
IMPLEMENTATION DIRECTIVE

OPERATIONAL PROCEDURES FOR USE AT CL2+

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INTRODUCTION

The Public Health Agency of Canada (PHAC) provides operational practices for use of biologicals at Containment Level (CL) 1 ([Canadian Biosafety Guideline](#), 2017; CBG) and CL 2, 3, and 4 ([Canadian Biosafety Standard](#), 2015; CBS). There may be cases where certain features of the biological (virulence or oncogenic factors) or protocols (volumes or intentional aerosolization) may increase or decrease the risk of exposure and thus necessitate changes in the operational procedures required. These operational procedures may be specified as a result of a local risk assessment performed by the Presidential Biosafety Advisory Committee (PBAC) or they may be specified directly from the government through [Biosafety Directives and Advisories](#).

The implementation of “containment level 2 plus” (CL2+) practices has changed over the course of time, predominantly due to the increased scope of knowledge pertaining to a particular biological or its use and due to data accumulated with respect to laboratory acquired infections (LAIs). The intent of the present PBAC Implementation Directive (PID) is to review and combine the various PBAC-approved and PHAC-mandated operational practices into one comprehensive document available to the McMaster research community.

In addition to external regulation by PHAC of operational procedures with respect to biologicals, these are also regulated by the Canadian Food Inspection Agency (CFIA). The PHAC regulates biologicals affecting humans and terrestrial animals. CFIA regulates biologicals causing foreign animal disease, aquatic animal pathogens, plant pathogens and emerging animal disease agents. Together these agencies effectively provide regulation and guidance for all forms of biohazardous work at McMaster University.

The operational procedures outlined below are in consideration of *in vitro* work only. If any *in vivo* work is to be undertaken with a biological to require CL2+, the *in vivo* work must also comply with CL2+ operating procedures.

CL2 OPERATIONAL PROCEDURES

Operational procedures for CL2 are outlined in the CBS and the CFIA documents *Containment Standards for Facilities Handling Aquatic Animal Pathogens* and *Containment Standards for Facilities Handling Plant Pests*.

ADDITIONAL OPERATIONAL PROCEDURES

In the addition to the above operational procedures, the laboratory must incorporate those procedures described below when handling the specified samples. In the event that an operational procedure listed below is in conflict with the Pathogen Safety Data Sheet (PSDS), a risk assessment will be performed by the PBAC and the Supervisor to determine which procedures are most appropriate to mitigate the risk of exposure.

CL2+ SPECIFIC SOPs

The Occupational Health and Safety Act requires, and thus has the force of law, that all *Supervisors be competent because of knowledge, experience and training*. As such, the Supervisor identifies risks, creates or reviews created SOPs and deems them appropriate to mitigate the identified risks within their laboratory and during the course of the work they organize. This is effectively the “sign off” on the SOPs by the Supervisor.

The PBAC requires that all laboratories newly working at CL2+ submit their Standard Operating Procedures (SOPs) while working in CL2+ mode for review and approval prior to initiation of the CL2+ work. These include Layout of the CL2+ area when in CL2+ mode, Laboratory Access, Biosecurity, PPE Required, Entry and Exit Protocols,

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Decontamination and Waste Handling, Housekeeping, Emergency Exit Protocols, Spill Protocols, Lab Specific protocols (experimental procedures done at CL2+), Use of a Biological Safety Cabinet and Lab Specific Procedures for Transitioning Between CL2+ and Other Containment Levels¹. Should there be any change to the CL2+ SOPs for a laboratory, for example an increase in culture volume, higher titers to be used or new procedures which generate aerosols, those modified or new CL2+ SOPs must be submitted to PBAC for review and approval before such changes are implemented.

ADDITIONAL OPERATIONAL PROCEDURES FOR USE WITH DIAGNOSTIC SAMPLES OF HIV, HEPATITIS C VIRUS AND WEST NILE VIRUS AND WITH MONKEY SAMPLES

These additional operational procedures are taken directly from a PHAC-generated document provided by the Pathogen Regulation Directorate. Most procedures listed are already required for work at CL2.

1. All activities should be conducted in a Biological Safety Cabinet (BSC).
2. If no BSCs are available or if it is not possible to use a BSC, workflow and manipulations with the pathogen must be designed to minimize the spread of contamination. These must include:
 - a. Identifying dedicated work areas and equipment for use with the pathogen
 - b. The use of absorbent material to cover work surfaces
3. A solid-front gown with tight-fitting wrists must be worn when infectious materials are directly handled and must be removed after completion of work and kept by the dedicated work area.
4. Personnel must have demonstrated proficiency in microbiological practices and techniques applicable to the select pathogen.
5. Leak-proof containers must be used to transport infectious material within the laboratory.
6. Centrifugation of infectious materials must be carried out in closed containers placed in sealed safety cups or rotors that are unloaded in a BSC. If it is not possible to use a BSC, sufficient time must be allowed for aerosols to settle before safety cups or rotors are opened.
7. Pathogen-specific disinfection and decontamination procedures must be in place.
8. Infectious agents stored outside the containment zone must be kept locked in leak-proof containers. Emergency response procedures are to take into account the existence of such infectious agents outside the containment laboratory.

