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**Biotron**

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# Standard Operating Procedures



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*Experimental Climate Change Research Centre*

**Insect Module**

**2019**

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# **BIOTRON INSECT MODULE: STANDARD OPERATING PROCEDURES**

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## **EMERGENCY CONTACT INFORMATION:**

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Facilities Management **x83304**

Campus Community Police **911**

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## **1. Introduction**

- a. The Biotron Insect Module (IM) is a facility for the rearing of, and experimentation on, insects. Because many imported or modified insects may have the potential to be agricultural, economic or environmental pests, the facility is to be managed as a Plant Pest Containment Level 1 (**PPC-1**), a level suitable for work with arthropods that require containment or quarantine. Note that this level of protection is different from the Biosafety Containment Level 1 system, in that it has been developed specifically for work on arthropods. As a result, the use of disease vectors carrying human pathogens may require additional containment or be inappropriate for use in the facility.
- b. These Standard Operating Procedures (SOPs) and rules are specific to the Insect Module of the Biotron and are in addition to any other rules covering overall use of the Biotron.
- c. All Occupational Health and Safety regulations under federal, provincial law or University regulations must be adhered to whilst using the Biotron. These laws and regulations take precedence over and are in addition to the SOPs outlined herein.

## 2. Training Requirements

- a. Access to laboratories in the Biotron require the following training courses (available [online](#)):
  - i. WHMIS – Workplace Hazardous Materials Information System
  - ii. Supervisor/Worker Health and Safety Training
  - iii. Laboratory Safety and Hazardous Waste Management
  - iv. Biosafety

*Note: i, iii, and iv are required to be updated every 3 years.*

- b. Access may be granted upon evidence that you have completed this training.

## 3. Orientation to the Insect Module

- a. **New Client Registration Environmental Chambers/Labs** must be completed with the Project Supervisor. The signed checklist must be provided to Biotron Administration before access can be granted to the IM.
- b. Separate **Autoclave Training** and/or **SOPs Review** will be provided by IM Laboratory Supervisor. A quiz will be administered following training and will need to be submitted to Biotron Admin within 24 hours for marking.

## 4. Responsible Personnel

- a. The Principal Investigator or approved supervisor (i.e. Graduate Student, RA) of a project is responsible for his or her personnel, and for ensuring that all these procedures are followed.
- b. While the Biotron Staff will work to maintain the integrity of the containment facility and the functioning of the Insect Module, they are not responsible for the maintenance of cultures or for cleaning up after users.
- c. Function and maintenance of the Biotron-owned equipment and chambers is the responsibility of IM Laboratory Supervisor. If you hear an alarm or there is an issue with Biotron-owned equipment or chamber, contact IM Lab Supervisor **immediately**.

## **5. Entering the Insect Module**

- a. Before entering the outer door to the Insect Module, please leave personal possessions, outdoor coats, bags, etc. in the lockers outside of the module.
- b. Make every effort not to come directly from the field or greenhouse into the Insect Module. If, however, it is unavoidable DO NOT wear the same footwear into the module, change before entering the Biotron IM.
- c. It is advisable to keep a pair of closed-toe shoes in the cabinets outside of the IM that are specifically dedicated for the module. If you do not have this, you **MUST** wear booties that are provided in anteroom prior to entering the inner door.
- d. Inspect yourself in the mirrors to check that there are no adhering arthropods.
- e. If you have long hair, tie it back. There are hair elastics available in the anteroom.
- f. Vacuum the outside of any containers and carts to prevent outside arthropods from coming in. Pay particular attention to the undersides of any containers or carts. A vacuum is located on the wall in the anteroom.
- g. Note that the doors are interlocking, and that the lights go off when the doors are open. If the lights do not come on when the door is closed, flick the switch – they will still turn off when the inner door is opened.
- h. Properly fastened and **Appropriate PPE** (personal protective equipment), as indicated in General Rules (p. 5), must be worn by all personnel, including visitors, trainees and others entering or working in the laboratory; suitable footwear with closed toes and heels, covered by booties, must be worn in all lab areas.
- i. General Laboratory practices are in effect (see General Rules on p. 5).

