# BI Biohazardous Work Policy – ETB 420

1. Refer to the BI Biohazardous Work Policy before reading this policy.
2. ETB 420 is the BI CL-2 MAMMALIAN culture lab.
3. ETB 425 is the BI CL-2 BACTERIAL culture lab, reserved for bacterial, viral and insect culture work.
4. Work with animal products may be allowed, following approval from the biosafety office, applicable ethics committees, and perhaps the central animal facility. See the BI Biological Technician for more information.
5. Work with laboratory animals is prohibited within BI facilities.
6. Work with anything higher than CL-2 is not authorized without approval from PBAC, the BI Biological Research Technician and BI Director.
7. All biological work must be captured in a McMaster Biological Utilization Protocol (BUP) and be approved by the BI Biological Technician. Refer to the BI Biohazardous Work Policy and contact the BI Biological Technician as needed.

## Training

1. BI users must have McMaster Biosafety Training prior to gaining access and working in a CL-2 culture lab. Refer to the BI Training Policy.
2. BI users are responsible for knowing the hazards and biohazards associated with their project and must review applicable biohazardous agents risk assessment(s) and supporting documentation (e.g. SDS/PSDS) with their supervisor.

## Access

1. Access to CL-2 culture labs is restricted to users with McMaster Biosafety Training who have completed on-site specific training with BI staff. Individuals without this training are considered visitors; refer to the BI Visitor Policy. Approval from BI staff is required in advance of bringing visitors into the CL-2 labs. Visitors require CL-2 specific PPE, which is available from BI staff upon advance request.
2. If entering a CL-2 lab with a visitor, the user and visitor must complete the BI Visitor’s Log, located in ETB 416, and indicate in the “Comments/Purpose of Visit” that entrance into a CL-2 occurred by writing “CL-2 425”.

## Emergency Procedures and Equipment

1. Following access into the CL-2 culture lab, individuals must familiarize themselves with emergency evacuation routes and other emergency procedures.
2. Users must be familiar with the use and location of emergency equipment, including the eyewash and shower station, spill kits, disinfectants and the panic button. Refer to the BI Emergency Map on the inside of the lab door.
3. Also refer to the outside door signage for emergency numbers.
4. Refer to the BI Emergency Procedures Policies.

## Biosecurity

1. Individuals working in the BI culture labs must follow the BI Biosecurity Policy and their own lab-group specific biosecurity policy as outlined by their supervisor. If conflicts exist between either Biosecurity policy (or SOP), then users must communicate this with the BI Biological Technician.

## PPE

1. BI Users must wear the appropriate PPE. Refer to the BI PPE Policy.
2. CL-2 culture labs require a separate lab coat, separate from CL-1 facilities, to remain in the lab, until decontaminated by BI staff.
3. Only one lab coat must be per hook or hanger.
4. Open wounds, cut, scratches must be covered with a waterproof dressing; such dressing may be found in most BI laboratories or can be requested from BI Staff.
5. CL-2 culture labs have gloves and masking tape provided.
6. All gloves must go into biohazardous solid waste. Refer to the BI Waste Disposal Policy – Biohazardous Waste.
7. Do not wear gloves on common surfaces. Common surfaces include computers, equipment touch screens, and handles.
	1. To clarify further, for example, when removing agents from an incubator, have one gloved hand to handle samples and one ungloved hand to touch the incubator door handle. Do not carry more items than can be safely handled; use a sample container or bin as needed.
8. BI users should wash hands frequently; after removing gloves, after handling infectious materials and prior to exiting the laboratory area.

## Code of Conduct

1. Food and drink, consumption and storage, smoking, applying cosmetics, and removing/inserting contact lenses are prohibited within the BI laboratories.
2. Users will keep work area clean and tidy.
3. Do NOT use other people’s supplies or equipment without PRIOR permission.
4. BI Users must have their own mechanical pipettes to remain in the CL-2 area until decontaminated.
5. Do NOT touch or move other people’s experimental setup or items without PRIOR approval.
6. Refer to the BI Code of Conduct Policy.

## Housekeeping and Disinfecting

1. CL-2 culture labs maintain 70% ethanol, bleach and Lysol wipes.
2. BI users should disinfect work surfaces before and after each work period with the appropriate disinfectant. Decontamination procedures depend on the nature of the agent; consult the appropriate SDS/PSDS, risk assessment and supporting documentation.
3. If additional disinfectant(s), deemed necessary from the biohazard agent(s) and/or material(s) SDS/PSDS are required, they must be provided and maintained by the user and kept in appropriate laboratory while the biohazard agents/material is in use. Refer to the BI Biohazard Work Policy.
4. Common surfaces are disinfected weekly with 70% ethanol by the BI Biological Technician and the lab cleaned monthly. Refer to the BI Housekeeping Policy.