ADDITIONAL OPERATIONAL PROCEDURES FOR USE OF LENTIVIRAL VECTORS

Lentiviral vectors to be handled using CL2+ operational procedures are those which are described in *PBAC Implementation Directive – Risk Assessment of Lentiviral Vectors*. These additional operational procedures are taken directly from a PHAC-generated document provided by the Pathogen Regulation Directorate. Most procedures listed are already required for work at CL2.

1. All activities must be conducted in a Biological Safety Cabinet.
2. A solid-front gown with tight-fitting wrists must be worn when infectious materials are directly handled and must be removed after completion of work and kept by the dedicated work area.
3. Personnel must have demonstrated proficiency in microbiological practices and techniques applicable to the select pathogen.

¹ Includes lab signage, lab access and lab decontamination steps

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4. Leak-proof containers must be used to transport infectious material within the laboratory.
5. Centrifugation of infectious materials must be carried out in closed containers placed in sealed safety cups or rotors that are unloaded in a BSC.
6. Pathogen-specific disinfection and decontamination procedures must be in place.
7. Infectious agents stored outside the containment zone must be kept locked in leak-proof containers. Emergency response procedures are to take into account the existence of such infectious agents outside of the containment laboratory.

ADDITIONAL OPERATIONAL PROCEDURES FOR USE WITH INFLUENZA VIRUS AND DIAGNOSTIC SAMPLES OF MYCOBACTERIUM TUBERCULOSIS

These additional operational procedures are taken directly from a PHAC-generated document provided by the Pathogen Regulation Directorate. Most procedures listed are already required for work at CL2.

1. All activities must be conducted in a Biological Safety Cabinet.
2. A solid-front gown with tight-fitting wrists must be worn when infectious materials are directly handled and must be removed after completion of work and kept by the dedicated work area.
3. Personnel must have demonstrated proficiency in microbiological practices and techniques applicable to the select pathogen.
4. Leak-proof containers must be used to transport infectious material within the laboratory.
5. Centrifugation of infectious materials must be carried out in closed containers placed in sealed safety cups or rotors that are unloaded in a BSC.
6. Pathogen-specific disinfection and decontamination procedures must be in place.
7. Infectious agents stored outside the containment zone must be kept locked in leak-proof containers. Emergency response procedures are to take into account the existence of such infectious agents outside of the containment laboratory.

ADDITIONAL OPERATIONAL PROCEDURES FOR HANDLING PATIENT SPECIMENS REASONABLY EXPECTED TO CONTAIN SARS-CoV-2 VIRUS

Procedures for handling patient specimens of this type are referenced in the PHAC [Biosafety Advisory Website](#). It is important to note that this applies only to patient specimens and not cultures or isolates of SARS-CoV-2 virus. Additionally, culture of these specimens to keep cells alive, to isolate cells or to isolate or propagate any microorganisms must be handled at CL3. This PBAC directive exceeds the requirements of the PHAC Advisory for handling patient specimens reasonably expected to contain SARS-CoV-2.

1. A lab coat, gloves, and eye protection are worn when handling primary specimens.
2. Centrifugation of primary specimens is carried out in sealed safety cups, or rotors, that are loaded/unloaded in a biological safety cabinet (BSC) or other primary containment device.
3. A certified BSC, or other primary containment device, is used for procedures that may produce infectious aerosols (e.g., pipetting [e.g., adding lysis buffer], preparing aliquots, diluting specimens, vortexing).
4. Respiratory protection (that provides a level of filtration of 95% or greater [e.g., N95]) is worn where aerosol generating activities cannot be contained within a BSC or other primary containment device.
5. Samples that are handled within a BSC or other primary containment device are moved to any analytic equipment (e.g., polymerase chain reaction [PCR] equipment) within a secondary closed container (e.g., gasketed, plates sealed with tape or flexible film).

TRAINING REQUIRED FOR CL2+

DOCUMENTATION OF TRAINING

All training must be documented² and the responsibility for maintaining the training record for any worker rests with the Supervisor to satisfy legislated requirements. The format of the training record may be in a format at the discretion of the Supervisor, however it must show that the worker is competent to carry out the tasks assigned.

All training documentation in the laboratory will be audited on a scheduled basis by the Research Compliance Auditor.

CORPORATE MATRIX

All persons are required to follow the training requirements outlined in the [corporate matrix](#).

If the work is located in a hospital, all personnel must take FHS Hospital Fire Safety training annually. This is irrespective of the Faculty in which the person works.

BIOSAFETY TRAINING

Provision of core *Biosafety Training* is the responsibility of the McMaster Biosafety Office. Maintenance of documentation of biosafety training is the responsibility of the Supervisor. Training is offered through Mosaic, under course code **BSLTRA**. Biosafety training must be updated annually. This consists of *Biosafety Update* also found through Mosaic, under course code **BSLUPD**.

Training information can be found on the McMaster Biosafety [website](#) under the tab marked “Training”.

Supervisors and workers that work with Risk Group 3 pathogens at CL2+ must complete specific annual update training found through Mosaic, “RG3 work at CL2+”. Please contact the [Associate Biosafety Officer](#).