## **6. Exiting the Insect Module**

- a. Before exiting the module, wash your hands (i.e. sink in Diet Making Room) and inspect yourself visually for any adhering arthropods. Remove your lab coat and eye protection (if wearing) and hang it on the “Lab Coats” rack close to the entrance. Do not remove your lab coat from IM. You must have a dedicated lab coat for the module.

- b. Walk through the door into the anteroom.
- c. Remember to not leave the IM wearing booties. Either recycle them into the white bin (to be autoclaved) or throw them out into the black bin or garbage (if have holes or are dirty), prior to leaving the IM lab.
- d. Inspect yourself in the mirror for adhering arthropods. Check the undersides of your shoes and under any flaps or collars.
- e. Vacuum the outside of containers, carts, around your shoes and any cuffs etc. A vacuum is located on the wall in the anteroom.
- f. Inspect yourself in the mirror again.
- g. Check carefully around the door for any wayward creatures.
- h. You are now clear to leave the Insect Module.

## 7. General Rules

- a. Keep all doors closed when possible.
- b. No food, gum, drink, application of makeup, lip balm or insertion or removal of contact lenses while inside the Insect Module.
- c. **Appropriate PPE**: If you are wearing shorts/skirt or have bare legs or open-toe shoes, DO NOT enter the lab. **Inside the lab, you are required to have covered legs, wear a lab coat, have long hair tied back** (so that it does not come in contact with insects) and wear eye protection (when needed\*).

*Note: \*Eye protection is necessary when working with toxic, poisonous, irritating or corrosive chemicals or when working with compressed gases. You must review SDS for each chemical prior to use.*

- d. Loss of containment, accidents, escapes and near-misses must be reported to the IM Laboratory Supervisor, who will ensure that the SOPs have been followed and will maintain a written record of these events. Please use the form provided on the white board or [here](#) to record these incidences.
- e. New reagents and chemicals.
  - i. If you are bringing in a new chemical/reagent into the IM, first check if it is already on the **Insect Module Chemical Inventory** (posted on the

- outside door to the IM and across from the lab coat hooks). If so, the SDS will be available inside the IM for review, as needed.
- ii. If the chemical is NOT in the Chemical Inventory, you MUST provide the chemical name and CAS number to IM Laboratory Supervisor to add to the inventory list as soon as possible. An SDS will be made available inside the lab once the inventory has been updated.
  - iii. When the chemical/reagent bottle is empty, please remove the Western bar code label and attach to the “Chemical Inventory – Barcode Disposal” sheet located by the exit door in the Diet Making Room. Be sure to change the location of the chemical in the [HECHMET chemical inventory system](#). If the location is not changed, you must attach the label to “Barcode Disposal” sheet in your lab.
  - iv. When you are done with the chemical, and will no longer be using it in the IM, please provide an update to the IM Laboratory Supervisor.
- f. Labelling of reagents and equipment.
- i. All reagents, materials, containers and instruments that are associated with a specific user/lab or project **MUST** be clearly labelled with the contents, user’s name and the date. Everything must be labelled with lab name, otherwise it will be discarded.
  - ii. Chemicals, whether or not hazardous, that are not in the original manufacturer’s packaging, must conform to Western regulations by having a clearly-labelled workplace label (white/purple label) with all the required information filled out.
  - iii. Any special-use equipment brought into the IM must be clearly labelled with lab name and date.
- g. Clean-up.
- i. Note that there is limited cleaning by custodial staff in the Insect Module. It is your responsibility to keep the module clean and tidy. If you made a mess, please clean it up.

