

Introduction

All students with projects that require the use of the Biomedical Engineering research labs must complete a WHIMIS and a Laboratory Biosafety course, which are offered by Dalhousie University, before being allowed to commence their research. 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 laboratory apparel. This begins with proper footwear and clothing. Laboratory labcoats should be worn when conducting experiments. Eye protection 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 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.

The Biomedical Engineering 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 scintillation counters 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 ensures 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.

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 and, when dry, 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, then 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. 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) and periodically check the balances 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 when you do 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, 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. Samples should be transferred to sealed, labeled containers so they don't get spilled or lost.
- 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. Stronger acids and bases or solvents may require the use of special gloves. 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. Leave freezer doors or tops open for as little time as possible to prevent the rapid build-up of ice.
- All biologic, medical or toxic waste should be disposed of properly. If you are in doubt, ask your advisor or a technical support person in the area.
- If the biologic waste requires autoclaving, then do it as expediently as possible DO NOT store biological waste (culture plates, culture flasks, pipettes, etc.) without prior sterilization by autoclaving.
- When using the laminar flow hoods, remove all your supplies from the hood, make sure you wash the working surface of the hood with 70% alcohol when you are finished, and that you have placed your used tips, flasks, scalpel blades, etc. in designated autoclave bags, "sharps" containers or beakers with dilute bleach. If you are unsure of the proper protocol, then ask.
- Keep cell culture incubators clean. Top up the water in the tray of the bottom of the incubator if it is getting low. Check CO₂ level on the accompanying gas tank and report if the gas level is getting low. This is everyone's responsibility. Don't leave it for someone else. Ensure that the CO₂ level in the incubator has recently been tested using a manual CO₂ tester (e.g. "Fyrite" tester) to match the reading on the incubator gauge (i.e. 5% or 10%).

- Don't leave old cultures in the incubator. Discard them appropriately.
- 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.
- All glassware, supplies, and equipment in the PCR and Radio Isotope area are to stay there. In the PCR area especially, don't bring in glassware, etc. as you may contaminate the area. If you don't have authorization to be in either of those areas, then don't be.
- Report spills or other incidents to the Safety Officer.
- **FINALLY, ABOVE ALL ELSE, WHEN IN DOUBT ASK!**

Cell Culturing/Laminar Hood Guidelines

- It is not wise to use the flow hood UV light in an effort to kill bacteria, when the hood is not in use, as it will burn out the bulb and it can, also, cause damage to eyes. The light is an ineffective way to sterilize the hood as it does not penetrate scratches or crevices. A 70% ethanol wipe down of all surfaces in a flow hood is a better choice.
- The hood should be left empty. Any spills of media or other solution in the hood should be immediately cleaned and wiped down with 70% ethanol.
- Turn on hood 30 minutes before you plan to use the hood.
- Wash the working surface of the hood with 70% ethanol.
- Only STERILE items are to be placed within the flow hood. The surfaces of pipette tip boxes, media bottles, culture flasks and plates, etc. (unless just removed from package) should be wiped with 70% ethanol before being placed in the hood.
- Work well within the hood.
- Any time your hands are out of the flow hood or you touch something outside of the flow hood, sterilize your hands with 70% ethanol.
- Never pass hands/arms over sterile bottle or culture vessel.

- Never return pipettes to media bottle if you've dispensed media into culture vessel containing cells.
- With sterile Pasteur pipette tip, suction off the droplets from the neck and inside of cap when starting a new media bottle.
- 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 bleach before disposal.
- Rinse vacuum line with 70% ethanol between culturing different cell lines and at the end of each session.
- The suction flask for waste should have bleach added before it 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 sterilize contents 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.
- Glass pipettes should be soaked in a weak bleach solution before disposal in a sharps/glass container.
- All culture flasks, plastic pipettes 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 biologic 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.
- 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.

Autoclaving

All 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 those that haven't been before use. You may even be required to prepare the media required for cell culturing and that, too, must be sterilized before use.

Empty glassware is autoclaved under unwrapped conditions. Pipette tips in boxes and surgical equipment wrapped in the appropriate material for sterilization are to be autoclaved under wrapped conditions. 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 has been high enough or held long enough to accomplish sterilization. The process must be repeated until stripes do appear.

Once the items have been autoclaved, all glassware and wrapped supplies should be dried in the 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 Toxic Waste

Toxic waste from all experiments/assays are to be stored in plastic waste containers available from Dentistry Stores. 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. 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 Dr. Martin Gillis at the Safety Office at 494-3380. Never dispose of toxic waste down the sink.

Disposal of Biological Waste

All biological waste from animal surgeries or cell culturing is to be placed in clear autoclave bags. This includes tissue, cells, plastic flasks, plates, pipettes, etc. that have come in contact with them. The bags are to be left open but must have autoclave tape attached somewhere on the outside of the bag. They, also, must have an indicator strip attached showing that the autoclave has functioned properly. The bags are 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 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. The bags are to be autoclaved for at least 20 minutes under the wrapped setting and brought to the autoclave biohazard collection site in the basement. **DO NOT STORE BIOLOGIC WASTE WITHOUT FIRST AUTOCLAVING IT.**

Disposal of Biological “Sharps”

All scalpel blades, hypodermic needles and other sharp materials that have come into contact with cells or tissue are to be disposed of in “Sharps” containers (available in Medical Stores in the Tupper Building). They are they are collected with the hazardous waste collection once a month.

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 lined cardboard boxes. Pasteur pipettes, including those used for cell culturing (those must be bleached first), can go into designated glass waste. When these containers are full, they must be securely taped shut and labeled to reflect that the contents is glass. They can then be placed into the large garbage bins outside the bay doors near Stores.

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 any 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.