Research Lab Policy & Procedures Manual

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INTRODUCTION

All students with projects that require the use of our research labs must first complete a WHMIS course. If the student is to work with a biological agent a Laboratory Biosafety course is also required. The Dalhousie University Environmental Health and Safety Office offers both courses. Although we make every effort to provide a safe environment for our students, ultimately, where graduate students often work unsupervised, the safety of the student is often their own responsibility. Students should ensure that they are wearing proper Personal Protective Equipment (PPE). This begins with proper footwear and clothing. Refer to the enclosed section for a detailed description of appropriate PPE and clothing/footwear.

Laboratory coats must be worn when conducting experiments. Eye protection is required and gloves might also be necessary. Some reagents require handling in fume hoods. Read labels and accompanying MSDS safety sheets and act accordingly. Food or beverages are never allowed in the laboratories and especially never in laboratory fridges. Make sure you check to see where showers, fire extinguishers, fire alarms, first aid kits, eyewash stations, spill control kits and the nearest exit are located. If you are unsure where any of this equipment is located or how to operate it, ask your supervisor or one of the technical support people in the area. Safety is your first priority.

The laboratories consist of many multi-user labs, as well as those that are assigned to individual faculty members. There are things such as pipettes, glassware, balances and pH meters that are common to most of the labs and then there are items such as plate readers or high-speed centrifuges that reside in a designated space. The location of a unique piece of equipment is often in a multi-user area but there are exceptions where there is equipment located in an individual faculty member's lab that everyone uses in order to accomplish their research goals. Whether your research takes you to a multi-user area or an individual faculty member's lab, the same courtesy and lab practices apply. The following Rules and Regulations help ensure that these laboratories are used efficiently and that everyone is able to conduct their research in an expedient way.

It also helps to ensure that your data is accurate and reproducible. Everyone's co-operation is **mandatory**.

GENERAL RULES AND REGULATIONS

When you are working in an area, it should be as clean when you leave as when you arrived.

All glassware is to be expediently washed then rinsed with deionized water. When dry, it is to be returned to its designated place. Do not leave dirty glassware in the sink.

Glassware, pipettes, stirring bars, etc. are to stay in the lab where you found them. If you need to take them to another lab in order to use a piece of equipment, return them when you are finished.

Do not remove equipment such as hotplates, pH meters, shakers, etc. from their designated space. Other people are depending on the use of this equipment and removing it may compromise their research. In extreme cases, where moving of equipment is necessary, ask permission and leave a note as to the current location of that equipment. It **MUST** be returned as soon as possible.

If you break a piece of equipment, report this immediately to the Research Safety Officer. Don't just walk away.

If you are unsure how to use the equipment, ask for instruction. Broken equipment causes delays.

Record the use of any piece of equipment in the Log Book accompanying it.

Do not remove the manual for any piece of equipment. Others may need to read it.

If you are using an analytical balance, make sure you clean up any spilled reagents. Many of the chemicals you use are poisonous and you don't want to compromise the health of others through sloppy lab practices. Use the cover for the balance (if present) to improve accuracy and periodically check the balance's calibration.

Always check the calibration on a pH meter before you use it. Ensure that the pH electrode is in good condition (e.g. filled with solution) and stored in either pH=4 or pH=7 buffer when not in use. Check the manual for the proper use of the meter and the electrode (e.g. the rubber stopper in filling hole may need to be removed before use).

If you open a new reagent, put a date on it and designate ownership.

If using someone else's reagents, ask permission and replace it if you use a significant amount.

If you use the last of a common stock such as HANKS, make up a new batch.

If you are storing your samples or reagents in fridges or freezers, make sure they have your name and the date on them and, for smaller items, that they are in a box or appropriate container. If the reagents or samples have expired, or are of no further use, then discard them appropriately.

Don't store your samples in test tube racks, beakers or glassware for any length of time as test tube racks, beakers and glassware are used by everyone and supplies are

limited. Samples should be transferred to sealed, labeled containers so they aren't mixed up with someone else's samples.

When using chemicals or reagents, make sure you read how to handle them safely. When shipped from the suppliers, all reagents come with handling instructions (i.e. MSDS information). Take the time to read them. This ensures your safety and the safety of others in the lab.

Never handle chemicals with latex surgical or examination gloves, as they are permeable to many chemicals. Nitrile gloves at minimum should always be used when handling chemicals. Double gloving and changing the gloves often reduces the risk of permeability. Stronger acids and bases or solvents may require the use of special gloves such as: **Ansell™ ChemTek™ Butyl Chemical Handling Gloves from Fisher Scientific**. These gloves are not stock items so they must be ordered ahead of time if their use is necessary. Always check MSDS information sheets when in doubt.