- ii. All bench space used must be cleared and cleaned immediately after use. If you must leave equipment set up, label with a name, date and contact information.
- iii. Media bottles (etc.) left to set or cure must be covered with cheesecloth and clearly labelled with (1) the media, (2) the user, (3) the date and time of production and (4) the date and time of expected clean up. Media curing should take no longer than 24 hours. Ideally, do this on a cart in the food storage room (Rm. #120B).
- iv. After use, all glassware must be cleaned and, when dry, the user is responsible for putting it away. DO NOT leave glassware on drying racks for more than 24 hours.
- v. After use, all benches and shelves used must be sprayed with bleach solution and wiped with a paper towel, followed by 70% ethanol and wiped with a paper towel.

## **8. Waste Management**

- a. All chemical waste must be dealt with as per Western regulations.
- b. All unused media waste, if non-hazardous, can be disposed of in the garbage. Please be mindful of potential leaks (double- or triple-bag media), and of the volume and weight of garbage bags.
- c. All used media waste must be autoclaved prior to disposal in the garbage. Follow Western Autoclave SOPs and instructions provided by Biotron Staff on operation of the autoclave (see **Appendix III: Autoclaving**; pp. 18-20).
- d. Clean the sink, by scrubbing all sides, and if using discard paper towels. If there is any organic material (i.e. food, media, etc.), do not throw them into the garbage but dispose into the autoclave bag located in the freezer (in Diet Making Room).
- e. All insect waste must be disposed of as per permit regulations either by:
  - i. Freezing all insect waste (including both the insects themselves and any media or plant material with which they have come into contact) for at least 24 hours before disposal in the garbage AND/OR

- ii. Autoclaving or preservation in ethanol insect species for which cold tolerance is unknown, and for all species imported to Canada under CFIA permits, as well as any species known to constitute an environmental, agricultural or economic risk.

*Please remove all waste from the module as soon as possible after treatment.*

## **9. Diet Making Room (120)**

- a. The Diet Making Room is a general-use room for users of the Insect Module to prepare media for insect cultures and perform experimental procedures associated with insects in culture in the facility that cannot be performed elsewhere. All rules and SOPs associated with the Biotron, and the Insect Module in general must be adhered to.
  - i. A cooking schedule is posted in the Diet Making Room and is arranged by the users. Be sure to adhere to the set schedule.
  - ii. The cold room is a shared space, keep it clean and tidy. If you require shelving space, contact IM Laboratory Supervisor.
  - iii. Contact IM Laboratory Supervisor if you have any issues with the autoclave. Be sure to clean up any mess that is made after autoclaving and dispose of any waste as soon as possible (as per Waste Management, p. 7).
  - iv. Balances, hotplates, glassware etc. belong to different labs. Do not appropriate them for specific project use, nor remove them from the Diet Making Room. Contact IM Biotron Labs with any issues. Please leave all equipment clean and tidy.
- b. All non-common use equipment must be labelled. Always request permission from the owner to use this equipment and observe the common courtesy of returning borrowed equipment clean and in working order.
- c. Storage for non-hazardous diet making components is available in a storage room off the exit anteroom (Rm. #120B). Keep this space tidy, avoid spills and keep food components in airtight (and insect-proof) containers whenever possible.

Label all components with the lab name and date, and coordinate with other users as there is limited space.

## **10. *Drosophila* and the Biotron Insect Module**

- a. As this is a general insect facility, *Drosophila* users must modify their habits to prevent escapes whenever possible. This is not only because we have an obligation to prevent all escapes from the IM, but also because flies can spread mites and microbes from cage to cage, and from room to room.
- b. The door to the Fly/Injection Room (Rm. #120C) must be kept closed except when entering and leaving. Turn the lights on immediately upon entering the room (*Drosophila* are usually positively phototactic).
- c. Measures must be taken to prevent unnecessary escapes. Escapees should be captured or killed, and Biotron Staff notified.
- d. There are traps, fly paper and an electric trap in the Fly Room. Please check on their state before you start work and replace/refill any traps that require it.
- e. Simple hygiene will prevent a build-up of flies (or transmission of mites):
  - i. Always wash benches and CO<sub>2</sub> pads down with 70% ethanol before and after use.
  - ii. Never leave food lying around. Unwanted fly food, old cultures etc. should be stored in the freezer and autoclaved before disposal, and then taken out of the IM as soon as possible thereafter.
  - iii. Regularly sweep floors and wipe benches.