## CL-1 & CL-2 items

1. CL-2 items require the use of a Biological Safety Cabinet (BSC).
2. Segregate CL-1 items from CL-2 as much as possible.
3. If segregation is not possible by using separate equipment, isolate CL-2 agents within the instrument by using a separate shelf or rack, for instance. Be sure to noticeably label the items as “CL-2”.
4. CL-2 items must be double-contained while in transport. This includes moving CL-2 items **within** the laboratory. For centrifugation, use bucket lids and seals; refer to the BI Centrifuge SOP. Refer to the McMaster Transfer of Dangerous Goods Training module.

## Spills

1. Biological spill procedures are captured in the BI Emergency Procedures Policies. Summarized spill procedures are posted in the CL-2 laboratory as reference; seek BI Staff assistance as needed during emergency situations.

## Waste Disposal

1. Refer to the BI Waste Policies.
2. Solid biological waste is disposed of into plastic bags. If working in a BSC, line the solid waste container with a plastic bag prior to working. When finished working, tie up the bag, spray the outside with 70% ethanol and dispose of into yellow-double lined biological waste boxes. Avoid putting biohazardous agents or plates directly in double-lined biological waste boxes due to the risk of aerosols/spores and the resulting foul odour; they should be sealed and/or contained in a plastic bag.
3. Anything that can puncture through a plastic bag, like pipette tips, should be contained in a puncture-proof container. When the container is full, cap it, spray the outside with 70% ethanol and dispose of the whole container in the yellow-double lined biological waste boxes.
4. Liquid biological waste must be decontaminated with the appropriate chemical decontaminant for the appropriate contact time. If working in a BSC, bring a beaker prefilled with decontaminant into the cabinet and dispose of liquid waste into the container. When finished working, spray the outside with 70% ethanol and bring to a sink for assessment of decontamination; add more bleach or provide more time for decontaminant contact as needed. When decontaminated, pour down the drain with copious amounts of water. NOTE: if liquid waste contains hazardous items, it cannot go down the drain and must be handled as hazardous waste; seek assistance from the BI Biological Technician.
5. Sharps must be disposed of into designated puncture-resistant sharps containers. The use of needles, syringes and other sharp objects should be limited. Never recap a needle. Bring sharps container in BSC if needed. When sharps container is ¾ full, seek assistance from the BI Biological Technician.
6. Glass contaminated with a biological agent is considered a ‘sharp’ and can be disposed of into sharps containers. However, if the sharps container is too small for the glass item, seek assistance from BI staff. If the biologically contaminated glass can be decontaminated, it may be disposed of as clean glass waste.
7. Any absorbable surface contaminated with biological agents should be disposed of as solid biological waste; including cardboard stored on the floor that could be contaminated from a biological spill.

## Transportation of Dangerous Goods

1. Users working with CL-2 agents should take McMaster Transportation of Dangerous Goods (TDG) Training.
2. TDG double-containment guidelines state that during transport, a biological agent should be contained in a labeled and sealed (leak-proof and secured with parafilm) primary container that is surface decontaminated and placed inside a biohazard-labeled, sealed, and shatter-proof secondary container, with enough absorbance material to contain the primary containers contents. The outside for the secondary container must be surface decontaminated as well.

## Inventory & Labeling

1. All hazardous materials stored in BI laboratories must be captured in the HECHMET inventory system.
2. All biological items must be captured in an approved BUP and specific inventory databases or binder locations communicated to the BI Biological Technician.
3. All lab items should be labeled according to GHS guidelines (e.g. substance name, owner, date, safety precautions, “refer to SDS”). If the labeling does not fit on small vials or tubes, contain those primary containers in a secondary container with the secondary container labelled appropriately.
4. Refer to the BI Substance Policy.

## Equipment Scheduling

1. In the mammalian culture lab, the only instrumentation that requires advance scheduling are the BSCs (BI:J4 & BI:HW).
2. Scheduled BSC usages are used by BI staff for billing purposes and to assess lab activities and associated risks on a daily basis.