When handling items from the ultra-low freezers (-45 or - 80°C) always use the thermal gloves provided. Minimize the time that freezer doors or tops are open to prevent the rapid build-up of ice.

All biohazardous or hazardous waste should be disposed of properly. Refer to the appropriate sections in this document for detailed instructions. If you are in doubt ask the Research Safety Officer in the building or consult the Dalhousie Biosafety or Dalhousie Chemical Safety officer. Their contact information is at the end of this document.

Sharps and broken glassware/used Pasteur pipettes should be disposed of properly. Again, refer to the relevant sections.

Report spills or other incidents/accidents to the Safety Officer. Refer to the relevant section(s) of this document for directions.

FINALLY, ABOVE ALL ELSE, WHEN IN DOUBT ASK!

Incubators

- Before using the cell culture incubators it is required that you have training on their proper use. Contact either your lab supervisor or the research safety officer for this training.

- To avoid contamination of cultures, incubators must be kept clean. They should be wiped down weekly with 70% ethanol. If there is a contamination of the incubator, an antimicrobial spray or bleach may be required. Make sure you disconnect the electricity before commencement. As both are toxic to cells, the incubator must then be rinsed with copious amounts of water to remove these toxins prior to resuming cell culturing. Ask for technical support if you discover a contamination in one of the incubators. Maintenance and cleaning of incubators is the responsibility of the users. It is recommended that there be a schedule of cleaning/maintenance beside each incubator.

- Top up the water in the tray at the bottom of the incubator with sterile water if the level is getting low. This will minimize evaporation of your culture media.

- Check CO2 level on the accompanying gas tank and report if the gas level is getting low. This is everyone's responsibility. If the incubator runs out of CO2 the pH of the media will change and the cells being cultured will die.

- Don't leave old cultures in the incubator. Discard them appropriately. Refer to the section on Disposal of Biohazardous Waste.

- Never turn off the CO2 when going in and out of the incubator regardless of whether the alarm keeps going off. It is too easy to forget to turn it back on resulting in loss of your cell lines.

Biosafety Cabinet/Cell Culturing Guidelines

- Proper training is required before using a BSC.

- We do not allow the use of the Biosafety Cabinet UV light in an effort to kill bacteria, if the hood is not in use; the light is an ineffective way to sanitize the hood as it does not penetrate scratches or crevices. A 70% ethanol wipe down of all surfaces in the BSC is a better choice. The UV light can cause serious damage to eyes and we want to avoid that risk.

- Turn on hood at least 30 minutes before you plan to use the BSC. This will allow time for the filters to clean the air within the cabinet. We prefer, however, that the BSC be left on at all times and not turned off. This ensures the air quality and is a more effective way to prevent contamination of your cultures.

- Wash the working surface of the hood with 70% ethanol. Although there may be more effective sanitizers you want to avoid exposure of toxins to your cultures or damage the filtration system in the BSC.

- Wear gloves and wash your gloved hands with 70% ethanol.

- The surfaces of pipette tip boxes, media bottles, culture flasks and plates, etc. should be wiped with 70% ethanol before being placed in the hood.

- Work well within the BSC to prevent contamination from outside air.

- Any time your hands are out of the flow hood or you touch something outside of the flow hood, re-sanitize your gloved hands with 70% ethanol.
- To minimize the number of times you remove your hands from the hood, collect all waste inside the hood and remove it at the end of your session.
- Never pass hands/arms over open sterile bottles or culture vessels.
- Never return pipettes to media bottle if you've dispensed media into culture vessels containing cells. This will prevent contamination of your media by the cells from your culture flasks.
- With a sterile Pasteur pipette, suction off the droplets from the neck and inside of cap when starting a new media bottle. This practice will lower the chance of bacterial contamination.
- Remove medium around the necks of culture flasks using a sterile pipette.
- Don't invert media bottles but ensure by swirling that all additives such as sera or antibiotics are well dispersed within the media.
- If media is contaminated, add at least 10% bleach by volume and allow at least 15 minutes contact time prior to disposal.
- Don't use someone else's autoclaved pipette tips, pipettes, etc. or media. You may contaminate their supplies and compromise their research. Use your own and label them accordingly.
- Rinse vacuum line with 70% ethanol between culturing different cell lines and at the end of each session.
- The suction flask for waste media should have bleach (10% of the potential final volume) added before the flask is used.