## **11. Escapes**

- a. Escapes from rearing containers will happen on occasion. Our goal is to confine escapees to the Insect Module, and to quickly catch them.
- b. Any insect you notice in the entry or exit anterooms must immediately be caught (for identification) or killed (as appropriate and possible).

- i. If you can identify the source of the arthropod, please place the container into the appropriate incubator and inform the owner. If not, place the container into the -20°C freezer.
  - ii. Please fill in and submit (hard copy or electronically) an Insect Escape form (available on the white board or [here](#)).
- c. If an insect you are working with escapes, please do everything you can to catch it immediately. There are insect nets available, as well as a shop vac. Do not hesitate to ask for help from anybody else in the Insect Module or to use the phone to call for help outside the IM\*\*.
  - i. \*\*Ensure that if you call someone in from outside, you are careful to prevent the insect from getting into the anteroom.
  - ii. Hang a ‘Warning: Escapee’ sign on the door to the exit anteroom.
  - iii. Once the insect is caught, please deal with it appropriately (think before returning it to your experiment!), and fill in an escape form, copied to the user (if you know who it is), their PI, and IM Laboratory Supervisor.
- d. If you cannot catch the arthropod, follow below steps:
  - i. Place the ‘Warning: Ongoing Escape’ signs on all entry and exit doors. This will inform other users to be particularly vigilant in their entry and exit procedures.
  - ii. Fill in an escape form and email it to the IM Laboratory Supervisor; use the subject line “Urgent: Ongoing Insect Module Escape”. A warning will be forwarded to all current users.
  - iii. Continue trying to catch the arthropod and be prepared to continue working with Biotron Staff until it is caught.

## **12. Moving Arthropods In and Out of the Insect Module**

- a. All insects must be moved in containers that are as escape-proof as possible, and tightly closed (see **Appendix I: Fruit Flies** and **Appendix II: Moths and Butterflies**; pp. 15-17).

- b. If insects have been imported under a permit, the package must not be opened in the Diet Making Room, but preferably in a walk-in chamber. The packaging material must be disposed of as though it were infested (i.e. autoclaved) or cleaned thoroughly.
- c. If the insect has been imported under a permit:
  - i. Submit an 'Imported Insect' form plus a copy of the permit to IM Laboratory Supervisor in order for the arthropod inventory to be updated (located on the wall of the Diet Making Room).
  - ii. When you have finished with the arthropods, contact IM Laboratory Supervisor so that the information can be updated.
- d. Please check all containers and cultures (inside and out) for unwanted individuals or hitchhikers before removing them from the Insect Module. You will then check them again as part of the normal exit procedure.
- e. If insect is a 'plant pest' imported under a permit, the package must be opened in a sleeved cage in the chamber to house the insect. The packaging must be disposed of as though it were infested (i.e. autoclaved).

### **13. Diseases and Parasites**

- a. Avoid the introduction of external parasites, mites, etc.
  - i. For *Drosophila*, adhere to standard anti-mite practices (i.e. anti-mite paper on shelves, benzyl benzoate-treated stoppers, etc.)
  - ii. Monitor all stocks (*Drosophila* and otherwise) regularly for mite infestation.
  - iii. If a culture with food mites must be used in the IM for unavoidable reasons or an infestation is found in a stock that cannot be discarded, the following protocols must be followed:
    - 1. Anti-mite paper must line all shelves in the incubator.
    - 2. Tight-fitting stoppers treated with mite repellent (benzyl benzoate) must be applied to all containers.

