Third draft – Sept 2008

The University of Western Ontario

The Biotron

Containment Level 3 (CL-3) Laboratory

Standard Operating Procedures

and

Users Manual

2008

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1. Introduction

The Biotron is an interdisciplinary, experimental climate change research facility located on the campus of the University of Western Ontario (Western) and dedicated to the elucidation of the impact of climate change and extreme environments on plants, insects and micro-organisms. Four of the research units (Microbiology, Insects, Transgenic Plants and Biomes) of the Biotron are designed and built to meet the requirements for Containment Level 2 (CL-2) laboratories. The Containment Level 3 Biohazard Laboratory (CL-3), which is located within the Containment Level 2 (CL-2) Transgenic plant unit at the Biotron will be used mostly for small scale research on plant pathogens.

CL-3 laboratories are unique laboratory facilities, which are designed and built to provide a safe environment for conducting research involving biological agents classified by the Biosafety Office of Public Health Agency of Canada (PHAC) as requiring Containment Level 3. Containment is provided at the primary level by biological safety cabinets and at the secondary level by specialized engineering features of ventilation design and facility construction.

1.1. Program Intent: Plum Pox Virus (PPV) Research by Agriculture and Agri-Food Canada, Molecular Farming, Microbiology for other research areas.

1.2. Pathogen List: **To be determined**.

However, the Biotron is an international facility open to academic, private, and government institutions and the CL-3 laboratory is designed and built to meet the requirements for any Level 3 pathogen that may be studied in the future.

The safe operation of the facility depends not only on the design requirements but also on strict adherence to the operating protocols and procedures outlined in this document by the specially trained personnel authorized by the University Biohazards Sub-Committee for work at Level 3.

This Users Manual contains information on access control, personnel training, medical surveillance and emergency response as well as Standard Operating Procedures for the conduct of research and waste disposal.

The following guidelines are adapted from the “Laboratory Biosafety Guidelines” 3rd Edition, 2004, published by the Public Health Agency of Canada. The Laboratory Biosafety Guidelines is used by the Canadian Foundation for Innovation (CFI) as requirement in their granting process.

2. Abbreviations

3. Emergency Contact Personnel and Decontamination Team

Alarms in the CL-3 laboratories of the Biotron are monitored by Western Environmental Services (WES) of the Physical Plant Department (PPD) on a 24hr basis and also by the University Police Department (UPD) Duty Officer. The CL-3 Biotron Call-in List (**Appendix A**) is used by WES and UPD to access a research representative or the BSO/BSC for emergency action. If necessary, the facility can be decontaminated on an emergency basis by the research representatives and the BSC.

4. Personnel and Responsibilities

4.1. Scientific Director / Executive Director of the Biotron – Responsible for implementation of policies and procedures for safe operation in the Biotron CL-3 laboratories to protect all personnel, the environment and the facility.

4.2. UWO - Biohazard-Sub-committee (BHSC) - Responsible for the operation of the CL-3 laboratories and safety of personnel. The University Biohazard-Sub-committee (BHSC) (which reports to the University Biosafety Committee (UBSC), reviews all research protocols used in the facility. This Sub-committee also reviews each application by research personnel to work in the facility. UWO-BHSC will ensure safety training and promote continuing education for un-experienced and experienced personnel, and that compliance with SOPs is being observed. UWO-BHSC will appoint a Biosafety Officer (BSO), who will report to UWO-BHSC under Terms of Reference (TOR) listed in **Appendix B**.

UWO-BHSC will also Chair the Biotron Biohazard Safety Committee, and will appoint, as /if necessary, suitable members to respond to questions of biosafety. For any Biological Emergency Response, UWO-BHSC will assume the role of On-Scene Commander, and take appropriate action.

The University is responsible, through Human Resources, Occupational Health and Safety (OH&S) and the Physical Plant Department (PPD), for maintaining the facility in safe running order. This includes the air handling system and alarms, all HEPA filters, electrical and plumbing system.

4.3. Biosafety Officer (BSO) – The BSO will be responsible for the enforcement of compliance with the SOPs developed for CL-3 laboratories as outlined in the TOR. The Biosafety Officer reports to the Scientific Director/Executive Director, the Safety Committee of the Biotron and Occupational Health and Safety (OH&S) any unsafe practices or concerns and works closely with the research personnel and the BHSC members to review work procedures, waste disposal practices and to facilitate communication between the groups. Where research personnel are employed by affiliated UWO institutions, the Biosafety Officer of that institution will also be consulted.

Other duties include:

4.3.1. Enforcement of immunization (if required).

4.3.2. Maintaining a current list of pathogenic agents under study at the Biotron

4.3.3. Maintaining records on testing for compliance with Health Canada guidelines on Biosafety

4.3.4. Conducting regular (quarterly) inspections of the CL-3 laboratories

4.3.5. Ensuring that training requirements and documentation are maintained.

4.4. Biotron Safety Committee (BSC) – The Biotron Safety Committee under the leadership of the Scientific Director/Executive Director and consisting of Scientific Director, Executive Director, Technical Director and all module leaders oversees the general operation of the facility.

4.4.1. This committee will be responsible for addressing any safety/biosafety concerns raised by any employee at the Biotron.

4.4.2. The committee will be responsible for reviewing and approving all projects which will require the use of the CL-3 laboratories, as well as developing, establishing and updating the SOPs.

4.5. Principal Investigators (PI) / Supervisors – Responsible for ensuring that appropriate safety orientation and training is conducted for Biotron staff, contractors, students, postdoctoral fellows or other persons under their supervision, whose duties require entry into CL-3 laboratories. The supervisor/PI will ensure that the person attends the appropriate training for CL-3 course at the first opportunity and any further training, that may be deemed appropriate.

Documentation of training for each individual must be forwarded to the BSO, and will include a mandatory reading of relevant manuals and SOPs.

4.5.1. Training in general techniques or techniques unique to a specific research project or area is the responsibility of the supervisor/PI and will include items as appropriate, such as:

4.5.1.1. Personal emergency response procedures (assistance alarms, communication equipment, fire alarms, fire extinguisher, emergency gas shutoffs, and procedures during and after normal duty hours).

4.5.1.2. Operations in Class II Biological Safety Cabinets (BSC).

4.5.1.3. Centrifugation procedures: use of sealed rotors and tubes, safety cups and rotor lifting devices.

4.5.1.4. Use of disinfectant traps and HEPA filters (on vacuum pumps, freeze dryers, centrifuges, and portable vacuum pumps).

4.5.1.5. Management of accidents (insect bites, self-inoculation with needles, infectious spills inside and outside BSC, centrifuge accidents, etc.).

4.5.1.6. Autoclave operation and preventive maintenance.

4.5.2. Forms to be Completed by Supervisor/PI for Work in CL-3 Facility

4.5.3.1. Biohazard Registry Form (BRF).

4.5.2.2. Position Hazard Form (PHF).

4.5.2.3. Accident/Incident Report Form (AIR).

4.5.3. The Position Hazard Form provides a means for the Biotron to communicate to the University and to the employee, known potential hazards in

the work place. The Position Hazard Form should be completed when a new employee is hired, when an employee is reclassified, when workplace hazards change, or when the position changes.

4.5.4. The supervisor completes the form and keeps a copy of this form on file, gives a copy to the employee, sends a copy to Occupational Health & Safety, and sends the original to Staff/Faculty Health Services in Room 25, University Community Centre. Staff/Faculty Health Services uses the form to determine the appropriate occupational health surveillance program for the employee.

4.5.5. When the supervisor gives the employee his/her copy of the form, it is an appropriate time to open discussion with the employee. The supervisor can review the hazards of the position and discuss proper procedures and precautionary measures. It is also a time for the supervisor to assess the employee's knowledge level of safe working procedures and to book the employee for training programs with OH&S.

4.6. First Responder – The designated First Responder is the link between personnel inside of CL-3 laboratory and outside, should an emergency arise.

4.7. Personnel – Must follow strict adherence to the CL-3 SOPs to ensure the safety of co-workers, all other personnel and the environment. Women assigned to BL-3 containment laboratories are required to notify UWO-OHSD

and the BSO as soon as pregnancy is suspected so that any risk can be avoided. Pregnant women will be assigned duties outside of a containment laboratory.

4.8. Students

4.8.1. Graduate students **will not be allowed to work unaccompanied** with any live Level 3 pathogens, infected plants and animals in the CL-3 laboratory of the Biotron at any stage of their academic career. They must be closely supervised at all times by their supervising faculty member/PI or an experienced technician or post-doctoral fellow.

4.8.2. Undergraduate students and summer students **are not allowed** to work in the CL-3 laboratory **under any circumstances**.

5. Contaiment Level 3 Laboratory Area

5.1. Security Guidelines

5.1.1. Purpose - The University of Western Ontario is committed to ensuring the safety of persons and security of property and information at the University, while maintaining a sense of openness for educational and research purposes. It is the essence of a University that independent research should be undertaken and proprietary information kept secret. Research often involves controversial issues, controversial results and varied interpretations. The research that will be conducted at the Biotron and especially in the CL3 laboratories may pose additional risk by those that wish to dispute, disrupt or acquire that research. Confidentiality standards instill confidence for a prudent

exercise of caution, good business practices, and the ongoing safety/security of persons, property and information.

5.1.2. Security Assessments – Persons - For the purpose of determining the extent to which a person may pose a risk, including corporate espionage or eco-terrorism, each person must undergo a security assessment before access level approval is granted. Access approval may range from no approval needed beyond that currently in place, to a high level of approval for higher level research areas. There are several classes of persons who may apply for access to the resources available in the CL-3 laboratories of the Biotron. They are defined as:

5.1.2.1. Employee means a person employed by The University of Western Ontario.

5.1.2.2. Contractor means a worker or any person who undertakes a contract for services with The University of Western Ontario. The term includes contractors, subcontractors, independent service providers, consultants, etc. and their staff, as well as agencies and agency referred personnel.

5.1.2.3. Temporary Visitor means an individual who has received permission in advance to tour the Biotron facility, or business representative while accompanied by a person approved by the General Manager, or unaccompanied.

5.1.2.4. Canadian Research User means an individual, including a researcher/ scientist, student, who is not an officer, director, or employee of The

University of Western Ontario and who is a Canadian Citizen ordinarily resident in Canada; or a permanent resident ordinarily a resident in Canada.

5.1.2.5. Foreign Research User means an individual, including a researcher/ scientist who is not an officer, director, or employee of The University of Western Ontario and who is NOT a Canadian Citizen ordinarily a resident in Canada; or a permanent resident ordinarily a resident in Canada.

5.1.3. In assessing the security risk, the Director, will oversee the Employment Related Security and Background Checks in collaboration with the Biotron General Manager, or designate. The Campus Community Police, with the information provided by the Director/General Manager, will oversee the conducting of the criminal screening of Employment Related Security and Background Checks program, as necessary.

5.1.4. Safety and Security Assessment – Persons - Biotron (Including CL-3 laboratory) access approval is based on the level of access required in combination with an evaluation for honesty, reliability and trustworthiness, where required, including:

General Employment Background References, Checking and Screening

Police Records Check including previous addresses and local occurrences

Educational Background review

5.1.4.1. Every person requiring access to the CL-3 laboratory of the Biotron must consent to a security assessment and complete the Application of Consent form, as provided by the Biotron Executive Director. The applicant will

meet with the Scientific Director, Administrative Officer and/or other Designated Official and provide photo identification that corresponds with the information on the application form. The Scientific Director, Administrative Officer and/or other Designated Official will verify the applicant's information and complete the necessary screening in accordance with corporate policy on background checks. Applicants seeking employment at the Biotron CL-3 laboratory are required to provide their police check security assessment as part of the application process.

5.1.4.2. Confidentiality: Prior to working in the Biotron CL-3 laboratory, employees, researchers, students and other workers are required to sign an agreement relating to maintaining the confidentiality (Confidentiality Agreement – **Appendix C**) of observations and other information acquired through access to the Biotron CL-3 laboratory, in accordance with expectations.