- Culture waste bottle should be emptied after each user is finished in the hood. Ensure enough bleach is added to the waste bottle to kill any organisms contained within and then dispose of contents with plenty of running water down the sink.

- Always wipe down the entire inner surface of flow hood with 70% ethanol after each use as well as media bottles, pipette boxes, etc.

- When finished using the Biosafety Cabinets, remove all your supplies from the hood, make sure you wash the working surface of the hood with 70% alcohol. Used tips, flasks, scalpel blades, etc. must be placed in designated autoclave bags, “sharps” containers or beakers with 10% bleach. If you are unsure of the proper protocol, then ask.

- If the biohazard waste requires autoclaving, then do it as expediently as possible. DO NOT store non-autoclaved, biohazard waste (used culture plates, culture flasks, pipette tips, etc.).

- Glass pipettes used for cell culturing should be soaked in a 10% by volume bleach solution for at least 15 minutes before disposal in a sharps/glass container. Bleach is an effective way to kill any potential pathogens that the pipettes might have encountered during the culturing process.

- All culture flasks, plastic pipettes/tips and cell or tissue waste are to be placed in autoclave bags. None of this waste goes out in the regular garbage. The autoclave bag being used to collect biohazard waste is to be kept in a plastic container with lid. If the waste is a designated biohazard, there should be a biohazard sticker on the plastic waste container.

- For more information pertaining to the use of Biosafety Cabinets please refer to the Canadian Biosafety Standards and Guidelines:

http://canadianbiosafetystandards.collaboration.gc.ca/cbsg-nldcb/index-eng.php?page=1

Autoclaving Cell Culture Supplies

All media, glassware, pipettes, tips, culture flasks, etc. for cell culturing must be sterile. If any of these items haven't been purchased in a sterile condition, then you must autoclave them before use. You may even be required to prepare the media necessary for cell culturing and that, too, must be sterilized before use.

Before using our autoclaves you must receive training. Improper use of an autoclave can result in severe burns.

The Primus autoclave in room 4235 is reserved exclusively for sterilization of supplies for cell culture.

There are specific programs for wrapped/unwrapped supplies and for liquids. Make sure you use the correct program. This is especially crucial for liquids as the wrong setting can result in the explosion of bottles or in the release of the contents from the bottles. It is **important**, too, to loosen the caps on bottles containing liquids to avoid explosion of the bottles during the sterilization cycle.

If in doubt, ask for assistance from the technical staff.

Anything being autoclaved must have autoclave tape attached to the outside. After autoclaving, if stripes don't appear on the tape, there has been a malfunction and the temperature has not been high enough or held long enough to accomplish sterilization. The process must be repeated. If the second run fails to result in stripes then this must be reported immediately to the research safety officer. The autoclave has biological monitoring done weekly and is certified yearly but on rare occasions malfunctions occur. For added assurance that the autoclave has functioned properly, attach a steam indicator strip somewhere in the middle of the load.

The Primus has a drying cycle so your supplies will be dry at the end of the run. Wait until there is a **green light** on the **start button** before opening the door. This is crucial to avoid getting a blast of steam/hot air. Use the high temperature gloves available to remove your supplies, as they may still be very hot.

Bottles containing liquids that have been sterilized should have their caps tightened before being stored awaiting use. Empty bottles should be tightened, as well, prior to being placed on the shelf.

It is extremely important **never** to autoclave volatile liquids.

For a detailed overview from the Public Health Agency of Canada on the use of autoclaves please refer to following link:

http://canadianbiosafetystandards.collaboration.gc.ca/cbsg-nldcb/index-eng.php?page=1

Disposal of Hazardous Waste

Hazardous waste from all experiments/assays are to be collected in 4 L, polyethylene waste containers available from Building Services. The accompanying labels are to be filled in as to the contents of the waste container and these containers are to be kept in the cupboards under the fume hoods until the monthly, designated hazardous waste collection day. This is usually the second Thursday of every month at 9:30 but the schedule is posted on the EH&S website. It is important NOT to mix waste reagents that could react with each other. If you are in doubt contact the Dalhousie Chemical Safety Officer (Contact information is at the end of this document).

At least two days prior to collection day, the Chemical Safety Officer requires you to fill out a Hazardous waste form and submit it to the Safety Office so they are prepared to handle the waste to be collected. These bottles are then to be brought to the bay doors near Dentistry Stores at the designated time and turned over to the safety officer who will be there. If you are unsure how to fill in the waste container labels, phone the Safety Office at 494-2495. Never dispose of toxic waste down the sink.