3. The incubator, including the external surfaces and the floor around it, must be wiped with 70% ethanol followed by 10% bleach every three days. Record of this procedure must be kept.
  4. All handling of cultures on lab benches must be performed on anti-mite paper and the surface wiped with ethanol and bleach (as above) immediately after use. Clean (in ethanol) any tools used (paintbrushes, forceps etc) before storage.
  5. A separate lab coat and gloves must be worn when dealing with the contents of an infested incubator and these must be changed before contacting non-infested incubators.
- iv. Be careful to avoid transmission of fungal or viral infections. Careful hygiene (as above) plus disposal of infected material (in ethanol or autoclaving) is essential. Deposit fungus-infected corpses into plastic bags or containers that have been misted with water to reduce aerosols of fungal spores.
  - v. The most common source of infestations and disease is plants or soil brought in from the greenhouse. Be very careful with such material and move it in cages or sealed containers.

#### **14. Use of Insecticides**

- a. We aim to avoid the use of insecticides in the Insect Module. If you plan to use insecticides as part of your research, please discuss this with the Biotron Staff first. Be prepared to develop detailed and specific SOPs that will prevent damage to other projects. Allow at least three-month lead time to give adequate time for this process.
- b. Use of insecticides for control of infestations will only be conducted under exceptional circumstances and will be subject to additional discussion among users and IM Laboratory Supervisor.

## 15. Labelling of Samples, Cultures and Materials

- a. All chambers must be clearly labelled on the outside with the following:
  - i. The User's name
  - ii. The name of the species
  - iii. Temperature, humidity and light conditions, including the times of any changes (i.e.: '12:12 L:D' must also include 'lights on 9am' or similar)
  - iv. Western and after hours contact details of at least three people who may be contacted in the event of an emergency. These people must have some idea of what to do about escapes, as well as be able to manage the cultures in the case of an emergency (e.g. loss of temperature control). Please discuss this with them and leave instructions on the door of your chamber.

*Note: Be sure to provide all the above information when filling out the **Biotron Insect Module Project Application** and remember to provide updates to IM Laboratory Supervisor, as changes are required. Biotron Staff will update the Chamber Information Labels as needed.*

- b. Each container and culture must be labelled with suitable information: your name/your lab name, date, species, etc., to allow them to be identified in the case of an emergency shutdown that requires the removal of cultures from your chamber.
- c. All materials, reagents, etc. in storage and elsewhere must be labelled with a name, contents and date. It helps to collate small items (spatulas, paintbrushes etc) onto a plastic tray to keep them together. In this case, the tray can be labelled. Reagent and chemical labels must also conform to WHMIS and Western regulations (see General Rules, pp. 5-7).

## 16. Changes to Project

- a. If your project changes (e.g. you start using different organisms or move to a different room), please complete a new **Biotron Insect Module Project Application** and **Insect Module Exit Checklist** forms and submit to Biotron

Administration. Do not move any current specimens until authorization has been given by Biotron Admin.

### **17. Project Completion**

- a. Ensure that you have removed or disposed of all your material, reagents, etc. from the IM.
- b. Clean out your incubator or chamber and wipe it down with 10% bleach and then 70% ethanol solution.
- c. Complete the **Insect Module Exit Checklist** and submit to Biotron Administration. Once specimens are removed and the form is submitted, the Biotron Staff will then be able to heat-treat the chamber prior to making it available for another user.

