

Lab Safety and Standard Operating Procedures

Faculty of Dentistry
And
School of Biomedical
Engineering

Introduction

- It is the requirement that students working in research laboratories at Dalhousie have WHMIS training
- We, also, require that students working with tissue, cell lines, microorganisms, viruses, etc. have Biosafety training.
- We make every effort to provide as safe an environment as possible but the safety of a student requires their co-operation.
- It is the objective of this lecture to provide information to students so they can work in a safe manner within our laboratories.

Personal Protection Equipment

- This begins with proper footwear and clothing.
- Long hair should be tied back.
- Labcoats must be worn when conducting experiments and removed before leaving the lab.
- Eye protection and gloves are also necessary. Gloves are to be removed before leaving the lab.
- Most reagents require the use of a fumehood.
- Read reagent labels and accompanying MSDS safety sheets and act accordingly. Also, review the safety manuals provided in all laboratories.



Basic Safety Rules

- Food or beverages are NEVER allowed in the laboratories and especially NEVER in laboratory fridges.
- Check to see where showers, fire extinguishers, fire alarms, spill control kits and the nearest exit are located.
- All laboratories are equipped with first aid kits and, on the sink faucet, an eye wash apparatus.
- 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.



- Our laboratories consist of many multi-user labs, as well as, those that are assigned to individual faculty members.
- There is equipment such as balances and pH meters that are common to most of the labs and then there are items such as plate readers or centrifuges that reside in a designated space.
- 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 Safe Lab Practices help ensure that these laboratories are used efficiently and safely 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**.

Rules and Policies

- 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, rinsed with deionized water and, when dry, returned to its designated place. Do not leave dirty glassware in the sink.
- Glassware, pipettes, stirring bars, etc. should stay in the lab where you found them. If you need to take an item to another lab in order to use a piece of equipment, then return it 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 the moving of equipment is necessary, either ask permission or leave a note as to its current location. It **MUST** be returned as expediently as possible.

Analytical Equipment

- If you are unsure how to use a piece of analytical equipment, ask for help. Broken equipment causes delays.
- If you do break a piece of equipment, report this immediately. Don't just walk away.
- Record the use of any analytical equipment in the accompanying Log Book.
- Do not remove the manual. Other people may need to read it.



Rules (con't)

- 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 could be compromising the health of others through sloppy lab practices. Use the cover for the balance (if present) and regularly 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 pH=4 or pH=7 buffer when not in use. Check the manual for the proper use of the meter and electrode.



Rules (con't)

- If you receive/open a new reagent put the date and P.I.'s name on it. When preparing a reagent, label contents.
- If it came with an MSDS sheet, put the sheet in the appropriate binder. If it didn't come with an MSDS sheet, make sure there is one in the binder.
- If using someone else's reagents, ask permission and replace them if you have used a significant amount.
- If you use the last of a common stock solution, 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, the P.I.'s name and the date on them. And for smaller items, such as test tubes or vials, ensure they are stored in a labeled box or appropriate container. If the reagents or samples have expired, or of no further use, then discard them appropriately.



More Rules.....

- Again, before using chemicals or reagents, read the MSDS sheets on how to safely handle them.
- 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 for more dangerous reagents is a good idea. Gloves should be changed often and removed before leaving lab.
- When handling items from the ultra-low freezers or from liquid nitrogen dewars, always use the thermal gloves provided. Thermal gloves should also be worn when removing glassware, waste, etc. from the autoclaves or samples from high temperature furnaces.



Rules...

- Report all spills or other incidents/accidents to Building Services They will require you to fill out an incident/accident report.
- If there is a biohazard, flammable or caustic spill, contain the area as best you can and get help. Your safety is more important. Call Emergency: 4109
- If there is a fire, close the door to that room, pull the fire alarm and go to the front door to meet the fire marshal/fire department
- **FINALLY, ABOVE ALL ELSE, WHEN IN DOUBT ASK!**



Cell Culturing/Biosafety Cabinets Guidelines



- Do not use the BSC UV light in an effort to kill bacteria when the hood is not in use. It is an ineffective way to kill bacteria and can cause damage to your eyes. A 70% ethanol wipe down of the hood is a better choice.
- The hood should be left empty when not in use.
- If the BSC is off, turn it on at least 30 minutes before you plan to use it. We do, however, prefer that you leave the hood on.
- Wash the working surface of the BSC with 70% ethanol.
- The outside surface of pipette tip boxes, media bottles, culture flasks and plates, etc. (unless just removed from packaging) should be wiped with 70% ethanol before being placed in the hood.
- Wear gloves and rinse the outside of the gloves with 70% ethanol. In order to prevent contamination, work well within the hood.