5.2. Access

5.2.1. – Biotron Building Access – Building hours of operation is from 8:30 a.m. until 4:30 p.m. from Monday to Friday. To enter the building after hours a security card, encoded by Physical Plant Department (PPD), is required. In order to attain security access to the CL-2 areas (Insect Module, Microbiology Module, Transgenic Plant Module, Biomes) of the Biotron, keys and/or security cards will be also issued and/or programmed accordingly. The following procedures must be followed:

5.2.1.1. Completion of the **"Building Access Form"** as located on the Physical Plant website at

http://www.uwo.ca/ppd/documentation/bldgaccessform.doc. The completed form is to be forwarded to the Biotron Administrative Officer (AO) located in Room 101 at the Biotron.

5.2.1.2. The Administrative Officer (AO) will cross-reference the applicant on the Building Access Form with the **Project Application Forms** (Staff Information section) which have been received. *(If the Project Application is not yet on file, the AO will follow up with the applicant.).*

5.2.1.3. Based on the information on the Project Application, the AO will then confirm with Health and Safety the applicant's WHMIS, Bio Safety and Lab Safety and/or other certifications which are required for the level of containment in which they will be working.

5.2.1.4. Once confirmed the AO will then log the information provided from the Building Access Form and Health and Safety into the **Building Access Log** (this log is kept in a secure file by the AO).

5.2.1.5. The AO will act as the initial signatory for the Building Access Form ensuring all the above criteria have been met. The Building Access form will then be forwarded on to one of the other authorized signing authorities. Once the proper signatures have been attained, the form is then forwarded to the Keys Office of the Physical Plant Department (PPD), where access and keys will be issued as per University policy.

5.2.1.6. There are to be **three** current signing authorities listed at all times at PPD for the Biotron, two of which are required on **all** Building Access Forms. At the completion of a project, the AO will electronically notify the applicant and

the PI to return any outstanding keys to PPD. The AO will also notify PPD of the termination of access for said individuals.

5.2.2. CL-3 Laboratory Access – Because rapid access to an infectious disease physician cannot be guaranteed at the hospital emergency department The University Biosafety Committee has decided that work with live virus in the CL-3 facility be conducted **ONLY** during regular work hours (8:30am- 4:30pm from Monday to Friday) when emergency medical coverage is provided by the University Staff/Faculty Health Services.

5.2.2.1. Approval to use the CL-3 facilities is given by the Biohazard Sub-Committee. All personnel, working in the facility must be approved by the Sub-Committee prior to commencing work in the facility. Completion of the Containment Level 3 access form and authorization by the BHSC is required.

5.2.2.2. Applications to use the CL-3 Laboratory - The CL-3 Laboratory is required for work using any of the agents listed as requiring Level 3 containment practices in the Public Health Agency of Canada publication "Laboratory Biosafety Guidelines: 3rd ed. 2004. (1) An application by a faculty member or principal investigator (PI) to conduct research in the CL-3 facility at the Biotron must be submitted and approved BEFORE the submission of the grant application which will support the work.

5.2.2.3. An application is initiated by completion of the University Biohazards Registry Form (BRF), which can be obtained from http://www.uwo.ca/ohs/. This form identifies the pathogen(s) to be used and will indicate the requirement for CL-3 laboratory and requires the approval of the

Biohazards Sub-committee. Details of protocols to be used will be required. Since space is limited in the facility, permission to use the facility MUST be obtained IN ADVANCE of submission of the grant application.

5.2.2.4. If Level 3 human pathogens are to be imported into Canada, an Import Permit will be required from Public Health Agency of Canada (PHAC) in advance. Import permits for CL3 agents will require that the facility has been certified by PHAC. The University Biosafety Coordinator must be notified of the intent to import a CL3 pathogen for use in the University CL-3 biohazard laboratory. The Biosafety Coordinator will notify the Biosafety Officer of the Biotron if the permit has been applied for by one of their employees.

5.2.2.5. If CL3 insect and/or plant pathogens are to be imported into Canada, an import permit will be required from the Canadian Food Inspection Agency (CFIA) in advance. This will require inspection of both the laboratory and animal housing facilities by the CFIA before the permit will be approved. The application must be submitted to the Biohazard Sub-Committee for approval before the import permit is applied for.

5.2.2.6. All protocols using insects in the CL-3 laboratory must be approved by the Animal Use Sub-Committee (AUS). Information can be obtained from Director, Animal Care and Veterinary Services.

5.2.2.7. If radioisotope use is proposed within the facility, additional permission from the Radiation Safety Committee will be required. Contact: The Radiation Safety Coordinator, HR, OH&S.

5.2.2.8. Applications for Research Personnel to Work in the Biotron CL3 Laboratory - All Personnel involved in the proposed Level 3 work must be approved by the Biohazard Sub-committee. The PI/Faculty member/Researcher supervising the research in the Level 3 facility should submit an application for each person in writing to the Director, OH&S.

5.2.2.9. Applications must contain a brief CV/Resume stating his/her research experience with microbiological agents and/ or cell culture and/or pathogens.

5.2.2.10. For graduate students and post-doctoral personnel, the length of time the individual has worked with the faculty member/PI who will be supervising the Level 3 work. Please note: new graduate students are required to spend at least 6 months gaining experience with non-infectious/pathogenic material before applying for permission to work at Level 3.

5.2.2.11. The application must also contain a point form summary of the protocol(s) to be used in the CL-3 laboratory. This must include details of decontamination procedures to be used if material is to be removed from the facility for continuation of work elsewhere. References will be required as to the efficacy of the decontamination procedures proposed. The Biosafety Coordinator must be notified of any subsequent changes to the protocols/agents used in the facility.

5.2.3. Personnel Training

5.2.3.1. All research personnel who plan to work in the CL- 3 laboratory of the Biotron MUST have attended the following UWO training sessions:

a. Biosafety Training

b. WHMIS

c. Laboratory Safety

5.2.3.2. The research supervisor/PI is responsible for ensuring that all the required training courses are attended. Training can be found at http:www.uwo.ca/ohs/. Training for specific work procedures will be the responsibility of the research supervisor in collaboration with the Biosafety Officer. Specific training on safety procedures in the CL-3 laboratory will be given after approval to use the facility is obtained.

5.2.3.3. Training for work at CL-3 will be given by the Biosafety Officer/Coordinator as individual sessions covering the requirements of the CL-3 Users Manual:

i. Layout of the facility and ventilation and alarm features

ii. Entry/Exit procedures

iii. Emergency exit procedures

iv. Fire Procedures

v. Spill and Accident Procedures

vi. Hazard awareness training for specific infectious and chemical agents to be used.

vii. Decontamination and waste disposal procedures

5.3. Biosafety

5.3.1. The responsibility for the safety of staff working in the CL-3 laboratory lies with the supervisors/PI using the facility.

5.3.2. A dedicated Biosafety Officer (BSO) who has a working knowledge and training of the laboratory practices and the BSC oversee and manage the safety/biosafety issues and develop a biosafety program**.**

5.3.3. The Biosafety Officer in close collaboration with the BSC and the PIs are involved in risk assessment of the research projects, and other biological safety matters such as:

5.3.3.1. Identifying training needs and assisting with the development and delivery of biosafety training programs.

5.3.3.2. Developing recommendations for procedural or physical modifications of the CL-3 laboratory.

5.3.3.3. Auditing the effectiveness of the biosafety program on a regular basis.

5.3.3.4. Participating in accident investigations within the CL-3 facility.

5.3.3.5. Coordinating and monitoring the decontamination, disinfection and disposal procedures for pathogenic/infectious materials in the CL-3 laboratory.

5.3.3.6. Coordinating the receipt, shipment and transport within the facility of pathogenic/infectious material according to the Workplace Hazardous Materials Information System (WHMIS) and Transportation of Dangerous Goods (TDG) regulations.

5.3.3.7. Establishing a record keeping and secure storage system for all pathogenic/infectious materials entering/leaving the CL-3 facility.

5.3.3.8. Coordinating emergency response activities.

5.3.3.9. Certification and recertification of the CL-3 laboratory.

5.3.3.10. Investigation and remediation of containment suite physical or operational failures.

5.4. Description and Physical Specification of the CL-3 Facility

5.4.1. Laboratory Design The special engineering and design features of the CL-3 laboratory are based on the recommendations of Public Health Agency of Canada (1). Key physical requirements are summarized as follows:

5.4.1.1. The facility is located on the G floor of the Biotron Building and is within the security Containment Level 2 area of the Transgenic Plant Unit of the Biotron. (See floor plan, Figure 1) and has controlled key card access.

5.4.1.2. At all times the laboratory is held at negative pressure with respect to surrounding areas to create directional air flow from outside areas into the laboratory. Air flow is designed to be from low hazard areas to high hazard areas.

5.4.1.3. The laboratory has a sealed exhaust system. All exhaust air is filtered through High Efficiency Particulate Air (HEPA) filters before discharge. To prevent the laboratory from becoming positively pressurized relative to the surrounding area the supply and exhaust air systems are interlocked. If the exhaust system shuts down, the air supply shuts down.

5.4.1.4. Hands free sinks and soap dispensers are present in each CL-3 laboratory rooms as well as in the change room.

5.4.1.5. The facility has a pass through autoclave connecting the CL-3 laboratory area (Room 20G) with the supporting CL-2 area (Room 20E).

5.4.1.6. Laboratory furnishings are kept to a minimum and are all readily disinfected surfaces for ease of decontamination.

5.4.1.7. All penetrations for services in the floors, walls and ceiling of the laboratory are sealed with a non-shrinking sealant.

5.4.1.8. Floor drains are sealed and traps are topped up monthly with disinfectant.

5.4.1.9. All doors into the CL-3 facility are posted with biohazard warning signs. Entry doors to the CL-3 laboratories are also posted with biohazard warning signs.

5.4.1.10. A sign indicating infectious agents must be on the laboratory doors where agents are used.

6. Operation of the Airlocks

6.1. Description of Operation A

Under standard conditions (CL3 laboratory is empty) **door 20F.1 (door from corridor to changing area)** is normally closed and locked (card reader LED is RED, indicator plate LED's GREEN – indicating nobody using change area). **Door 20G.1-1 (door from lab vestibule to gowning area) –** door normally closed and unlocked (indicator LED's GREEN)

Access - *IF NOBODY IS IN SHOWER - Access to changing area is by valid card at door 20F.1.

- Access to gowning area by turning lever at 20G-1.1

- Once inside changing / gowning area, button located next to each door may be activated to lock out card reader at 20F.1 and to lock the lever leading into gowning area. LED's on all indicators to turn RED.

Egress - Free egress by inside lever at either door (** see D.O.O. #B for exception)

- Turning inside lever at either door unlocks outside lever at 20G.1-1and re-enables card reader at 20F.1.

- All LED's to change back GREEN.

Hydro outage - Doors to continue to operate as above on battery back up.

Battery - Battery in access control system equipment & door hardware power supply to provide continuous power (uninterrupted).

Back up loss of power - Upon loss of hydro and battery power, door 20F.1 will allow access by key and free egress and 20G.1-1 to remain closed and unlocked

Power restoration - Doors to automatically return to normal operation.

Fire alarm - To operate the same as during 'loss of power'.

Notes - Separate airlock for doors 20F.1 and 20G.1-1 as noted in Description of Operation B

6.2. Description of Operation B

Normal - 20F.1 (door from corridor to changing area) - door normally closed and outside lever locked – magnetic lock unlocked. Door **20G.1-1 (door from lab vestibule to gowning area) –** door normally closed and unlocked – magnetic lock unlocked.

Operation - Any time the door from the corridor to the changing area is opened, the magnetic lock at the door from the lab vestibule to the gowning area will lock and cannot be opened from either side until the corridor door is closed.

Any time the door from the lab vestibule to the gowning are is opened, the magnetic lock at the door from the corridor to the changing area will lock and cannot be opened from either side until the gowning area door is closed again. The purpose of this is to prevent air from flowing from the CL3 lab vestibule area directly to the corridor.

Hydro outage - Doors to continue to operate as above on battery back up.

Battery - Battery in door hardware power supply to provide continuous power (uninterrupted)

Back up loss of power - Upon loss of hydro and battery power, doors to remain unlocked and allow free access and egress.

Power restoration - In order for maglocks to work within the airlock interlock, a manual reset switch must be activated to restore power for maglocks.