**18. These SOPs are subject to change. ALL USERS MUST REVIEW AND FOLLOW NEW SOPs WHEN THEY ARE INSTITUTED.**

*Written by Brent Sinclair and Jeremy McNeil (13 March 2008)*

*Last Modified/Updated by Julia Nowak (3 September 2019)*

## **APPENDIX I: FRUIT FLIES (*DROSOPHILA* AND RELATIVES BEING REARED ON ARTIFICIAL MEDIA)**

1. When flies are being used in the Biotron Insect Module (IM), please put out traps (a bottle with funnel and yeast-water mixture) to catch any escapees. These traps must be checked and cleared regularly.
  - a. Yeast-water mixture: 1 tablespoon of active dry yeast, 4 tablespoons of sugar, 350 mL of water (e.g. [MSU Extension](#))
2. All vial flipping and fly management is to be done inside a sleeved cage to prevent mass release.
3. Never leave open media containers, fruit or other potential breeding sites (i.e. organic material) available in the IM.
4. Please monitor all stocks (*Drosophila* and otherwise) regularly for mite infestation.
  - a. If a stock is found to be infested, please inform your Project Supervisor and follow the protocol below for handling infested stocks (see Diseases and Parasites, p. 11).
5. All waste (including vials and bottles to be disposed of, and the contents of morgues and traps) that has contacted *Drosophila* must be frozen at -20°C (in the Diet Making Room freezer), then autoclaved prior to disposal in the regular garbage (see **Appendix III: Autoclaving**; pp. 17-19).

## **APPENDIX II: MOTHS AND BUTTERFLIES**

1. All material brought into the Biotron Insect Module (IM) must be in closed containers that are clearly marked with the appropriate rearing information (species, origin, date rearing was started).
2. All material must be held in cages during rearing, even when within designated areas.
3. Manipulation of adults (removal of eggs, adding sugar water, etc.) must be carried out in the specific chambers where the animals are being reared.
4. All eggs must be surface-sterilised in dilute bleach solution for 5 minutes in the Clean Laboratory (Rm. #120D) and then stored in closed containers (in the assigned fridge or incubator) until required.
5. Larvae must be set up for rearing in the Clean Lab. Make sure that the work surface is cleaned with bleach and ethanol both prior to and after the transfers are made. ENSURE THAT NO LARVAE ARE ALLOWED TO ESCAPE. Follow Escapes procedure (p. 9) if there are escapees.
6. The larvae, always in properly closed containers, must then immediately be transferred to the appropriate rearing room.
7. If larvae are to be reared on vegetation rather than artificial diet then, bring the plant material (e.g. cut leaves) into the IM in zip lock bags or closed containers. Manipulations for replacing diet will be done in the clean lab.
8. If the insects are to be reared on whole plants, then all vegetation must be inspected to ensure they are clean and then placed in closed cages before being brought into the IM.

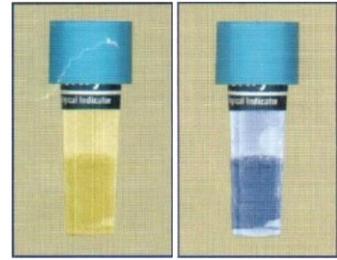
9. All pupae/chrysalids must be collected from rearing containers in the Dirty Manipulation Lab (Rm. #120G). If they are being reared individually on artificial diet and there is evidence of serious fungal contamination or other pathogens, **DISPOSE OF THE MATERIAL IMMEDIATELY, WITHOUT OPENING THE CONTAINER, BY AUTOCLAVING** (see **Appendix III: Autoclaving**; pp. 17-19). If there is a real shortage of biological material then take the closed containers **OUTSIDE** of the IM, remove the pupae/chrysalids and surface sterilise them before bringing them back into the IM.
  
10. Once collected the pupae should be surface-sterilised in bleach solution for 5 min, placed in proper closed containers and returned to the appropriate rearing conditions
  
11. Never leave open cages with insects in the IM.
  
12. All waste material (diet, remaining vegetation, etc.) must be frozen at -20°C (in the Diet Making Room freezer), then autoclaved prior to disposal in the regular garbage.

## APPENDIX III: AUTOCLAVING

*Prior to using any of the autoclaves in the Biotron, you must receive appropriate Autoclave Training from IM Laboratory Supervisor.*

1. Each autoclave cycle must be logged in the Autoclave Log Book with your information and the cycle which you are running.
  