- Any time you touch something outside of the flow hood, re-rinse your gloved hands with 70% ethanol.
- Never pass hands/arms over media bottles or culture flask.
- With a sterile Pasteur pipette, suction off any droplets from the neck and inside the cap when starting a new media bottle. Also, remove any media around the neck of culture flasks. This helps reduce the risk of bacterial contamination.
- Any spills of media on the hood surface should be immediately wiped away and the area rinsed with 70% ethanol.
- Don't invert media bottles but ensure by swirling that all additives such as sera or antibiotics are well dispersed within the media.
- If the media is contaminated, add bleach to it before disposal.
- Rinse vacuum line with 70% ethanol between culturing different cell lines and at the end of each session



- The suction flask for media waste should have bleach added before being used to collect media.
- Ensure enough bleach is added to the flask to kill any cells or bacteria present... at least 10 %bleach.
- Empty the bleached waste flask after use and rinse with lots of running water. Keep the flask in a non-breakable container.
- Always empty the BSC and wipe down the entire working surface with 70% ethanol after each use.
- Any scalpel blades or needles are to be disposed of, after use, in “sharps” containers. Do not overfill these containers and never put your hands inside.



- Glass pipettes after being used in cell culturing should be soaked in a 10% bleach solution. Bleach kills everything. The pipettes are too sharp to go in the autoclave bags and don't really fit in the "sharps" containers. The solution can then be decanted off and the pipettes placed in a plastic lined, well taped cardboard box and disposed of in one of the large garbage bins outside the cargo bay.
- All culture flasks, plastic pipettes, tips, etc. and cell or tissue waste is to be placed in biohazard, autoclave bags. None of this waste goes out in the regular garbage. The autoclave bag being used to collect the biologic waste is to be kept in a plastic container with lid and there should be a biohazard sticker on the container.



- The BSC and incubator should, also, have a biohazard sticker affixed to the outside.
- Note: never turn off the CO₂ supply 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 the death of all cells contained in that incubator.

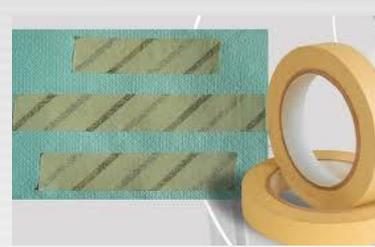


Incubators

- Keep cell culture incubators clean by regularly wiping the interior with 70% ethanol. Top up the water tray at the bottom of the incubator with sterile water if it is getting low. Regularly check the CO₂ level on the accompanying gas tank and report if the gas is getting low. This is everyone's responsibility.
- The CO₂ gauge should be calibrated on regular intervals to make sure the gauge is registering the correct gas level.
- Don't leave old cultures in the incubator. Discard them appropriately.



Autoclaving



- All glassware, pipettes, tips, culture flasks, surgical equipment, etc. used for cell culturing must be sterile. If any of these items haven't been purchased in a sterile condition, then you must autoclave them.
- You may be required to prepare the media needed for cell culturing and that, too, must be sterilized.
- Make sure you receive training on how to safely use the autoclave prior to its use. Improper use can cause serious burns.



- Empty glassware is autoclaved under the “unwrapped” setting.
- Pipette tips in boxes and surgical equipment wrapped in the appropriate material for sterilization are to be autoclaved under the “wrapped” setting
- Any media or other liquids must be autoclaved under the “liquid” setting. It is important to have the cap slightly loosened so the contents don’t cause the bottle to explode.
- Anything being autoclaved must have autoclave tape attached to the outside.
- After autoclaving, if stripes don’t appear on the tape, then there has been a malfunction and the temperature wasn’t high enough or held long enough to accomplish sterilization. The process must be repeated until stripes appear.