Please refer to the following links for detailed instructions on preparing waste for collection and for the requisite waste collection form:

http://www.dal.ca/content/dam/dalhousie/pdf/dept/safety/Chemical/Procedures_Monthly_Chemical_Waste_Disposal.pdf

http://www.dal.ca/dept/safety/programs-services/environmental-protection/chemical-waste-collection.html

Disposal of Biohazardous Waste

All biohazard waste from cell/bacterial culturing should be in clear autoclave bags. This includes tissue, cells and plastic flasks, culture plates, and pipettes/tips, etc. that have come in contact with potential pathogens. We require clear bags so it will be clearly visible whether the bag contains sharps.

No sharp items such as hypodermic needles, scalpel blades or Pasteur pipettes should be placed in these bags. Refer to the Disposal of Biological "Sharps" section on the following page of this document for instructions for their disposal.

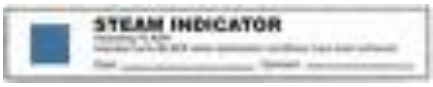
Again, prior to the use of any autoclave it is mandatory to have received training. Please arrange with the Research Safety Officer for training. Records will be kept in the Joint Occupational Health and Safety Committee server folder. The following URL provides a good overview of proper autoclave use:

http://canadianbiosafetystandards.collaboration.gc.ca/cbsg-nldcb/index-eng.php?page=1

Biohazard waste should never be stored in the autoclave room (4235). It is brought in only when the Amsco autoclave used for biohazardous waste is available. The waste containing autoclave bag must always be in a leak proof container. Plastic garbage cans are perfect for this use. Leak proof containers should be provided by the laboratory supervisor for each lab doing research that produces biohazard waste.

The bags are to be left open and must have autoclave tape attached somewhere on the outside of the bag. These bags should not be more than two thirds full. They, also, must

have an indicator test strip attached on the inside of the bag in order to determine at the end of the run that the autoclave has functioned properly. **The indicator test strip is mandatory when autoclaving biohazardous waste.**



The bags are placed in an autoclave tray containing from 2 – 5 cm of water. The open bag and the water in the tray allow for an adequate amount of steam to enter the bag and kill any pathogens present. If the autoclave tape does not have black stripes or the test strip shows insufficient heat at the end of the autoclave run, then redo the run.

Please note: Biological spore tests are done to ensure that the autoclave is functioning properly so it is unlikely you will have any trouble. In the event of a malfunction, contact the technical support person in the research area.

The bags are to be autoclaved for ***at least*** 30 minutes under the wrapped setting (121 degrees celsius) and brought to the autoclave biohazard collection site in the loading dock. Make sure you have an orange tag with the date and PI's name attached. The tag should also have the steam indicator strip showing a successful run attached. ***DO NOT STORE BIOLOGIC WASTE WITHOUT FIRST AUTOCLAVING IT.***

Please refer to the Canadian Biosafety Standards and Guidelines for more information on autoclaves, handling of biohazard waste and biological testing:

http://canadianbiosafetystandards.collaboration.gc.ca/cbsg-nldcb/index-eng.php?page=1

Disposal of Biological "Sharps"

All scalpel blades, hypodermic needles and other sharp materials are to be disposed of in "Sharps" containers (available in Medical Stores in the Tupper Building). They are collected with the hazardous waste collection once a month.

Disposal of Glass Objects

Glass is to be placed in either a Styrofoam container or a plastic lined, cardboard box before being discarded in the regular garbage. Pasteur pipettes, including those used for cell culturing (those must be bleached first), can go into designated glass waste containers. When these containers are full, they must be securely taped shut and labeled to reflect that they contain glass. They can then be placed into the large dumpsters outside of the loading bay doors.

Use of Analytical Equipment

All analytical equipment has an accompanying logbook. It is necessary to sign these books when using the equipment. If you are unsure how to use the equipment, ask for instruction from the designated contact person for that piece of equipment. There may be special instructions posted near a piece of equipment and these instructions are to be followed. It is important to leave the equipment in a clean and operational condition. If for instance, you have adjusted the plate reader to read luminescence, then it has to be returned to the normal operational state before you leave. This is a courtesy for the next person. The equipment should be routinely maintained but if you experience any problems, contact the designated person for that piece of equipment.