2. For the first waste load of each week the Autoclave Sterilization Testing Procedure (below) MUST be followed and documented in the log book.
  - a. Take **one** (1) ampule from box and place it in the load to be autoclaved; ensuring that it is centred to the load and easily retrievable. Autoclave load as per usual (see “When you are ready to autoclave”, p. 18).
  - b. After the load has been autoclaved, remove the ampule from the load once it has cooled. Be careful.
  - c. Once removed from the load, secure the cap of the ampule by compressing it using ampule crusher (this is a black cylinder with a blue cap). Instructions are on the wall in the IM on how to use this apparatus.
  - d. Take a **NEW** ampule from the box (this must be from the same box & lot number as the one in the load) and crush it in the same manner above using the ampule crusher. You should see broken glass in the ampule if crushed properly. Mark the lid with a “C” for control to easily identify it in the incubator.
  - e. Place both ampules in the incubator for 24 hours. Ensure the incubator is plugged in and the light is lit up.
  - f. **REMEMBER** to log all your actions in the book provided: date the load was run, test results, etc.
  - g. After 24 hours, remove both ampules from the incubator and review the colours of the contents of the ampules. You should see the following:

- i. The “C” or control ampule (NEW – unsterilized) **should be yellow or cloudy** = positive (+) for live spores (pictured on the left)
- ii. The autoclaved (sterilized) ampule **should be a blue-gray** colour and clear = negative (-) for live spores (pictured on the right)