Fire alarm - Power to maglocks and electric locks to be lost, must be reset manually after fire alarm system is reset.

Notes - Doors are also set up for a privacy interlock – see Description of Operation A. *Pull stations within CL3 area are directly tied to magnetic lock power and upon activation of any of these pull stations all magnetic locks will release / unlock

6.3. Description of Operation C

Normal - Doors are normally closed and unlocked.

Access to airlock from lab vestibule – Only possible if door from airlock to lab is closed.

Access to lab from airlock – Only possible if door from gowning area to airlock is closed

Egress to airlock from lab – Only possible if door between lab vestibule and airlock is closed.

Egress to lab vestibule from airlock – Only possible if the door between the lab and the airlock is closed.

Hydro outage - Doors to continue to operate as above on battery back up.

Battery - Battery in door hardware power supply to provide continuous power (uninterrupted).

Back up loss of power - Upon loss of hydro and battery power all doors at airlock to be unlocked and allow free access and egress.

Power restoration - In order for maglocks to work within the airlock interlock, a manual reset switch must be activated to restore power for maglocks.

Fire alarm - Power to maglocks and electric locks to be lost, must be reset manually after fire alarm system is reset.

**Notes - ***Pull stations within CL3 area are directly tied to magnetic lock power and upon activation of any of these pull stations all magnetic locks will release/unlock.

7. General Containment Level 3 Procedures

Containment Level 3 (CL-3) applies to a laboratory that handles agents/pathogens requiring CL3. These agents may be transmitted by the airborne route, often have a low infectious dose to produce effects and can cause serious or life threatening disease. This type of containment must have a specialized design and construction, whit emphasis not only on primary barriers to protect the individual, but secondary barriers to protect the environment. CL-3 laboratories must undergo annual performance, testing and verification. Additional features to prevent transmission of Risk Group 3 organisms are appropriate respiratory protection, HEPA filtration of exhausted laboratory air and strictly controlled laboratory access. All work must be performed in biosafety cabinets. Personal protective equipment (PPE) is very specific, including solid front clothing and dedicated footwear, for use only within the facility.

7.1. Administrative – Two notices must be posted at the entrance of CL-3 facility. These should be strategically placed so as not to confuse or distract another researcher, firefighter, etc.:

7.1.1. A **"CL-3 Infectious/Pathogenic Agent Summary"** is a concise notice of the infectious/pathogenic agent to be used, immunization requirements (if necessary), and persons to be contacted in an event of emergency.

7.1.2. A **"Log In/Log Out"** form with name of the investigators going inside, project and the time they have entered and excited.

7.1.3. A **"Weekly Schedule"** to inform users of who else will be inside CL-3 and when, will be posted by the Biosafety Officer, and will be accessible over the network.

7.1.4. Within CL-3 laboratory, precise and simple instructions will be posted for: Entry Protocol, Exit Protocol, Biological Emergency Response Protocol (including spills), Fire Alarm Emergency Response Protocol, Autoclaving Loads and Cycles to be used.

7.1.5. No laboratory notes are to be removed from CL-3 laboratory unless sterilized. Results and comments must be sent outside electronically.

7.2. Laboratory procedures

7.2.1. Biological safety cabinets will be used for all procedures with infectious/pathogenic substances. No work involving infectious/pathogenic material will be conducted in open vessels on an open bench.

7.2.2. Latex, nitrile or plastic examination ("surgical" or "medical") gloves must be worn in CL-3 laboratory. Double gloves should be used so that the outer pair can be continually discarded and replaced. An appropriate disinfectant must be available for surface decontamination of gloves within each biosafety cabinet.

7.2.3. Certain laboratory procedures must be confined to the biosafety cabinets. Such procedures include, but are not limited to: a) high speed blending, mixing and grinding; b) agitation; c) filtration; d) centrifugation in unsealed table-top centrifuges; e) sonication.

7.2.4. Plastic, disposable items should be used whenever possible. Consideration will be given to the elimination of sharps and points by using plastic items, blunt-end needles, rounded-point scissors, mortar and pestle instead of glass tissue grinder, etc.

7.2.5. All work surfaces must be decontaminated after use, and immediately after following any contained spill or release of infectious/pathogenic material.

7.2.6. Laboratory gas/ethanol burners must be turned off before personnel leave the laboratory, and during any absence from the laboratory. Open flames of any type must not be left unattended.

7.2.7. Whenever feasible, supplies should be uncrated in the “clean” area to avoid the necessity for decontaminating empty carton, boxes, and packing material.

8. Entrance Procedures

Persons shall be authorized to enter a Containment level 3 (CL-3) laboratory of the Biotron only if their presence is required on the basis of program/project or support need, and required immunization (if necessary) and appropriate training are completed. Such persons will be made aware of the

potential hazards and will comply with all entry and exit procedures as defined in this SOP.

8.1. Before entry into CL-3 facility:

8.1.1. Have work scheduled the previous week with the BSO.

8.1.2. Arrange an approved First Responder to monitor from the outside.

8.1.3. The log in/out sheet (**Appendix D**) must be signed, dated and the time noted.

8.1.4. The magnehelic gauges/air pressure indicators must be checked for normal negative pressure (PPD - What is the normal negative pressure for the CL-3 laboratory in the Biotron?).

8.1.5. The steam to the autoclave must be checked.

8.2. Entry into the Facility

8.2.1. The doors to the facility are to be kept locked at all times, whether the facility is in use or not.

8.2.2. Card Access will be issued only to the principal investigators (PI) who have permission from the Biohazard Sub-Committee (BHSC) to use the facility. Application forms for a card access are obtained through the Director, Occupational Health and Safety (OH&S). The PI is responsible for controlling access to the facility for approved personnel in their research group.

8.2.3. Entry is by special entry code ONLY and is for ONE person at a time. Others are required to wait their turn.

8.2.4. There is only one change-room in the CL-3 facility. I case female and male persons should work together at the same time, the entry can occur at 20 min (at least) intervals.

8.2.5. Entry to the facility for all personnel is through the change-room. The Safety Officer must be notified before large equipment is moved into or out of the CL-3 laboratory. PPD will also be notified in the event the doors are to be opened to prevent false alarms.

8.2.6. The CL-3 laboratory is located within the Containment Level 2 Transgenic Plant Unit of the Biotron and is divided into three areas according to hazard:

8.2.6.1. Low / Intermediate hazard Level 2 - change room (Room 20F) and waste treatment room (Room 20E).

8.2.6.2. Intermediate hazard area: CL-3 access gowning area (Room 20G), and interlock entry areas (20H and 20J).

8.2.6.3. High Hazard CL-3 area: CL-3 Lab 1 (Room 20H-1) and CL-3 Lab 2 (Room 20J-1).

8.3. Clothing

8.3.1. Entry into change room - Remove street clothing (except underwear) and change into surgical pants and shirts. These surgical clothes may be reused (by the same individual) provided that they are not contaminated. If there is any doubt, discard the clothing into the autoclave bag and use clean ones. Leave street clothing in designated locker in the change room. All watches,

jewelry, wallets etc. should be left in the change room. These surgical greens may be used only in the biohazard facility. They are not to be worn in any of the adjacent rooms or corridors.

8.3.2. Put on disposable latex gloves and a hair net.

8.3.3. Put on designated shoes (kept in the facility). Shoes must be closed toe with a non-slip sole. Leather sneakers are suitable.

8.3.4. Put on disposable shoe covers over shoes. This reduces the possibility of a splash or spill onto the shoes requiring them to be discarded.

8.3.5. Street shoes may be used only if occasional entry to the waste treatment room (Room 20E) is required. Disposable shoe covers must be worn over shoes.

8.3.6. Glasses may be worn, but a head strap for the arms of the glasses should be used.

8.3.7. Proceed into the gowning area (Room 20G).

8.3.8. Put on disposable solid front wrap-around gown. These gowns are kept in room 20G). This gown is not to leave the inner area unless it has been autoclaved.

8.3.9. The gown may be reused for up to one week unless it becomes soiled or contaminated. Dispose of soiled gowns into biohazard waste in room 20G??).

8.3.10. Hang gowns on the pegs provided in room 20G??. Personnel must only wear their own gown and the pegs are identified with the wearer’s name to avoid confusion.

8.3.11. Gowns from the two CL-3 laboratories (20H-1 and 20J-1) must be kept separate.

8.3.12. Put on a second pair of latex gloves. Make sure that the cuff of the top glove goes over the cuff of the overgown. Tape in place if required.

8.3.12. Put on second pair of shoe covers over first pair.

8.3.13. Masks, head covers and safety glasses are mandatory. A full face shield must be worn if there is any possibility of splashing during the procedure.

8.3.14. Enter into CL-3 laboratories (20H-1 and 20J-1) trough airlocks 20H and 20J.

9. Exit Procedures

9.1. Temporary Exit from 20H-1 and 20J-1 laboratories.

9.1.1. Before leaving, ensure that all live pathogens are secured in the incubator or contained in the Biosafety Cabinets (BSC) which is operating.

9.1.2. In CL-3 (20H-1 and 20J-1) laboratories remove shoe covers and outer gloves and discard into biohazard bin for autoclaving.

9.1.3. Wash inner gloved hands (do not remove inner gloves). Exit to 20G immediately.

9.1.4. In room 20G, remove wrap around gown and hang on your peg. Never leave CL-3 area wearing overgown.

9.1.4. Leave through interlock/shower area.

9.1.5. At entry to change room: remove shoe covers and gloves and discard into biohazard bag outside change rooms. Enter change room.

9.1.6. Wash hands and bare arms. (There is no sink in the change room!?)

9.1.7. If returning to CL-3 Laboratory put on clean gloves and shoe covers before returning from change rooms.

9.1.8. On returning to room 20G, put on overgown, outer gloves and shoe covers. Never leave inner area wearing outer gown or outer gloves or shoe covers used in inner area.

9.2. Movement Between Rooms 20H-1 and 20J-1

Movement between rooms 20H-1 and 20J-1 should be avoided. Over-gowns and additional shoe covers and gloves specifically for use in 20H-1 and 20J-1 are kept in interlock areas 20H and 20J.

9.2.1. Overgowns, shoe covers and over gloves worn in 20H-1 laboratory must not be worn in 20J-1 laboratory if different projects/procedures are involved.

9.2.2. Exit procedures for the 20H-1 laboratory followed by entry procedures for the 20J-1 laboratory and vice versa must be carried out as above.

9.3. Exit from CL-3 Facility on Completion of Work

9.3.1. Ensure that all pathogens are secured in the incubators.

9.3.2 Ensure that all biohazardous material is disposed of into autoclavable bags. These should be sealed for transport to autoclave, opened to let steam in and then autoclaved at the end of each days work or as needed.

9.3.3. Wipe down all work surfaces with 70% Ethanol containing 1% detergent.

9.3.4. When the above steps have been completed, exit from laboratory as described in 9.1.

9.3.5. Wash hands and lower arms thoroughly in change room. (There is no sink in the change room?!).

9.3.6. Remove surgical pants and top. If reusing these items, store them in a separate locker in yellow area designated for lab clothing clearly marked with your name. **Street clothes must be kept in a separate locker.** Each individual will therefore require two lockers. Lockers must be labeled clean (for street clothes and dirty for lab wear)

9.3.7. Showering before leaving the facility is optional unless a spill has occurred or if working with animals with a separate SOP requiring showering out.

10. Access by Non-CL-3 Trained Personnel

Visitors, service and contractor personnel must follow all regulations in force for the CL-3 laboratory at the Biotron. In addition, they will be required to be accompanied by trained Biotron personnel for a limited period of direct supervision inside the CL-3 laboratories. When authorized maintenance personnel must enter a CL-3 facility, warning will be given of any unusual situation that may be hazardous, such as spills or leaks.

11. Biohazard Spill

Spill kits are available in rooms 20H-1, 20J-1 and 20G.

11.1. Spill of Biohazardous Infectious/Pathogenic Agent inside Biological Safety Cabinet.

11.1.1. Keep the biological safety cabinet turned on. The spill and any aerosols generated will be contained by the air circulation through the HEPA filter in the cabinet.