Spills

Spills can happen in any laboratory environment. It is important to be prepared for these events. Equipped Chemical Spill Kits are located in room 3110 on the third floor of the Dentistry Building and in the hallway outside the autoclave room (4235) on the fourth floor. They contain absorbent pads, respirators, heavy gloves, overalls, etc. A small spill may be easily contained and dealt with. The URL below provides you with a Chemical Spill Response Guide.

http://www.dal.ca/dept/safety/programs-services/chemical-safety/chemical-spill-response-guide.html

If it is a significant spill or there is a chance of toxic exposure, burns, fire or explosions call Dalhousie Emergency Response at 4109. Close the door to the affected area, evacuate everyone in the surrounding area and go to the front door to meet the response team. A significant flammable spill likely will require the fire department. Pulling the fire alarm will result in the immediate evacuation of the building.

If it is a biohazard spill, most pathogens are inactivated by bleach. If it is a large spill or if it is in a level II lab call Emergency Response at 4109.

Above all, do not take unnecessary risks. If unsure of what to do, ask for assistance.

Fires

Although there are fire extinguishers at regular intervals throughout the research area in the Dentistry Building, without fire training we do NOT recommend that you fight fires. Instead, evacuate the room where the fire has started and close the door in an effort to contain the flame. Sound the fire alarm and go immediately to the front door to meet with the chief fire warden and/or fire department. You have valuable information as to the location and type of fire they are going to encounter and they need that information.

Proper Clothing, Footwear and PPE

It is a Dalhousie University policy that all people working in our laboratories wear lab coats and safety glasses. When working with chemicals we require you to wear nitrile gloves at minimum. With harsher reagents, heavy duty rubber gloves might be required or double gloving. Check MSDS sheets for recommendations on that particular reagent. Gloves must also be worn when working with a biological agent. Long hair must be pulled back so it doesn't hinder your vision. Sandals or open toed shoes are not permitted. Bare legs are also not permitted in our labs, therefore, no skirts or shorts are to be worn.

When choosing a lab coat, please consider the following guidelines:

Fabric – 100% cotton is superior to synthetic blends for fire-resistance, however, synthetic blends are considered to be acceptable for most of the activities that occur in the Faculty of Dentistry.

Sleeves **-** Lab coats must have long sleeves to protect the upper and lower arms. Cuffs are recommended. Coats should never be worn with the sleeves rolled up.

Closures **-** Front closing with snaps, buttons or zippers. Snaps are recommended since they can be removed quickly in the event of fire, chemical, radiological or biological spills. Coats must be completely closed when working in a lab.

Pockets and Slits **-** Lab coats with pockets conveniently placed on the outside are recommended, rather than side-slits that allow easy access to pockets on your clothing underneath.

Length **-** Lab coats must extend to, or slightly below the knee. Any exposed skin below the lab coat must be covered.

Please note **-** Day to day laundering of Lab coats is the responsibility of the individual. The clinic will launder a lab coat **only** if it has been contaminated with hazardous materials (biological, chemical, radiological). If your coat becomes contaminated it must be placed in a plastic bag which has been labeled to identify the contaminant, and deposited in the appropriate laundry bag in Dental Stores. Please ensure your name is on the coat with permanent ink to ensure you receive your coat back from the laundry service. The turn around time for this process is approximately two working days.

Accident/Incident Reporting

All incidents that may have resulted in an injury or accidents whether they are cuts, burns, spills or damage to the building and/or equipment must be reported. Accident/Incident forms can be obtained from Building Services. When the forms are

completed, please return them to Building Services. We need that information to make the building a safer place.

Important Contact Information

Maxine Langman, Dentistry Research Safety Officer
Email: Maxine.Langman@dal.ca
Phone: 902-494-2359

Steve Beaton, Dalhousie Chemical Safety Officer
Email: Steve.Beaton@dal.ca
Phone: 902-494-1934

Pamela Gallant, Dalhousie Biosafety Officer
Email: Pamela.Gallant@dal.ca
Phone: 902-494-4452

Tammy Chouinard, Dentistry Building Manager
Email: t.chouinard@dal.ca
Phone: 902-494-4813

Sandra Pereira, Biomedical Engineering Administrative Assistant
Email: Sandra.Pereira@dal.ca
Phone: 902-494-3427

Denise Lynds-Brown, Applied Oral Sciences Administrative Assistant
Email: Denise.Brown@dal.ca
Phone: 902-494-1675

Dalhousie Security Services
Phone: 902-494-6400

In Case of Emergency call **4109**