recombinant Research.

26. Raizada Lab-Specific, On-Site Training Acknowledgement and Compliance Agreement Signature Page

I, _____ (print), hereby acknowledge that I have read and understood and will comply by all 25 sections of the “Raizada Lab Biosafety Containment Level 2 (CL2) and General Safety Training Manual” (Updated March 2021). Furthermore, I acknowledge that I have completed the on-site lab-specific training (remote by video during Covid) during which this manual was discussed page by page. I also acknowledge that I have a copy of this manual.

The training was conducted by: _____ (print) on _____ (date).

Signatures:

_____ (trainee) Dated: _____

_____ (trainer) Dated: _____

_____ (P.I.) Dated: _____

Appendix I - Lab Signage, Posted SOPs, Forms and Log Sheets Posted in Crop Science Room 310

1. Lab Safety/biosafety manual on blue cabinet in binder below Safety Board + public version on lab website + electronic copy to each lab member + **Appendix I** (List of Lab signage/logsheets) + **Appendix II (FORM-** Dept of Plant Ag Training Checklist – posted on Lab Website)
2. Emergency phone numbers on Safety Board and near phone in lab office
3. Monthly CL2 lab inspection checklist on Safety Board + posted on lab website
4. Entry/exit logbooks: entrance to P.Paul's Lab and hallway exit + posted on lab website
5. Signage on entrance closest to shower and entrance from Paul's Lab that all non-Raizada Room 310 visitors must sign in/out in logbook even if using the autoclave (no exceptions)
6. Sign on entrance nearest to lab office, stating that this door can no longer be used (one already there for Covid)
7. Post BIOHAZARD SYMBOL + CL2 lab entry/exit procedures ON ALL 3 DOORS
8. Official biosafety CL2 signs on freezers
9. No food poster on all fridges
10. Food only on fridge poster for room 311 (Covid only)
11. #5 Biological Spill Response Plan – UofG template
12. Lab-specific biological spill response plans posted for each type of spill
13. Biosafety Spill poster above CL2 Work Zone cabinet noting its contents
14. "DO NOT ENTER" biohazard/biological spill signs for 3 doors (6 copies)
15. Room Maps: (A) Lab CL2 Containment Map including (B) Room 422 map on Safety Board; (C) Room 310 general lab safety/hazards/equipment map on Safety Board; (D) Emergency egress route on Safety Board on all 3 exits
16. GHS hazard pictograms signs – 6 throughout lab especially media room and where other chemicals are stored, as well as Safety Board
17. Looking for an MSDS/Safety Data Sheet? Poster + big sign of URL link/name to SDS database; also posted near computer in Room 311
18. Equipment SOP -- SOP for use of Room 310 biosafety cabinet (BSC), taped to BSC cabinet
19. Equipment SOP - SOP for cleaning BSC front grille and catch pan of biosafety cabinet, taped to BSC cabinet
20. BSC Front Grille and Catch Pan Cleaning Sheet Log posted on BSC + posted on lab website
21. BSC cabinet safe use poster – Govt of Canada
22. Loss or Failure of Containment Poster UofG posted on BSC
23. Emergency Response Plan (post on both lab doors)
24. Chemical spill kit contents and chemical exposure SOP – post near chemical spill kit cabinet
25. Medical Assessment decision tree + medical treatment locations (Safety Board)
26. Equipment SOP –posted on equipment – autoclave
27. Equipment SOP –posted on equipment – gas cylinder
28. Equipment SOP –posted on equipment – dry ice
29. Equipment SOP –posted on equipment – 2 copies - downstairs liquid N tank and near dewars in Room 312
30. Chemical safety quick tips and chemical exposure response –2 copies - post Rm312 and Safety Board
31. Segregation of lab chemicals sign on p29 in Room 311 and under chemical hood and on Safety Board – 3 copies
32. Table: Disposal of Different Types of Waste" on Lab Safety Board
33. Biohazard waste large sign that is laminated on large waste bin
34. "No sharps poster" at biohazard waste and broken glass container

35. Desk poster for door of Room 311 also noting eating/drinking policy
36. Biohazard waste poster series
37. Aerosol hazards waste poster in CL2 area or BSC – PHAC
38. Proper biosafety in the lab including PPE poster- Govt of Canada
39. Autoclave log sheets posted on autoclave (handled by Chris Grainger)
40. Room 310 CL2 entry/exit requirements for lab personnel
41. Handwashing poster at each sink x3
42. Biohazard spill antimicrobial spectrum of disinfectants poster
43. Good sanitation practices for plants (CFIA)
44. **FORM**- Incident Report Form posted on Safety Board + posted on lab website
45. NIOSH Pocket guide to chemical hazards (400 pages, posted on lab website under Lab Safety)
46. Equipment SOP - Chemical Fume Hood SOP (attached to fume hood)
47. **FORM**- Agreement on Biosafety Form (template) – posted on lab website
48. **FORM**- On-Site Training Signature Form – posted on lab website
49. **FORM**- Annual Refresher Emergency Response Certification – posted on lab website
50. **FORM**- Annual Raizada Biosafety Emergency Spill Drill and General Lab Safety Meeting and Clean-up - posted on lab website
51. Potential Human Pathogens + Link to Pathogen Data Safety Sheet Database (Govt of Canada) – post on Safety Board
52. Equipment SOP – Posted on equipment: CL2 Electroporator SOP
53. Equipment SOP –posted on equipment – Incubator/shaker- 2 copies
54. Equipment SOP –posted on equipment – Microscope
55. Raizada Lab Chemical Inventory (posted on Safety Board + Server)
56. Poster of Existence of Raizada Lab Microbe Inventory (Secure Server)
57. CL2 Disposal SOP: liquid culture and agar/phytagel: posted on BSC
58. Equipment SOP –posted on equipment – centrifuge – 2 copies
59. Transporting live biohazards in the building – SOP
60. CL2 Plant Growth Containment Plan (binder 2 copies on blue cabinet below Safety Board + protocol 63 for disposal)
61. CL2 general biosafety precautions poster (on Safety Board or CL2 workzone)
62. CL2 biohazard disposal SOP (posted in CL2 Work Zone)
63. CL2 biohazard disposal of plants and associated waste (posted in CL2 Work Zone – print 3 copies – 2 go to #60 binders)
64. General lab safety – quick tips (post on Safety Board)
65. Responding to flooding (post near autoclave)
66. Quick overview of medical incidents list – post on Safety Board or Lab Office
67. Biosafety Permits on Safety Board

Appendix II – Department of Plant Agriculture Training Checklist (at end)