11.1.2. Cover the spill with absorbent paper towel or pad to absorb the liquid and gently pour Bleach (chlorine) or Wescodyne onto the pad being careful not to splash or create additional aerosols.

11.1.3. Allow 20 minutes contact time for decontamination of the material.

11.1.4. Using tongs or forceps carefully discard the contaminated towel into the tray of Wescodyne inside the cabinet. Immerse the pad into the disinfectant. Finally wipe the entire work surface of the cabinet with Wescodyne or Sporicidin and discard wipes into disinfectant tray.

11.1.5. Discard contaminated gloves, wash hands thoroughly, and replace with a clean pair.

11.1.6. Place disinfectant tray into an autoclave bag inside the cabinet, seal it, then double bag with a clean bag and autoclave immediately.

11.2. Spill of a Biohazardous Agent outside the Hood.

11.2.1. Clothing NOT contaminated.

11.2.1.1. Leave the room immediately and warn others in the facility to leave immediately or not to enter.

11.2.1.2. After 30 minutes (to allow for the aerosols created by the spill to settle or clear), put on coveralls, boots and gloves and return with spill kit. Cover the spill with absorbent spill pad or paper towel. Carefully pour undiluted Bleach (chlorine) onto pad and leave for 30 minutes.

11.2.1.3. Carefully pick up pad with tongs or forceps and place pad into biohazards bag and autoclave.

11.2.1.4. Discard contaminated gloves and replace with clean ones.

11.2.1.5. For large volume spills i.e. over 50 ml sprinkle Chlorabsorb powder onto liquid to reduce spread.

11.2.1.6. Leave the room for 30 minutes to allow for decontamination. Scrape up Chlorabsorb powder with two pieces of cardboard (in spill kit) and place into biohazard bag for autoclaving. Wipe up the floor or bench carefully, using fresh disinfectant. If you use a mop, sterilize or thoroughly decontaminate the mop head afterwards.

11.2.2. Clothing contaminated.

11.2.2.1. Warn others in the facility of the spill and ask them to leave immediately or not to enter.

11.2.2.2. Request assistance if required. Ensure assistant is suitably protected.

11.2.2.3. Immediately remove contaminated clothing.

11.2.2.4 If the spill has penetrated to the skin, strip and use the emergency shower. Discarded clothing should be placed in a bag for autoclaving. The clothing must be autoclaved before being removed from the facility. After

decontaminating yourself, leave the inner area and go to the change room, and re-shower. Put on a clean surgical scrub suit, coveralls, boots and gloves and return to the inner area to complete the clean up of the spill. Disinfect the water on the floor from the shower with Bleach (chlorine) and after 30 minutes release the floor drain to allow the water to drain. Wash the floor and any other contaminated surfaces with fresh disinfectant.

11.2.2.5. Remove contaminated clothing and autoclave. Boots must be disinfected with Bleach (chlorine).

11.2.2.6. If widespread contamination has occurred in the laboratory, notify the BSC and PI immediately. The laboratory will be shut down, all viral cultures discarded and the facility will be decontaminated as described below.

11.2.3. Fracture of tube containing biohazardous agent in the centrifuge.

11.2.3.1. If you can tell from the noise of the centrifuge that one of the tubes has broken, switch off the machine, but leave it SHUT for 30 minutes to allow aerosols to settle. Leave the facility during this time and advise other workers in the facility of the problem and instruct them also to leave.

11.2.3.2. If you discover the breakage when unloading the centrifuge, hold you breath, close the lid and leave the facility for 30 minutes to allow the aerosol disseminated into the air to clear.

11.2.3.3. After 30 minutes return to clean up the spill in the centrifuge. Using tongs or forceps, remove all broken part of the tubes or cups and place into an autoclave bag. Be especially careful if there are any sharp edges on the fractured tube.

11.2.3.4. Any removable parts of the centrifuge e.g. cups are to be autoclaved, if possible, and inside of the machine must be thoroughly washed out and decontaminated with Wescodyne and ethanol mixture. (50% Wescodyne using 95% ethanol)

12. Chemical Spill

12.1. Preparation for chemical spill is essential.

12.2. All personnel must know and state the hazards of the material(s) before start working with it in the CL-3 laboratory. Review the Material Safety Data Sheet (MSDS).

12.3. All personnel working in the CL-3 facility must know the exact location and type of spill kit available and know how to use it.

12.4. Hazardous chemical materials are kept to a minimum in the CL-3 facility to avoid hazardous situations in the containment areas. Chemicals are limited to ethyl or propyl alcohol and disinfectant concentrates. Permission to take other chemicals into the CL-3 facility will only be given after submission of the protocols using chemicals to the Biosafety Officer.

12.5. Protective clothing – gloves and eye protection must be worn when dispensing or diluting hazardous chemicals. Goggles, face shields and spill kits are available in rooms 20H-1, 20J-1 and 20G.

12.6. Hazardous materials are controlled by the Workplace Hazardous Materials Information System (WHMIS) and information regarding the safe handling procedures and first aid measures will be available on the supplier label

and on the Material Safety Data Sheet (MSDS). All employees are responsible for following the requirements of WHMIS, and have the appropriate training and safety equipment (eye goggles, face shield, spill kit, etc.) available inside rooms 20H-1, 20J-1 and 20G.

12.7. Absorb the spilled chemical using the absorbent pads in the spill kit. Place used pads into a disposal bag and call OH&S for disposal instructions.

12.8. In the event of a chemical exposure, follow the first aid measures (12.6. below) and seek assistance from the OH&S and/or Staff/Faculty Health Services.

12.9. First Aid for Chemical Spills

12.9.1. For skin or clothing exposures and emergency drench showers are available in rooms 20H-1, 20J-1 and 20G. Flush exposed area thoroughly with water for 5-10 minutes. For eye and mouth exposures eye wash stations are available in rooms 20H-1 and 20J-1. Flush thoroughly for 10 minutes. Report the exposure to the supervisor/PI in charge of the project. Contact the Biotron BSO and Staff/Faculty Health Services if required.

12.9.2. Contact OH&S regarding disposal of chemical wastes from the CL-3 facility. If the emergency drench shower has been used, consult with OH&S for disposal instruction before opening the floor drain.

12.10. Spill Reporting Procedure

12.10.1. All spills must be reported to the PI and Biosafety Officer as soon as possible after the spill.

12.10.2. The supervisor/PI must complete the Accident Investigation Form (AIR) and return it to OH&S as soon as possible.

12.10.3. The supervisor/PI must conduct an accident investigation and report and submit this to OH&S with the AIR.

13. Radioisotope Spill

13.1. Decontaminate the infectious/pathogenic/chemical agent first as described in 11. and 12. (above). Then clean up the radioactive spill as recommended in the safety manual that accompanies your license to work with radioactive material and as described in the protocol approved by the Biohazard Sub-Committee.

13.2. Place decontaminated clean-up materials into a biohazard bag and seal. Double bag this with a clean bag and store this.

13.3. Notify the supervisor/PI, the Biosafety Officer and Radiation Coordinator immediately.

13.4. Do NOT autoclave the bag of waste as this may result in release of radioactive products into the autoclave.

13.5. Disposal of the radioactive spilled material will be by incineration AFTER the radioactivity level has been checked to be below the Scheduled Quantity for the isotope and must be verified by the Radiation Coordinator.

14. Fire Safety/Alarm Procedures in the CL-3 Facility

The CL-3 laboratories are designated “burn-out area”. Base fire fighting personnel will not enter these areas to put out or control fires, due to the biological hazard. Instead, they will limit their efforts to other areas of the Biotron building and allow fire to consume the CL-3 laboratories. If there is a fire within CL-3 laboratory, a judgment call must be made. If the fire is small enough to control, the staff will attempt to do so with the available extinguishers. If the fire is too large to control, the staff must pull the fire alarm and leave immediately. If time permits, a quick decontamination should be performed.

14.1. Small Fires

14.1.1. Don’t try to fight the fire yourself unless the fire is minor and you feel confident in the use of the fire extinguisher.

14.1.2. If the fire is small e.g. a small ethanol fire, pull the fire alarm and then use the fire extinguisher, which is mounted on the wall of the laboratory.

14.1.3. Report the use of the extinguisher to your supervisor/PI, Biosafety Officer and the Fire Prevention Officer (FPS) immediately after the fire extinguisher has been used.

14.1.4. Secure the pathogen cultures/material and replace in incubator. Leave other clean up until later. Leave the biosafety cabinet running.

14.1.5. The used extinguisher must remain in the facility. Contact the Biosafety Officerr to arrange for surface decontamination of the used extinguisher prior to removal of the extinguisher by FPS and a replacement extinguisher.

14.2. Large or Uncontrolled Fires

14.2.1. If the fire is large or you are unsure, pull the fire alarm and **leave the room immediately**. Call 911 (campus phone) from a safe area for assistance.

14.2.2. The panic button alarm is located on the wall of room 20F. Activate the panic button on leaving the CL-3 area. Remain in a safe area and call UPD at 911 to advise security as to the nature of the alarm.

14.2.3. Leave the building immediately by the stairs. Do not use the elevators.

14.2.4. Do not attempt to secure viral/pathogen or other cultures or materials and equipment. Your first priority is for your own and co-worker safety.

14.2.5. Discard contaminated clothing in the laboratory only if you have time. Otherwise, leave facility and building in lab clothes.

14.2.6. Wait in the area immediately in front of the Biotron Building to advise firefighters and/or Emergency Response Incident Commander about the condition of the CL-3 laboratory and any biohazards.

14.2.7. Identify yourself to the incident commander as a Biohazard CL-3 facility worker. Lab clothes can be removed and bagged for autoclaving at the marshalling area in front of the Biotron Building.

14.2.8. In the case of a building fire alarm with no evidence of fire in the CL-3 facility: secure the cultures as above, leave the Biosafety Cabinet running and evacuate the building immediately by the stairs.

15. Medical Emergency Procedures

15.1. Accidental Exposure to Infectious Agents

The procedure described below must be followed in case of accidental exposure to infectious/pathogenic material in CL-3 laboratory. Any potential exposure to infectious/pathogenic material in the CL-3 facility requires immediate treatment followed by counseling for the injured person and possible administration of triple drug therapy in order to decrease the risk of infection. The latest evidence indicates that prophyllactic drug treatments (if required) should be given as soon as possible and at least within 2 hours of exposure to be maximally effective. It has been agreed that the University Staff/Faculty Health Services will serve as the emergency treating department for all CL-3 workers regardless of their employer. The following procedure must be followed at once by all workers in the CL-3 facility in the event of an exposure to infectious/pathogenic material in the CL3 facility.

Any of the following situations will be considered as a potential exposure:

15.1.1. Needlestick, cut or puncture wound from items contaminated or possibly contaminated with infectious materials. If any doubt exists as to whether the skin was broken, treat as for an exposure until counseling has been obtained from Occupational Health Physician.

15.1.2. Bite or scratch from experimentally infected insect/animal.

15.1.3. Splash of infectious/pathogenic liquids to eyes, mouth or nose or onto unprotected skin.

15.1.4. Accidental penetration of infectious material through defects or tears in disposable gloves such that skin contamination has (or may have) occurred. If any doubt exists as to whether the skin or other exposed body surface e.g. mucous membrane has been contaminated, treat as for an exposure until counseling has been obtained from the Occupational Health Physician.

15.2. First Aid

15.2.1. Wash the exposed area immediately for 5 minutes with soap and water. Cleanse puncture wounds or cuts as thoroughly as possible. Use the scrub brushes provided at each sink.

15.2.2. If skin penetration has occurred encourage bleeding by massaging the

surrounding area to express blood.

15.2.3. If wound/skin contamination is on the hand, remove the gloves on the affected hand. Remove outer glove on unaffected hand or hands and re-glove for administration of first-aid treatment.