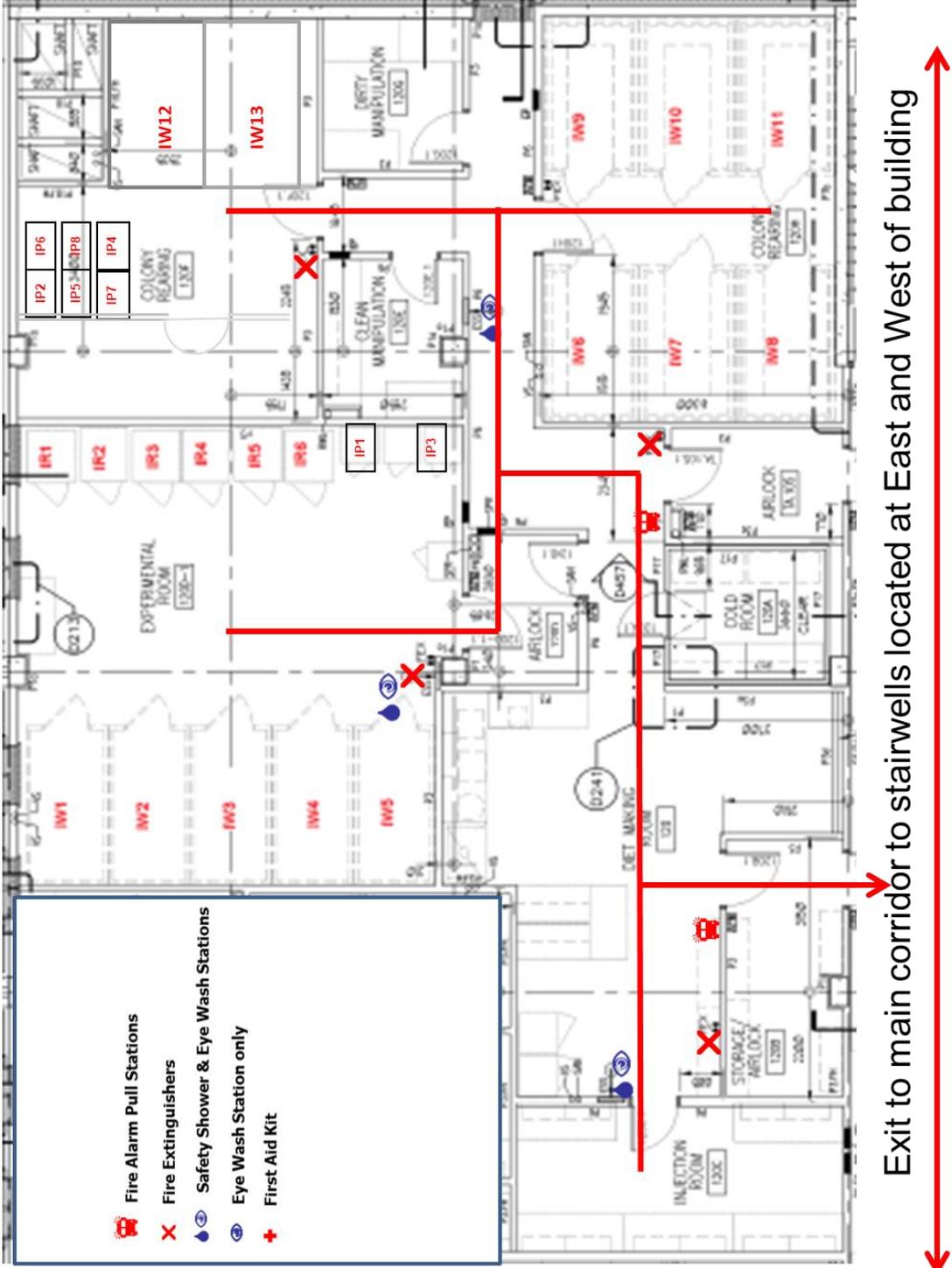


- h. If you see this result, then the autoclave is operating correctly. **If this is not the result attained, please contact the IM Laboratory Supervisor immediately.** Discard both ampules in the provided sharps container after results have been collected.

**DO NOT discard autoclaved waste until results of the Sterilization Test are confirmed.**

3. When you are ready to autoclave:
  - a. **DO NOT** autoclave liquids containing bleach, formalin or glutaraldehyde.
  - b. If you are sterilizing liquids or have liquid waste, ensure that you are running a liquid cycle. Use hard or gravity cycle for solid waste or when sterilizing glassware. Usually a 60-min cycle is sufficient for sterilizing or autoclaving waste. Select cycle by clicking on ‘Select Cycle’ button and scroll to appropriate program. Press ENTER and then OK.
  - c. Anything that is going into the autoclave must be inside a plastic autoclave bin (available on the shelves to the left of the autoclave). Bags should be loosely sealed.
  - d. Close the autoclave door and press ‘seal’ button. Double-check that the correct cycle is showing on the top left of the autoclave display before starting the cycle. When ready, press the green ‘START’ button on the autoclave.
  - e. If the door is not properly sealed, the autoclave display will show this, and the autoclave will not start. Ensure the door is sealed prior to starting.
  - f. Once the cycle has started, the autoclave may be left unattended until the end of the cycle.

- g. The autoclave display will read “COMPLETED” and the time will read “0” when the cycle is done. Press ‘unseal’ button and once the pressure is zero, crack the door open and wait for steam to dissipate before fully opening the door. Stand to the side of the door and use CAUTION when opening the door. Use insulated autoclave gloves when removing contents from the autoclave. Leave on the floor out of the way of other users.
  - h. When cooled, pack the waste into a garbage bag. Tie the bag and label with “Treated Biomedical Waste” label. You must write your name, location of the autoclave (Biotron 120), and date.
4. After autoclaving, remember to do the following:
- a. Dispose of waste as soon as possible and avoid leaving it in the Diet Making Room for extended periods of time.
  - b. After putting the autoclaved waste into garbage bags and labelling, ensure that they are not leaking (if so, double-bag). Once packed, place the garbage bags onto the cart (not the floor) outside of the IM. *Do not overload garbage bags as they can get too heavy.*
  - c. Clean autoclave bins and any drips on the floor after use. Leave bins upside down to dry on shelf to the left of the autoclave.



Above is the Biotron Insect Module floor plan showing locations of fire alarm pull stations, fire extinguishers, safety showers and eye wash stations, as well as emergency exits.