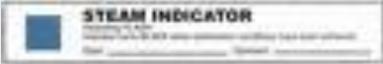


- Once the items have been successfully autoclaved, all glassware and wrapped supplies should be dried in a drying oven to remove excess moisture.
- Bottles containing liquids that have been sterilized should have their caps tightened before being stored awaiting use.
- Empty bottles, after the excess moisture has been removed, should be tightened, as well, prior to being placed on the shelf.

Disposal of Hazardous Waste

- Liquid, toxic waste from all experiments are to be stored in polystyrene bottles available from Dental Stores.
- The accompanying labels are to be filled in as to contents of the waste bottles and the filled bottles stored in cupboards under the fume hood until the monthly designated hazardous waste collection day.
- These bottles are then to be brought to the bay doors near Dental Stores at the designated time and turned over to the Dalhousie Safety Officer who will be there.
- If you are unsure how to fill in the waste container labels phone Stephen Beaton at the Safety Office: 494-1934. Also, he must be given at least 2 days advance notice via an electronic form of any waste you have to be collected.
- Never dispose of toxic waste down the sink.

Disposal of Biological/Biohazard Waste

- All biological/biohazard waste from animal surgeries or cell culturing is to be placed in clear autoclave bags contained in leak proof containers.
- Note: nothing sharp like scalpel blades or Pasteur pipettes are to be included as this could cause injury to those people handling the bags.
- The bags are to be left open and must have autoclave tape and an indicator strip attached. 
- The bags are to be placed in an autoclave tray containing at least an inch of water.
- The open bag and the water in the tray allow for an adequate amount of steam to enter the bag and kill all pathogens present.

Disposal (con't)

- 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.
- The bags are to be autoclaved for at least 30 minutes under the “wrapped setting” and then brought to the autoclaved biohazard collection site in the cargo bay area.
- **DO NOT BRING DOWN UNAUTOCLAVED BIOHAZARD WASTE.**

Disposal of Biological “Sharps”

- All scalpel blades, disposable hypodermic needles and other disposable sharp instrument, regardless of whether they have come into contact with cells or tissue, are to be placed in “Sharps” containers.
- These are available in Medical Stores in the basement of the Tupper Building.
- When the container is around three quarters full, seal the lid and bring the container to the cargo bay area and store with the autoclaved biohazard waste.



Disposal of Glass Objects

- No glass is to be disposed of in the regular garbage
- It is to be placed in either Styrofoam containers or cardboard boxes.
- Pasteur pipettes, including the ones used for cell culturing (those must be bleached first) can go into the designated glass waste containers or boxes.
- When these containers are full, they must be securely taped shut and labeled to reflect that the content is glass.
- They can then be placed into the large garbage bins outside the bay doors at the rear of the building.



Use of Shared Analytical Equipment

- All shared equipment has an accompanying logbook.
- It is necessary to sign these books when using the equipment.
- If you are unsure how to operate this equipment, ask for help.
- There may be special instructions posted near some of this equipment and these instructions must 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.
- If you experience any trouble contact the designated person for that piece of equipment.



Emergency Response

- Before you start any laboratory procedure, assess the risks and take precautions i.e. fumehoods, gloves, protective eyewear, etc.
- Wear protective clothing and proper footwear. It is easier to dispose of clothing than decontaminate the person.
- If there has been a spill contact your supervisor/designated person.
- First aid to the an injured person takes precedence over decontaminating the lab area.
- All injuries/serious incidents/accidents must be reported.
- In case of emergencies call 4109 then, if necessary, 911.

Some Final Biosafety Remarks

- If you are working in a Level 2 Biohazard lab, the pathogens, human cells/blood, bacteria, etc. are to remain in that lab.
- Nitrile gloves are to be removed before leaving the lab. They are not to be worn in the hallways.
- Equipment should stay in those labs.
- Biohazard stickers should be attached to all areas/equipment where the pathogen, etc. is exposed.
- Under no circumstances should food or drink be brought into those labs.



- Anyone who doesn't have WHMIS training, please register for the next available course. If you are going to be working with tissue, cell lines, or pathogens please register for Biosafety training.
- Don't be afraid to ask questions.
- Remember to RETURN YOUR KEYS and clean up your mess at the end of your stay with us

Thank you