15.2.4. If required, there are first aid boxes in rooms 20H-1, 20J-1 and 20G. These contain band aids and gauze to stop bleeding. (see Appendix 2)

15.2.5. For mouth or eye splashes, rinse in eye wash or sink for 10 minutes.

15.2.6. Contact Staff/Faculty Health Services, UCC room 25 (x5471) as soon as possible forfollow up.

15.2.6. Leave facility in the usual way as outlined in section II D and go to Staff/Faculty Health Services.

15.3. Communication with Occupational Health Services

15.3.1. All employees must report the accident IMMEDIATELY on completion of First Aid procedures to UWO Staff/Faculty Health Services (S/FHS) or have a co-worker report the incident while First Aid procedures are underway. The performance of First Aid procedures should be the first priority. Use the phone nearest to the accident site. S/FHS will immediately notify the Occupational Health Physician who will be available for counseling and follow-up care. Phones in CL-3 facility are location in rooms 20H-1, 20J-1 and 20F.

Staff/Faculty Health Services: ext. **5471**

15.3.2. The supervisor of the injured person must be notified as soon as possible about the accident so that an accident investigation can be conducted. Exit From facility and go Directly to Staff/faculty Health Services – UCC room 25.

15.3.3. Follow standard Exit procedures as in the User's Manual section 9.

15.3.3.1. Remove outer gloves (if still wearing them) and outer shoe covers and discard.

15.3.3.2. Wash hands.

15.3.3.3. Proceed to room 20G.

15.3.3.4. Remove over-gown and discard.

15.3.3.5. Proceed to hallway outside change room. Discard inner shoe covers and gloves.

15.3.3.6. Enter change room.

15.3.3.7. On entry into change rooms: Wash hands.

15.3.3.8. Remove surgical scrub suit and put on street clothes.

15.3.3.9. Go immediately to S/FHS in UCC room 25.

15.3.3.10. Identify yourself to the OH receptionist who will immediately arrange for consultation with the Occupational Health Physician. The OH Physician will determine, in collaboration with the patient, an appropriate course of management and will arrange for the collection of a blood sample and provision of post-exposure drug therapy as indicated.

15.3.3.11. Medical history and accident particulars will be collected by the OH physician during counseling or by the OH nurse afterwards.

15.3.3.12. Follow-up medical appointments will be arranged by discussion between the OH physician and the injured person.

15.4. Report(s) for Accidents/Incidents in the CL-3 laboratory

The OH Physician will contact the supervisor/PI or the injured person and the Biosafety Officer who will jointly conduct an accident investigation and complete the University Accident Report Form (AIR) within 48 hours. If the injured worker is employed by an affiliated institution, the AIR for that institution must also be completed. The Safety Officer for that institution will also take part in the accident investigation.

15.4.1. After First Aid has been completed call Staff/Faculty Health Services and inform them of the incident and ask to see the Occupational Health Doctor immediately for counseling.

15.4.2. Notify the supervisor/PI and BSC as soon as possible after any incident which may have resulted in a personal exposure to infectious agent.

15.4.3. The supervisor/PI must complete the University Accident Report Form as soon as possible and return it to DOHS.

15.4.4. The University Accident/Incident Report Form (AIR) are found in **Appendix ???.**

15.4.5. The PI and BSC must conduct an investigation into the accident/incident and a written report must be prepared and reviewed by the Occupational Health Physician. Recommendations for accident prevention must be made if possible. The Biosafety Officer of an affiliated institute will also be involved in an accident investigation, which involved an employee from that institution.

16. Alarms

16.1. The CL-3 facility is continuously monitored 24 hours a day by the Western Environmental System (WES). The control system monitors the following parameters:

Labs/corridor differential pressure

CL-3 panic buttons

Exhaust fan(s) and HEPA filters **(PPD to identify the fans.)**

16.2. All the above monitoring points are connected to the indicator panel in the corridor (see attached diagram) and to the control panel in the mechanical room and to the University Police Department (UPD).

16.3. The fans are set up to run continuously and to start up automatically after:

power fluctuation or shutdown

negative pressure reset

any cleared alarm or trouble condition

16.4. There is no alarm panel reset. The system will return to normal operation automatically as soon as the alarm/trouble cause is removed.

16.5. Alarm Condition

16.5.1. An alarm condition will occur when the panic button in CL-3 is activated or the labs cannot maintain the negative pressure.

16.5.2. A panic button alarm is announced by continuous born sounding and the “panic button activated” indicator on the panel in the hallway. This alarm can be silenced and reset by toggling the “Alarm Disable Switch” (**See Appendix ??)**

16.5.3. Lab pressure alarm is announced by short (5 second) horn signal, a continuous sone-alert signal and the corresponding red lamp on the panel in the hallway. This will continue to exist for as long as the pressure at any point inside the facility is positive. This alarm can also be disabled by the same toggle switch.

16.6. Door Contact Alarm (PPD to provide detailed information.)

16.7. Fire/Emergency Alarm (PPD to provide detailed information.)

16.8. Biological safety Cabinet Alarm

17. Mechanical System Failure

17.1. Air Handling Failure

17.1.1. Failure of either the air supply system or one of the exhaust air systems will activate an alarm, which sounds in the hallway and is heard in the inner laboratories. Indicator lights show, on the wall-mounted panel outside room 20F, which system is malfunctioning. All alarms are transmitted to the University Police Department (UPD) as well as WES, and thus, the UPD should always be contacted in the event of an alarm.

17.1.2. If the air supply system fails, as indicated on the wall mounted indicator system, the exhaust system will continue to run. Therefore, there is no immediate hazard.

17.1.2.1. Stop all laboratory work, secure all cultures/pathogens, seal all biohazard bags containing biohazardous waste, and leave the facility in the usual manner. Phone Western Environmental Services (WES) at ext. 8738 and the UPD emergency number 911 and inform them of the problem and dispatch will notify the ppropriate personnel to handle the problem.

17.1.2.2. If either one of the exhaust systems fails, the supply air system also shuts down. In this case, the air inside the room(s) served by that exhaust system will remain stagnant. Check the indicator panel to determine the extent of the shut down.

17.1.2.3. Immediately cease work. Leave the facility quickly, discarding contaminated clothing in the usual manner. Leave the Biosafety Cabinets working and call the WES (8738) and UPD emergency number 911 for assistance.

17.1.2.4. Notify the sipervisor/PI and the Biosafety Officer as soon as possible.

17.1.2.4. When ventilation is restored return to the facility and secure cultures/pathogens.

17.2. Power Failure and Emergency Generator

Equipment essential to the operations in CL-3 laboratories is on emergency power (independent electrical generator). These include: lights, biological safety cabinets, freezers, refrigerators, the air handling system.

17.2.1. In the event of a facility power failure the following sequence will occur:

17.2.1.1. Emergency lighting (battery operated) will immediately activate in the laboratory.

17.2.1.2. The Emergency Generator (which runs on diesel fuel) will start to operate. All electrical systems in the CL-3 Facility are served by a backup emergency generator. This will maintain all lights, incubators and biological safety cabinets. This generator starts automatically with a short delay of 16-20 seconds. During this time the battery operated lights will operate but all other electrical equipment will not operate.

17.2.1.3. If emergency lighting activates: The biological safety cabinets will not function. If working with virus in the cabinet, immediately withdraw arms slowly from cabinet work space, leave the laboratory and wait in room 20G until the emergency generator starts. Wait 2 minutes for any aerosols to clear and

then re-enter laboratory and secure cultures. Leave the facility in the standard manner and call WES at ext. 8738 or UPD at 911 for information.

17.2.1.4. Do not work in the facility while it is under emergency power.

iv. When the power returns the ventilation systems will start up again automatically using regular power supply.

17.2.1.5. Notify the supervisor/PI and the Biosafety Officer, immediately if there has been a power failure during experimental work.

17.2.1.5. The emergency generator is checked weekly by PPD.

18. Pest Control

Outside insect pests should not be a problem in the CL-3 facility due to the sealed window and the isolated location of the CL-3 laboratory within the CL-2 transgenic Plant Unit on the G floor of the building. If insects are detected in the facility please notify the BSC immediately. A pest control program consisting of non-toxic traps and bait will be used.

19. Natural Disasters/Vandalism or Activist Intrusion

19.1. The University of Western Ontario has an Emergency Response Team who is trained to co-ordinate on-site emergency response services in the event of a campus emergency. In case of a major emergency this response team can be activated by calling the University Police Department (UPD) at 911.

19.2. The CL-3 Biohazard facility is located on the G floor of the Biotron Building and within the secure Containment Level 2 area of the Transgenic Plant

Unit. (see Appendix 7: Facility plan) and will be covered in the Emergency Response for these areas.

19.3. If an emergency situation is declared which may involve the biohazard facility, such as a severe weather or tornado warning, bomb threat or other need for emergency evacuation of the Biotron Building, any personnel who may be working in the CL-3 facility must be notified so that they can leave the facility safely. This will be done by University Police who will notify all laboratories of the need to evacuate. If the lab has an individual in the CL-3 facility the supervisor/PI will notify the person by phone of the need to evacuate.

19.4. If an evacuation is called with enough warning time, personnel working with live pathogens in the facility will secure the pathogenic material by replacing containers in the incubators, discarding any wastes from cultures into disinfectant tray in the biological safety cabinets and will then leave the facility in the standard manner.

19.5. In the event of immediate hazard, the fire alarm will sound and personnel must exit the facility immediately as described (above) for a large immediate fire hazard. The marshalling area is in front of the Biotron Building.

19.6. Exit the building by the stairs. Do NOT use the elevators

19.7. Assemble in the marshalling area in front of Biotron Building. Identify yourself and your place of work and provide any information they may have on the status of the lab to the incident commander. Arrangements will be made by the incident commander to discard lab clothing into bags for autoclaving later.

20. Medical Surveillance Program

20.1. After approval by the University Biohazards Sub-committee has been received for a person to work in the CL-3 Facility, the supervising faculty member/PI must complete the University Position Hazard Communication Form (Section V) for the individual who has been approved for Level 3 work indicating which microbiological agents/pathogens are to be used and the containment level.

20.2. Staff/Faculty Health Services will contact the individual for an initial health review and counseling before work in the CL-3 facility has commenced. Appropriate immunization will be discussed and titres verified if necessary.

20.3. Medical reviews will be conducted annually and at the final termination of work in the facility.

20.4. A blood sample to be stored and used as a baseline reference will be required.

20.5. NO Medical TESTING will be done without the express permission of the individual concerned.

20.6. The initial health review must be completed before work in the CL- 3 Facility is started.

20.7. Annual check-ups will be performed to monitor the health of the individual for the duration of the work in the CL-3 facility.

20.8. A medical appointment and exit blood sample is required when an individual ceases to work in the CL-3 facility or leaves the University.

20.9. All potential exposures to infectious agents in the CL-3 facility must be reported immediately to the Staff/Faculty Health Office.

20.10. The Occupational Health Physician will see the person who has received the potential exposure as soon as possible and will provide counseling on post-exposure procedures and the advisability of triple drug therapy.

20.11. An Accident/Incident Report Form must be completed by the research supervisor as soon as possible after the accident.

21. Housekeeping Duties and Cleaning in the CL-3 Facility

21. 1. Laundry

21. 1. 1. All lab clothing used in CL-3 laboratory MUST be autoclaved before being sent to the laundry. Laundry bags are kept in room 20G and 20E. Used lab clothing should be placed into these when discarded and full bags must be taken to the autoclave and sterilized. The bags can be placed into the autoclave from room 20E, it is not necessary to enter room 20G to do this. A full bag of laundry requires 1 hour sterilizing time.

21. 1. 2. After autoclaving, the laundry will be sorted and bags are to be taken to the Dental/Medical Stores Room, Lower Ground Floor Dental Sciences Building. Clean laundry is also picked up from here. This is the responsibility of the research group.

21.2. Daily maintenance:

21.2.1. Check autoclave steam pressure.

21.2.2. Check air pressure indicators.

21.2.3. Check disinfectants and refill if necessary.

21.2.4. The biological safety cabinets must be wiped out with 70% alcohol daily when in use. This is the responsibility of the research group.

21.2.5. The sinks in laboratory rooms must be wiped down with disinfectant, at the end of the day's work. This is the responsibility of the research group.