Training records will be audited by the Research Compliance Auditor.

LAB SPECIFIC STANDARD OPERATIONAL PROCEDURES TRAINING

Provision and documentation of lab-specific SOPs training (which includes the operational procedures while in CL2+ mode) is the responsibility of the Supervisor. These SOPs include:

- Layout of the CL2+ area when in CL2+ mode
- Laboratory Access
- Biosecurity
- PPE Required
- Entry and Exit Protocols
- Decontamination and Waste Handling

Lentiviral vectors to be handled using CL2+ operational procedures are those which are described in *PBAC Implementation Directive – Risk Assessment of Lentiviral Vectors*.
r’s training is to create a training record for each worker and themselves.

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- Housekeeping
- Emergency Exit Protocols
- Spill Protocols
- Lab Specific CL2+ protocols
- Use of a Biological Safety Cabinet
- Lab Specific Procedures for Transitioning Between CL2+ and Other Containment Levels

and shall be created and signed off by the Supervisor. Some sample SOPs and training records can be found on the Biosafety website in the section entitled “SOPs, Docs, Forms & Templates”. Guidance from the Biosafety Office and the PBAC is readily available. More SOPs for equipment can be found on the FHS Safety Office website:

http://fhs.mcmaster.ca/safetyoffice/safety_equipment.html

This training is to be documented in the training records and will be audited by the Research Compliance Auditor.

N95 MASK TRAINING AND FIT TEST

An N95 mask is used during biohazardous spill cleanup in CL2+ protocols. Those responsible for spill cleanup must be trained in and fitted for the use of an N95 respirator. Information is found in the [McMaster Respiratory Protection Program](#).

Please contact the relevant safety office to arrange for N95 respirator fit testing. For those persons in FHS, please contact the FHS Safety Office (x24956; gummelc@mcmaster.ca) and for those persons not in FHS, please contact EOHSS (x24352; eohss@mcmaster.ca).

FHS N95 Respirator training requires an annual update to be completed online through Mosaic, course code FHSN95. Fit testing is required to be done every 2 years. This training and fit testing is to be recorded in the training records and will be audited by the Research Compliance Auditor.

SPILL CLEANUP PROCEDURES TRAINING

Provision and documentation of CL2+ spill cleanup procedures training is the responsibility of the Supervisor. Using the guidance documents that can be found on the Biosafety website in the section entitled “SOPs, Docs, Forms & Templates”, the spill cleanup procedures shall be modified by the Supervisor to reflect the specific procedures implemented in their CL2+ laboratory. Special attention is to be made with respect to the choice of disinfectant and its minimum contact time. Once approved by the PBAC, these CL2+ spill cleanup SOPs are then used as the content for training in CL2+ spill cleanup procedures. This training is to be documented in the training records and will be audited annually by the Research Compliance Auditor.

REASSIGNING A LABORATORY FROM CL2 TO CL2+

For the laboratory which was formerly a CL2 lab, who wishes to convert to CL2+ requires a number of tasks to be completed:

- Updated Biohazard Utilization Protocol (BUP) for resubmission to PBAC
- CL2+ SOPs to be generated and approved by PBAC
- CL2+ SOP training and documentation

- N95 Training and Fit testing
- All CL2 requirements up to date including annual lab audit

REASSIGNING A LABORATORY FROM CL1 TO CL2+

For the laboratory which was formerly a CL1 lab, who wishes to convert to CL2+ requires a number of tasks to be completed:

- Updated BUP for resubmission to PBAC
- CL2 training
- CL2+ SOPs to be generated and approved by PBAC
- CL2+ SOP training and documentation
- N95 Training and Fit testing
- All CL2 requirements up to date including annual lab audit

REASSIGNING A LABORATORY FROM NON-BIOHAZARD WORK TO CL2+

For the laboratory which was formerly a non-biohazard lab, who wishes to convert to CL2+ requires a number of tasks to be completed:

- Complete BUP for submission to PBAC
- CL2 training
- CL2+ SOPs to be generated and approved by PBAC
- CL2+ SOP training and documentation
- N95 Training and Fit testing
- All CL2 requirements up to date including annual lab audit

MECHANISM TO MONITOR PID EFFECTIVENESS

- Use of CL2+ operational procedures, #BUPs
- User feedback, qualitative quantitative

CONTACT INFORMATION FOR ASSISTANCE

Please use the following contact for assistance in training options and implementation of the above procedures.

Dr. Jen Robertson, University Biosafety Officer

- robertjv@mcmaster.ca
- X23453
- HSC 1J11A

Kristen Carrigan, Associate Biosafety Officer

- carrigan@mcmaster.ca
- X23453

- HSC 1J11A

Carol Carte, Research Compliance Auditor

- carte@mcmaster.ca
- X22950
- HSC 1J11A

Faculty of Health Sciences Safety Office

- fhsso@mcmaster.ca
- X22402
- HSC 1J11A

Environmental and Occupational Health Support Services (EOHSS)

- ehss@mcmaster.ca
- X24352
- GH 304