## Equipment Usage

1. Refer to equipment SOPs for specific operating instructions.
2. If you require instrument settings that are not default settings, you must notify the BI Biological Research Technician and consult other room users IN ADVANCE to ensure your work does not negatively affect others.
3. Equipment in contact with biohazardous substances will be labeled with a biohazard symbol. Refer to the BI Equipment Policy – Biohazardous if handling biohazardous agents or using equipment exposed to biohazardous agents.
4. Most biohazard equipment will be routinely decontaminated and/or cleaned by BI Technician. Refer to the specific equipment SOP or the BI Laboratory Housekeeping Policy for further information.

### Autoclave

1. BI autoclaves, located in ETB 435, are for sterilization ONLY; they are not approved for decontamination.
2. Autoclaves require specific training and scheduling before use.
3. Refer to the BI Autoclave SOP.

### BSC

1. Leave BSC on half-power when not in use.
2. With the sash closed, sterilize the BSC with ultraviolet (UV) light for 15 minutes prior to and after working as needed. NOTE: UV light only sterilizes surfaces in direct contact.
3. To turn the BSC onto full power, open the sash to the designated height. Allow airflow to stabilize for 5 minutes. Do not use the BSC, if it is alarming.
4. Disinfect the BSC work surface with 70% ethanol prior to and after working.
5. Ensure appropriate waste containers are in the BSC and prepared properly prior to working. Clean out or seal waste containers appropriately following work.
6. Disinfect items as they enter and leave the BSC.
7. Only one person may work in a BSC at a time. Use good culture practices and work in the BSC from clean to dirty. Avoid clutter. Do not block airflow grills. Refer the McMaster Biosafety Office poster.
8. Report untidy BSCs and spills to the BI Biological Technician.
9. If the BSC fails while in use, contain biological agents, close the sash, and report concerns to the BI Biological Technician immediately.

### Cell Countess

1. Data obtained on the countess may be transferred electronically via USB drives.
2. When using cell countess consumables, fill in the log sheet appropriately.

### Centrifuges & Vortex

1. Be weary of aerosol production when agitating, centrifuging, or vortexing.
2. Ensure centrifuge integrity is assessed prior to use.
3. For the large benchtop centrifuge, lids and O-rings are available for use if needed.
4. For the microfuge, ensure the lid is tightly closed.

### Cryostorage unit

1. Be weary of liquid nitrogen (worse case: burn) when opening and utilizing the Cryostorage unit.
2. Always wear the appropriate PPE, including face shield and cryo-gloves.
3. Refer to the BI Liquid Nitrogen SOP.

### Incubator - CO2

1. The CO2 incubators are set for 37oC and 5% CO2, with 95% humidity.
2. BI staff will refill the humidity pan as needed.
3. Label items appropriately (e.g. user name, agent name, date and containment level) when incubating.
4. Disinfect items as they enter and leave the incubator.
5. Report any contamination and spills to the BI Biological Technician.

### Microscope

1. An inverted microscope, with 4x and 10x objectives, is available for verifying cell culture health and contamination. Leave the unit in “Auto” and turn ON with front ON/OFF switch.
2. Do not clean the microscope; for spills, seek assistance from BI Biological Technician.

### Water bath

1. Be weary of hot temperatures (worst case scenario: burn).
2. Use the temperature pre-sets or set to desired temperature.
3. Use autoclaved water for water bath. Autoclaved water is prepared by the BI Biological Research Technician.
4. Inform the BI Biological Technician if water requires changing (i.e. debris or contamination).

## Lab Entry & Exit Protocols

1. Ensure personal belongings are stored outside the lab area. Any items brought into the lab space should be kept to a minimum and handling of such items should be in a manor to avoid cross-contamination with hazardous and biohazardous items (see below).
2. Ensure you have the appropriate lab-area attire (e.g. pants, closed-toed/heeled shoes) before entering and starting work. Refer to the BI PPE Policy.
3. Prior to entry, read the door signage for any location-specific entry protocols.
4. Upon entering, don lab coat and other PPE, as required.
5. Only wear clean gloves when donning/removing eye/face protection or respiratory protection. Clean equipment if necessary and return to storage location or discard in the appropriate waste channel.
6. Before leaving, verify that cold unit doors are closed, and unnecessary equipment is turned off.
7. Upon removing gloves, discard in solid biohazardous waste stream.
8. Upon removing lab coat, return to lab coat storage area.
9. Wash hands with soap before leaving.
10. Exit laboratory ensuring door closes and locks behind exiting.