21.2.6. Prior to exit, add disinfectants to all drains.

21.2.7. Prior to exit turn on UV light in the biosafety cabinets.

21.3. Weekly maintenance:

21.3.1. Test eyewash units.

21.3.2. Sweep and mop floors in Laboratory rooms 20H-1, 20J-1, airlock rooms 20H, 20J and 20G at least once a week with 2% Bleach, Virkon or Beaucoup disinfectant, while they are in use and more often if necessary. This is the responsibility of the research group. Buckets and mops are provided for this purpose. Floors in the hallways and the change rooms are also the responsibility of the research group, except during the annual shut-down, when the facility will be decontaminated and thoroughly cleaned by caretaking staff. Hallways should be cleaned on a regular basis as required.

21.3.3. All surfaces in laboratory rooms (benches, doors, fridges phone handles, etc.) should be wiped weekly with disinfectant.

21.3.4. Swipe test for radioactivity (if applicable).

21.3.5. Clean shower area.

21.3.6. Autoclave out waste from wastepaper baskets.

21.3.7. Restock items (gloves, scrubs, towels, etc.).

21.3.8. Refill soap dispensers.

21.4. Monthly:

21.4.1. Check fire extinguishers.

21.4.2. Test function of alarms.

21.4.3. Smoke test Biological Safety Cabinets.

21.4.4. Perform autoclave bioindicator test (spore test).

21.5. Decontamination Procedures Prior to Scheduled Shut – Down

21.5.1. Operating procedures in the Biotron CL-3 facility allow open manipulations of infectious/pathogenic agents only inside the biosafety cabinets in room 20H-1 and 20J-1. Other procedures take place outside the cabinets ONLY in closed containers, for example, in the centrifuge or in the incubator. Thus the potential for contamination in the suite is minimal.

21.5.2. Before any maintenance personnel are allowed into the facility, the entire suite is will be washed and disinfected with proven effective disinfectants under the supervision of the Biosafety Officer and a supervisor/PI, so that there will be no possibility of any of the research infectious/pathogenic agents remaining alive on any of the surfaces or equipment inside.

21.5.3. The cabinets and filters will be professionally decontaminated with formaldehyde gas by an outside contractor.

21.5.4. Decontamination Process - Standard Operating Procedures for decontamination of each room in the Biotron CL-3 facility will be described. Each item in the room will be signed off as it has been decontaminated by the Biosafety Officer and supervisor/PI. Finally the entire facility will be declared to have been decontaminated and will be signed off by the Biosafety Officer and the Chair for the Biohazards Sub-Committee as being safe for workers to enter.

22. Disinfectants

22.1. Only disinfectants approved by the Biosafety Officer are used in the facility. These will be approved on the basis of reported research and proven effectiveness as reported in the literature.

22.2. Chemical disinfection is used as a primary decontaminant in the facility to ensure that materials, which have to be removed from the biological safety cabinet for autoclaving, are surface decontaminated. Chemical disinfection is also used to decontaminate work surfaces, floors and sinks.

22.3. The following disinfectants are approved for use in the CL-3 biohazard facility:

a. Chlorine (Bleach)

b. Wescodyne (Iodine)

c. Ethanol, 70% with 1% detergent e.g. Hibitane

d. Coldspor (Glutaraldehyde)

e. Beaucoup (Phenolic)

f. Sporicidin (Phenolic)

23. Waste Management

All laboratory wastes from the CL-3 facility must be effectively decontaminated before leaving the facility. All wastes from the facility must be incinerated for final disposal.

23.1. General - No live cells, virus or live plant/insect/animals may leave the facility. If extracted biochemical products of cells, virus or plant/insect/animal organs are to be removed for further biochemical procedures in other laboratories, written documentation must be provided to the Biosafety Officer that decontamination methods which reliably inactivate the infectious/pathogenic agent have been appropriately carried out before the material leaves the CL-3 Facility. References from the literature or experimental data will be required.

23.2. Autoclaving

23.2.1. The CL-3 facility is equipped with an Getinge pass-through autoclave situated between the CL-3 room 20G and theCL-2 room 20E. The autoclave is maintained by the Physical Plant Department of the University and is serviced and inspected twice a year.

23.2.2. Autoclaving is the preferred method for decontaminating materials which are to leave the facility. E.g. all waste cultures and medium, plastic ware, disposable clothing and paper towels.

23.2.3. Bags of combustible wastes must be sealed in the laboratory, carried to the autoclave and then the seal loosened in such a manner that penetration by the steam into the load is allowed. Do not overfill or compress bags.

23.2.4. Loosen caps on bottles and tubes.

23.2.5. Autoclave bags must be supported at all times, in a rigid, leak-proof, autoclavable tray.

23.2.6. Do not over-fill the chamber as this can lead to incomplete sterilization.

23.2.7. Containers with waste products from viral or cell culture must be kept in the biological safety cabinet until they are to be autoclaved. The container must have enough Wescodyne disinfectant in it to immerse all disposable plastic ware and dilute all growth medium.

23.2.8. When the discard container is to be autoclaved it must be placed inside a biohazard bag while inside the biological safety cabinet. This bag must be loosely sealed with autoclave tape.

23.2.8. Place bag containing waste container into a plastic autoclave tray and carry to autoclave.

23.3. Incineration

23.3.1. All autoclaved solid waste items should be finally disposed of by incineration. The incinerator is located in Room D6022 in the Health Sciences Animal Care Unit.

23.3.2. After autoclaving, the waste should be transferred to a clean black garbage bag in room 20E. The bags are taken out of the facility on completion of work in the facility.

23.3.3. The outer bag must be labeled according to the University regulations for incinerated waste. This consists of a red label containing the name of the PI. The CL-3 origin of the waste and the waste class – Yellow A2 or Orange A3 as per incinerator instructions. The incinerator room is open Mon-Fri 8:00 am to 4:30 pm. At other times the waste material can be stored in the facility after it has been autoclaved and re-bagged.

23.4. Sharps Disposal

Sharps must be disposed directly into a sharps container inside the BSC. The container must be sealed and bagged before being taken directly for incineration.

24. Operation of Equipment

24.1. Policy on Equipment Use

This policy applies to all users of equipment in the CL-3 laboratories of the Biotron. It pertains particularly, but not exclusively, to the Biotron owned and/or maintained major equipment, such as Biosafety Cabinets (BSCs), autoclave, centrifuges, fridges and freezers, computers, etc. The concerns addressed by the policy are:

- SAFETY in operation of the equipment
- TRAINING in use of the equipment

• PERMISSION to use the equipment, including users from OUTSIDE the Biotron

• MAINTENANCE of the equipment (responsibilities and costs)

• LIABILITY for cost of damage to equipment (repair, replacement)

No one will operate a piece of equipment without appropriate training and specific approval. “Training” on a piece of equipment such as the autoclave or a Biosafety Cabinet involves, for example:

(a) reading the Standard Operating Procedure or sections of the equipment manual, and/or taking a specific short course or training seminar;

(b) going through the operation with someone who is competent in its use - several times if warranted;

(c) recording the training (having your personal record sheet “signed off” by your trainer and/or having your name added to a list of users)

It is the responsibility of the advisor/ supervisor/ PI to ensure that all students, technicians, researchers and visitors are aware of the training requirements and receive the necessary training before using equipment. The advisor/ supervisor/ PI are also responsible for costs that may be levied for equipment maintenance or repair.

To ensure equipment is returned in good repair, all equipment, used in the CL-3 laboratories must be SIGNED OUT and CHECKED BACK IN by authorized staff (BSO/General Manager/Technical Director). Users will be responsible for the equipment until it is signed back in, and it should not be passed on to another person without being signed in and checked. For safety

reasons, the use of centrifuge rotors must be precisely monitored and recorded, and these must be checked in after each use.

If it becomes necessary to repair or replace equipment, charges may be assigned to users. The charges may be based on:

(a) the reason for the repair/replacement (e.g. careless damage or loss);

(b) extent of the previous use (based on sign-outs and time used). Such user-cost charges may also be assigned for some maintenance costs (oil, bulbs, etc.) if required.

If a prospective user is NOT a member of the Biotron, and not working in close collaboration with a specific research laboratory in the Biotron, then that person must have written (signed) permission to use that equipment. The permission will come from the BSO/General Manager/Technical Director; it will be for a specified time period or number of uses, and it will not be transferable to anyone else but the designated user. Cost recovery will apply and the request to use the equipment must indicate that the user (or research supervisor) will accept responsibility for costs/ damage. If extensive training or supervision is required and can be arranged, a time-charge may be applied.

All users of equipment will:

(a) record their use in the log/ sign-out books;

(b) report problems with the equipment immediately;

(c) leave the equipment and work area clean and in good order;

(d) be willing to assist with routine maintenance (e.g. oil changes, cleaning).

24.2. Biological Safety Cabinets (BSCs) - Every employee working in a BSC must be trained in its correct use and have a good understanding of the different types of cabinets and how they work.

24.2.1. Classes and Characteristics of Biological Safety Cabinets - In the BL-3 laboratories of the Biotron Type IIA2 and Type IIB2 safety cabinets are used. Class II cabinets are designed for personnel, product and environmental protection. They are designed for work involving microorganisms in containment levels 2, 3 and 4 laboratories and are divided into two types (A and B) on the basis of construction type, airflow velocities and patterns, and exhaust systems. Within type (A), there are two subtypes, A1 (formerly designated type A) and A2 (formerly designated type B3). Within type (B), there are two subtypes, B1 and B2. Class II cabinets are most commonly used in biomedical research laboratories because of their characteristics.

24.2.2. Class II, Type A2 Cabinets (Figure 2)

• Cabinet air may be recirculated back into the laboratory or ducted out of the building by means of a "thimble" connection (see figure) (i.e., a small opening around the cabinet exhaust filter housing) whereby the balance of the cabinet is not disturbed by fluctuations in the building exhaust system. The thimble must be designed to allow for proper certification of the cabinet (i.e., provide access to permit scan testing of the HEPA filter).

• Maintain a minimum average face velocity of 0.5 m/s (100 ft/min).

• Have ducts and plenums under negative pressure.

• Is suitable for work with minute quantities of volatile toxic chemicals and trace amounts of radionuclides.

24.2.2. Class II, Type B2 Cabinets (Figure 3)

• Does not recirculate air within the cabinet.

• Maintain a minimum average face velocity of 0.5 m/s (100 ft/min).

• Hard-ducted through a dedicated duct exhausted to the atmosphere, 100% of cabinet air, after passage through a HEPA filter; contain negative pressure plena.

• Suitable for work with volatile toxic chemicals and radionuclides.

The exhaust canopy must allow for proper BSC certification. An alarm should be provided that is audible at the cabinet to indicate loss of exhaust flow from the building exhaust system. The cabinet internal fan should also be interlocked to shut down when the building exhaust system fan fails, to prevent pressurization of the cabinet.

24.2.3. Use of the Cabinet

24.2.3.1. Procedures when preparing for work in the BSC:

1. Turn off UV lights if in use and ensure that the sash is in the appropriate position.

2. Turn on fluorescent light and cabinet blower, if off.

3. Check the air intake and exhaust grilles for obstructions.

4. If the cabinet is equipped with an alarm, test the alarm and switch it to the "on" position.

5. Confirm inward airflow by holding a tissue at the middle of the edge of the viewing panel and ensuring that it is drawn in.

6. Disinfect the interior surfaces with a suitable, non-corrosive disinfectant.

7. Assemble all materials required for the procedure and load them into the cabinet; do not obstruct the air grilles; the working surface may be lined with absorbent paper with plastic backing; segregate "clean" items from "contaminated" items.

8. Wait 5 minutes to purge airborne contaminants from the work area.

24.2.3.2. Procedures for working in the cabinet:

Wear the appropriate protective clothing and gloves.

1. Perform operations as far to the rear of the work area as possible.

2. Avoid movement of materials or excessive movement of hands and arms through the front access opening during use; when you do enter or exit the cabinet, do so from straight on; allow the cabinet to stabilize before resuming work.

3. Keep discarded, contaminated material to the rear of the cabinet; do not discard materials in containers outside of the cabinet.

4. Do not work with open flames inside the cabinet.

5. If there is a spill during use, surface decontaminate all objects in the cabinet, disinfect the working area of the cabinet while it is still in operation (do not turn the cabinet off).

24.2.3.3. Procedures upon completion of the work:

1. Allow the cabinet to run for 5 minutes with no activity.

2. Close or cover open containers before removing them from the cabinet.

3. Surface desinfect objects in contact with contaminated material before removal from the cabinet.

4. Ensure that all materials are placed into biohazard bags within the cabinet.

5. Using a suitable non-corrosive disinfectant (e.g., 70% ethanol), disinfect interior surfaces of cabinet; periodically remove the work surface and disinfect the area beneath it (including the catch pan) and wipe the surface of the UV light with disinfectant.

6. Turn off the fluorescent light and cabinet blower when appropriate (some cabinets must be left on at all times; if you are unsure, check with your cabinet certifier, safety officer or building maintenance personnel).

7. Turn on the UV light if appropriate (do not turn on when people are working close by); UV must be tested to ensure that it is

emitting a germicidal wavelength (ask your cabinet certifier to perform this test).

24.3. Autoclave

The BL-3 laboratory is equipped with a GETINGE (model / capacity???) double-door autoclave. All users are trained in the correct use of this autoclave. Records are maintained of this training. The autoclave is equipped with interlock doors, and the clean end (operating end on BL-2 side – room 20E, see the floor plan on Figure 1) can only be unlocked (opened) after a complete cycle has been run. Either doors will be unlocked at the end of a cycle and can be opened, but once the dirty side door (BL-3 side – room 20G, see the floor plan on Figure 1) is opened, the clean side door can not be opened unless a new cycle is completed. The treatment of liquid and solid waste and the transport of waste material within the BL-3 laboratory, the Transgenic Plant Unit and the Biotron are described within the protocols for each laboratory. Procedures to be followed in the event of a failure of the autoclave are also described in the specific protocols.

The first person that will enter BL-3 laboratory should first, make sure that the autoclave is turned on and has the required steam pressure. The last one leaving BL-3 may be required to load the autoclave and initiate the required cycle.

24.3.1. Operating of Autoclave

24.3.1.1. Operating instructions are posted by the autoclave in rooms 20E and 20G.

24.3.1.2. All personnel must familiarize themselves with the operating procedures and receive training on the operation of the autoclave from the BSO or authorized personnel before operating the autoclave for the first time.

24.3.1.3. Since this autoclave is a pass-through type, both doors must not be opened at the same time. There is a visual warning on both control panels indicating when the opposite door is open.

24.3.1.4. A chart recorder is provided with the autoclave and will print out a record of each run. These must be checked after each run to confirm completion of a successful run and stored and held on file for at least 6 months. This is the responsibility of the research group/PI using the BL-3 laboratory.

24.3.1.5. Autoclave indicator tape should be used with every cycle as a check that the load has been subject to autoclaving. (**NOTE this is NOT an indicator of sterility**).

24.3.1.6. Cycle Times – Standard programs have been installed and security coded on the touch pad of the autoclave for the sterilization of all materials coming out of the BL-3 laboratories. **Autoclave programs must NOT be altered without permission and approval from the BSO.** The standard cycles used for decontamination of wastes from BL-3 laboratories will be 60 min (121°C) on LIQUIDS cycle or 60 min (121°C) on GRAVITY cycle for liquid or solid wastes respectively. Combustible waste should be placed in biohazard bags 26x36 ins. One liter of water should be routinely added to all bags of scrubs/coveralls being autoclaved.

After completion of each cycle, the door must be cracked and the chamber vented for 10 min. The rack with the autoclaved material must be removed from the autoclave and allowed to cool for 30-60 min at room temperature.

24.3.1.7. Infectious or toxic materials should not be placed in the autoclave for sterilization the next day.

24.3.1.7. If the autoclave malfunctions, place a warning sign **"DO NOT OPEN, BL-3 LOAD IN PROGRESS"** on the clean end door in room 20E.

24.3.2. Verification of Autoclave Cycles

24.3.2.1. The effectiveness of all types of autoclave cycles in use must be verified using biological indicators. All Protocol Weekly. The research or Biotron group who uses the autoclave first that week is accountable for performing the verification testing. Record keeping for the testing is maintained in a binder in room 20E.

24.3.2.2. To test the efficacy of the autoclave cycle an ampoule or spore strip containing a biological indicator e.g. *Bacillus stearothermophilus* must be included in a sample of each type of load processed. The ampoule (or spore strip) must be recovered and incubated in the incubator provided. A control ampoule, which is not autoclaved must be incubated with each test. The lot # and expiry date of the ampoules/spore strips used and the date of the testing must be recorded.

24.3.2.3. No growth should be detected for any test ampoule as detected by change in color of the growth medium from purple to yellow.

24.3.2.4. If growth is detected the waste must be reprocessed with a longer cycle time until no growth of medium is observed. This new cycle time must then be used for any future load.

24.3.2.5. The Biosafety Officer must be notified of any failure of autoclave cycle.

24.3.3. Disposal of Autoclaved Items

24.3.3.1. Liquids

Loads containing liquids and wescodyne/alcohol disinfectant must be allowed to cool inside the closed autoclave. Do not open the door to the autoclave until cool to allow vapours from the disinfectant to condense. All liquid wastes will be non-biohazardous after autoclaving. The culture wastes containing liquid disinfectant and plastic ware should be carried, in a container, to the sink in Room 6011. The liquid waste should be drained out of the container and disposed of down the drain and flushed after with water. Only liquid waste that is liquid at room temperature may be poured down the drain. The waste plastic must be double bagged, properly labeled and taken for incineration. Care must be taken to ensure that the bags are drained completely to avoid leakage of disinfectant.

24.3.3.2. Radioactive liquids

The appropriate method of decontamination for radioactive biohazards waste must be discussed with the BSC and RSC before the research protocol is approved. The biohazard component is decontaminated first by appropriate chemical disinfection and then the radioactive wastes are disposed of as appropriate for the activity level and particular isotope.

24.3.3.3. Solid combustible waste

Bags of autoclaved waste must be double bagged with clean garbage bags appropriately labeled and taken for incineration. The outer bag must be labeled with an incineration label stating the name of the PI, the L3 origin of the waste and waste class either A2 or A3 as per incinerator instructions. (Appendix 13) These instructions are posted on the wall or 20E. It is the responsibility of the research group to take the bags of waste to the incinerator as required.

24.3.3.4. Radioactive combustible wastes

Disposal method must be approved by the BSC and RSC before the protocol is approved. Wastes below the Scheduled Quantity for each isotope may be bagged and taken directly for incineration.

24.3.4. Autoclave Maintenance and Servicing – The operation and performance of the autoclave must continually be monitored and any problems or concerns reported immediately to the BSO and/or General Manager. In addition to the servicing conducted by a qualified technician (usually at the time of performance testing) in accordance with the manufacturer's instructions, the users should conduct the following inspections and maintenance of the equipment:

24.3.4.1. Every time the autoclave is used:

a. Check the steam pressure.

b. Report any faults or warning indicators to the BSO an/or General Manager.

c. Keep the outside and inside of the autoclave clean.

24.3.4.2. The drain opening in the autoclave chamber should be inspected monthly to ensure that it is not blocked. Unscrew the drain screen and backflush to remove debris. Reinstall the drain screen after cleaning. This is the responsibility of the Biotron staff.

24.3.4.3. The autoclave will be performance validated, tested and certified using a 12 point thermocouple test twice per year: Once during the annual shutdown and decontamination of the facility and after 6 months of use. This will be carried out by a qualified technician and it is the responsibility of the Biosafety Officer in collaboration with Physical Plant Department (PPD) to schedule the autoclave maintenance and performance validation. Copies of the test certificate are held by the BSO in the master copy of this SOP.

24.3.4.4. The users are responsible for cleaning the chamber of the autoclave as required.

24.3.4.5. Any problems with the autoclave must be reported to the Biosafety Officer immediately.

24.4. Telephones, Faxes and Computers

These devices are used for transmittance of data outside the BL-3 laboratories. When handling these devices, to prevent contamination and cross-contamination one should first wipe the hands with a cloth/paper towel soaked in a mild disinfectant. At the end of their use, the surfaces that were touched should also be wiped with cloth/paper towel dipped in mild disinfectants, water, and then wiped dry. Keyboard covers must be used, which allow the keyboard to be used with the cover in place.

24.5. Refrigerators and Freezers

24.5.1. For the storage of micro-organisms and plant material in freezers, screw-capped sterile cryo-vials will be used.

24.5.2. There will be no ice-machine within the BL-3 laboratories of the Biotron. If one does need ice. It will have to be brought in before-hand.

24.5.3. In the event of a breakdown, most freezers are will insulated and will keep specimens frozen for 1-2 days. A decision will have to be made on whether the frozen samples are expendable, if there are duplicate samples stored elsewhere. If the frozen samples are not expendable dry ice can be added to the freezer to keep the samples frozen, or a spare freezer can be taken into the BL-3 laboratory and the faulty freezer decontaminated and removed for repair. For refrigerators, in case of a breakdown, the similar principles as that noted for freezers will be followed.

24.6. Vacuum Pumps

Vacuum pumps are required for the removal of agar plugs from a plate, for filtration or clarification of liquids, for the removal of culture media fluid and in some pieces of equipment. Vacuum pumps obtained for BL-3 laboratories are either oil-less or work on a system of aspirators that use water in reservoir. A small amount of disinfectant could be added to the reservoirs during of after the vacuum procedure. The water can also be drained and autoclaved after each vacuum procedure has been completed. When using a vacuum pump, it is important to note the following:

24.6.1. Filtration must be done inside an operational BSC.

24.6.2. Between the flask storing the liquid and the vacuum pump, there should be another flask acting as a reservoir trap partially filled with disinfectant and a sterility filter, to prevent aerosolization of infectious material.

24.6.3. The connections should be secure and the vacuum pump operational before any liquid is filtered.

24.6.4. One should be aware that the pores of a filter will clog with bacteria or debris in the media, and that the flow rate may slow or stop completely. One should monitor this flow rate and preferably filter several small volumes rather than a few larger volumes.

24.6.5. When disconnecting the system, one must first break the vacuum gradually by slowly pulling off the tubing at the connection of the sample flask. Only after this is done is the vacuum pump turned off (doing otherwise will cause a back-flow of oil into the flask still under vacuum).

24.7. Other Equipment……

25. Laboratory Specific Protocols

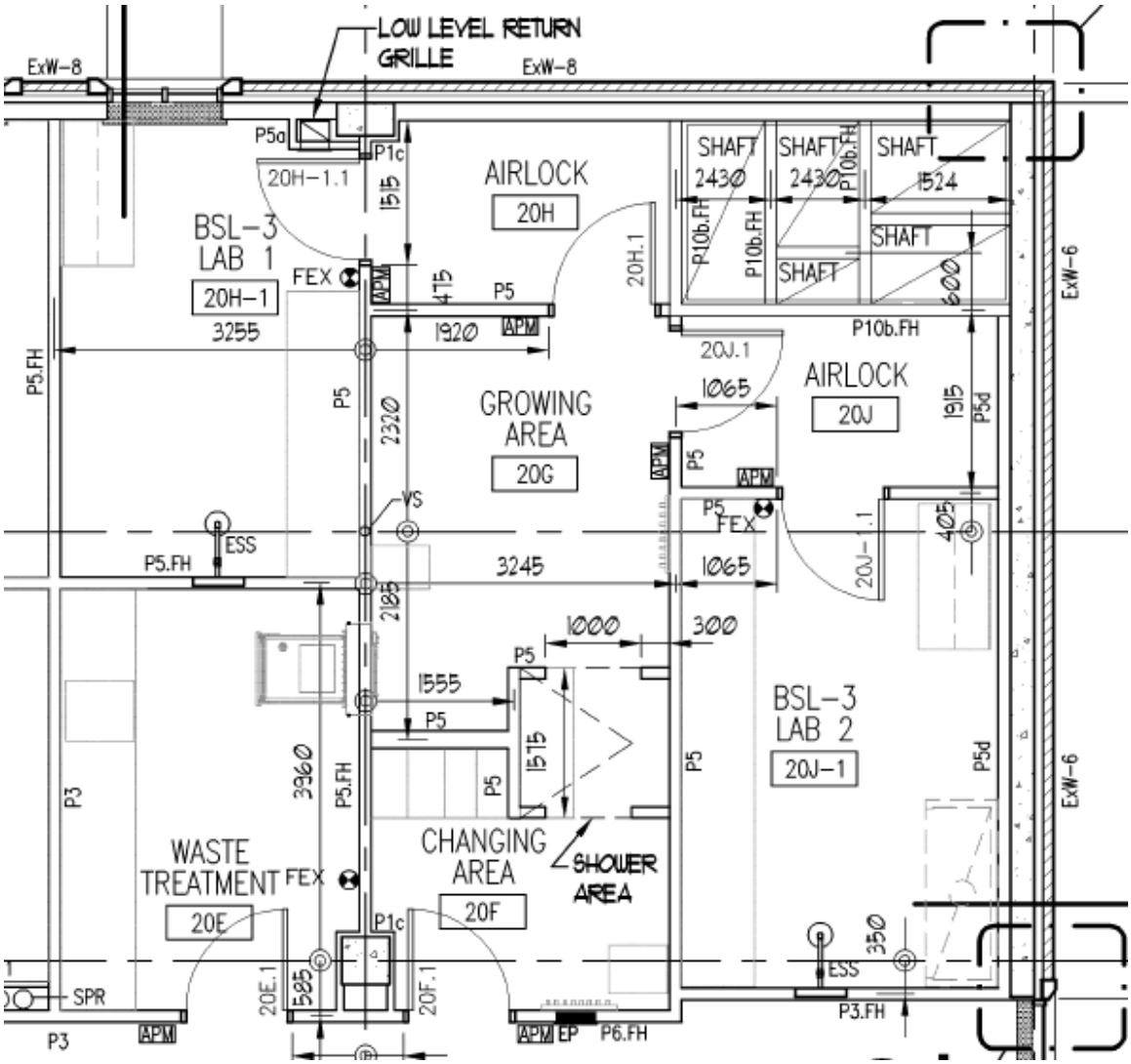
25.1. Protocol 1….

25.2. Protocol 2….

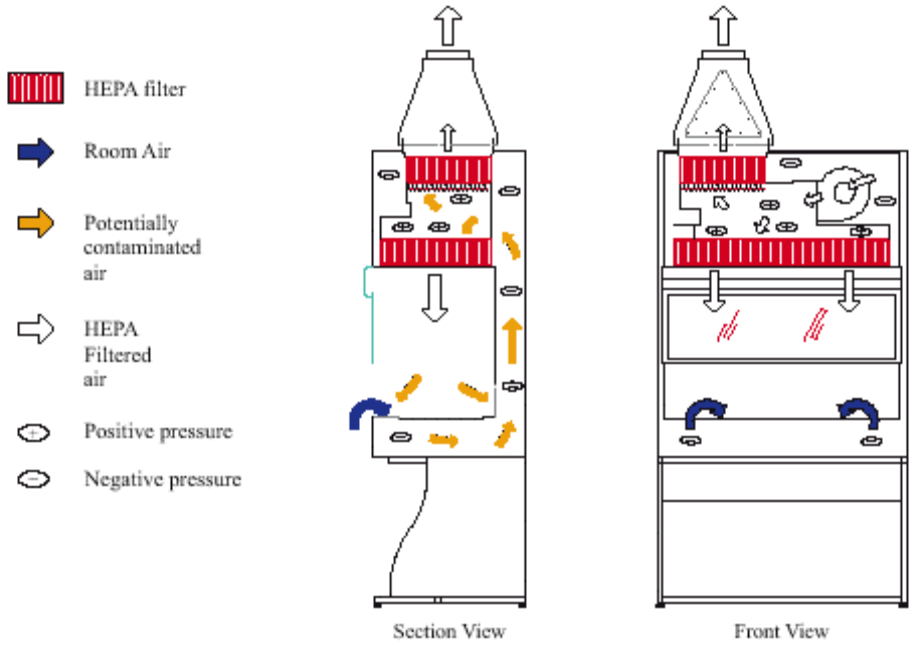
25.3. Etc…..

26. Figures:

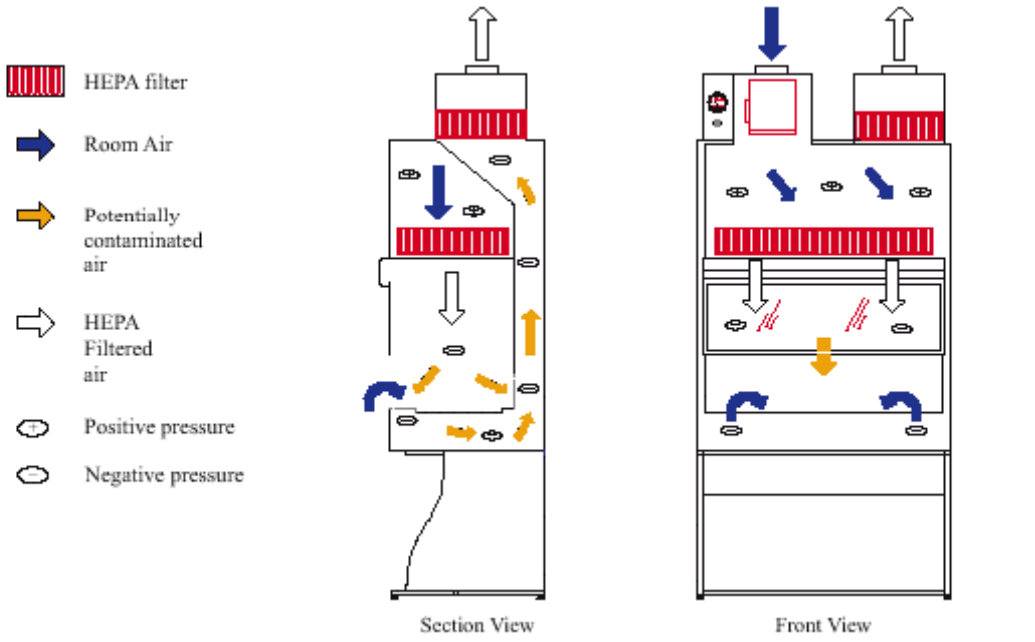
26. 1. Figure 1.



26. 2. Figure 2. - Class II, Type A2 Bio-safety Cabinet



26. 3. Figure 3. - Class II, Type B2 Bio-safety Cabinet



27. Appendix

27.1. Appendix A – Biotron Call-in List (to be determined)

Name	Position	Phone No (work)	Phone No (home)	Mobile Phone

27.2. Appendix B – TOR of the Biosafety Officer

Terms of Reference – Biosafety Officer

Reporting: The Biosafety Officer will report to the Director of the Biotron or designated person, the Biotron BSC and UWO-BHSC.

Objective: To ensure safe day to day operations in The Biotron Level 2 (CL-2) and Level 3 (CL-3) Containment laboratories, and that The Biotron is in compliance with all governing guidelines and regulations.

Duties: Develop, recommend, advise and implement biological safety policy and procedures in consultation with BSC and UWO-BHSC and will be a member of the BSC.

1. Authorized by UWO-BHSC to inspect safety procedures carried out in The Biotron CL-2 and CL-3 laboratories.

2. Enforces immunization requirements (if necessary) and updates immunization lists as required.

3. Implements control procedures for visitors to CL-3 laboratories.

4. Maintains a current schedule/list of microorganisms/pathogens under study in each of the CL-2 and CL-3 laboratories.

5. Maintains liaison with UWO medical authorities to ensure that only persons which are medically fit are permitted entry into CL-3 laboratory.

6. Is notified of any physical repairs or maintenance to the CL-2 and CL-3 laboratories, and notifies the users of action that affect their operations.

7. Coordinates and provides biosafety training of individuals allowed access into the CL-2 and CL-3 laboratories.

27.3. Appendix C – Confidentiality Agreement Form

BIOTRON

CONFIDENTIALITY AGREEMENT

Date:_____

I, _____ acknowledge and agree that I
Print Name
will observe and comply with the laws of Canada and Ontario and except as may be required by law or otherwise expressly permitted through my employment with the University of Western Ontario. I will not disclose or give to any unauthorized person any information, obtained from observations, research or otherwise, that comes to my knowledge or possession by reason of my work at, visit to or affiliation with the Biotron. Information includes but is not limited to scientific information, business, financial, legal, marketing, technology, intellectual property, document, material or data.
If I have any questions as to whether any particular information is subject to this Confidentiality Agreement, I will not seek direction from my supervisor.

_____ _____

Signature Witness

27.4. Appendix D – Project Application Form

Print Form

biotron
experimental climate change research

Attn: Biotron Administrator
1151 Richmond Street N., London, ON N6A 5B8
T: (519) 661-2111 ext. 82288 F: (519) 661-4162
web: www.biotron.uwo.ca

UNIVERSITY of GUELPH Western

Project Application

Date: _____

Lead Investigator Information

Name:	
Address:	
Email:	
Faculty/Dept. or Company:	
Phone:	
Fax:	

Requested Project Start Date: _____

Length of Project in Weeks: _____

Project Information

Project Title:				
Brief Project Description & Methodology:				
Research Project Objectives:				
Biohazards:	Indicate all biological hazards to people or other organisms in the building, including:			
	micro organisms, insects, recombinant DNA, volatile chemicals, toxic chemicals, radioactive isotopes,			
	drugs, etc. Attach copies of any forms required by Research Western in regard to these hazards.			
What Level of Containment is required?	◯ Level 1	◯ Level 2	◯ Level 3	◯ Unknown
If unknown, please explain				
Deliverables:				
Estimated Project Budget:				
Funding Sources:				
Stakeholder Benefits, Impacts, etc.:				

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Staffing Information

Number of Staff:	
Team Members:	
Are all Team Members WHMIS certified (or equivalent)?	○ Yes ○ No
	Certificate (or equivalent) is required.

Modules & Equipment

Earth Sciences:	☐ Climate Chamber (-35 to 40C) ☐ Ion Chromatography
	☐ Gas Chromatography ☐ Mass Spectrometry
	☐ Inductively Coupled Plasma ☐ X-ray Fluorescence
	☐ Atomic Absorption
	Describe sample type & analysis required
Microbiology:	Peltier Incubator - # Required
	LN2 Sample Storage - # of Boxes Required
Transgenic Plants:	Walk-In Chambers - # Required (5 to 40C) (max. 6)
	☐ Seed Germinator
	Medium Reach-In Cabinet (5 to 40C) (max. 2)
Insects:	Colony Rearing Chambers - # Required (5 to 40C) (max. 10)
	☐ Walk-In Colony Rearing Chamber (-10 to 40C)
	Walk-In Experimental Chambers - # Required (5 to 40C) (max. 5)
	Medium Experimental Chambers - # Required (5 to 40C) (max. 6)
	Small Experimental Chambers - # Required (5 to 40C) (max. 8)
	☐ Extreme Temperature Chamber (-73 to 200C)
Plants and Algae:	High Light (5 to 40C) (max. 4)
	Standard (5 to 40C) (max. 10)
	CO2 (5 to 40C) (max. 3)
	Low Temperature (-40 to 40C) (max. 2)

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Modules & Equipment		
Biomes:	Biomes (max. 6)	
https://www.biotron.uwo.ca/biomes.html	Nursery (max. 2)	
Imaging:	☐ Upright (Zeiss Axioskop2)	☐ Stereo (Zeiss Stereo V12)
	☐ TEM 1 (Philips 420)	☐ TEM 2 (Philips CM10)
	☐ SEM (Hitachi S-3400N)	☐ Confocal (Zeiss LSM510 Duo vario2)
	☐ Upright (Zeiss Z1 Imager)	

Please forward the Project Application to:
Attn. Biotron Administrator
Fax: (519) 661-4163 or
Email: chay5@uwo.ca
Your application will be reviewed and you will be contacted within 48 hours of receipt.

Thank you for considering the Biotron.

Office Use Only	
Received on:	Reference #
By:	
Notes:	

27.5. Appendix D – Biotron CL-3 Entry / Exit Log Sheet

Biotron CL-3 Entry / Exit Log Sheet

DATE	NAME	PROJECT	LAB 1 (20H-1)		LAB 2 (20J-1)		PROTOCOL (PATHOGEN)	COMMENTS
			Time in	Time out	Time in	Time out		