Lawson Health Research Institute Biosafety Manual

For Containment Level 1 and 2 Laboratories 2017

Chapter 1: Introduction to Lawson's Biosafety Program and Manual 5
1.1 Definitions 6
1.2 Abbreviations 7
1.3 Regulation of Biological Material within Lawson 8
1.4 Responsibilities 8
1.5 Crimnal Code of Canada 11
Chapter 2: Regulatory Agencies, Guidelines and Standards
2.1 Public Health Agency of Canada 12
2.1.1 The Human Pathogens and Toxins Act/Regulation (HPTA/HPTR) 13
2.1.2 Importing Human Pathogens 13
2.1.3 Importing Animal Pathogens 14
2.1.4 Importing Plant Pathogens 14
2.1.5 Export Requirements for Biological Materials 14
2.1.6 Transportation of Human Pathogens 15
2.2 Canadian Food Inspection Agency 15
2.3 Laboratory Animals 16
2.4 Waste Management 17
2.5 Autoclaves 17
2.6 Biological Safety Cabinets 18
2.6.1 Class I BSC 19
2.6.2 Class II BSC 19
2.6.3 Class III BSC 20
Chapter 3: Program Organization and Administration 21
3.1 The Lawson Biosafety Oversight Committee 21
3.2 Lawson Biohazards Sub-Committee (for the Review of Biohazardous Agent and Gene Therapy Protocols) 24
3.3 Lawson Biohazardous Agent Permit Process 27
Chapter 4: Laboratory Biosafety 29
4.1 Biosafety Training 29
4.2 Personal Protective Equipment 30
4.2.1 Glove Removal (Doffing) 32

4.2.2 Hand Washing 33
4.3 General Laboratory Safe Work Practices 33
4.4 Working with Laboratory Animals 35
4.5 Human Pathogens 36
4.5.1 Human Bloodborne Pathogens 37
4.5.2 Universal Blood and Body Fluid Precautions 37
4.6 Medical Surveillance and Immunoprophylaxis 39
4.7 Medical Procedures and Incident Reporting 40
4.8 Spills 41
4.9 Access/Security Controls 42
4.10 Containment Level 2 Requirements 43
4.11 Containment Level 2+ Requirements 43
Chapter 5: Biological Material 45
5.1 Classification of Biological Agents 45
5.2 Prions 45
5.3 Recombinant DNA and Interfering RNA 46
5.4 Animals 47
5.4.1 Animal Tissues 47
5.4.2 Cultured Animal Cells 48
5.5 Blood and Body Fluids 48
5.5.1 Fixed Tissues and Tissue Sections 48
5.6 Viral Vectors 49
Chapter 6: Biological Agent Risk Groups and Containment Levels 51
6.1 Risk Factors 51
6.2 Risk Groups 51
6.2.1 Risk Group 1 (low individual and community risk) 51
6.2.2 Risk Group 2 (moderate individual risk, low community risk) 52
6.2.3 Risk Group 3 (high individual risk, low community risk) 52
6.2.4 Risk Group 4 (high individual risk, high community risk) 52
6.3 Containment of Biological Hazards 52
6.3.1 Aerosols 53

6.3.2 Large Volumes and High Concentrations....53
6.3.2 Effluents and Waste....53
6.3.3 Pipetting....55
6.4 Biosafety Containment Levels....55
6.4.1 Containment Level 1 (CL1)....55
6.4.2 Containment Level 2 (CL2)....57
6.4.3 Additional Requirements for Containment Level 2 Plus Work....59
6.5 Animal Biohazard Containment Facilities....60
6.5.1 Animal Escape....61
References....61
Appendix A: Lawson Biological Safety Cabinet Safe Work Practice....62
Appendix B: Lawson Autoclave Safe Work Practice....66
Appendix C: SWP for working in Containment Level 2 Labs / Tissue Culture Labs / BSC work....70
Appendix D: Lawson SWP for Persons Working with Lentivirus-based Vectors *in vitro*....75
Appendix E: Lawson Biosecurity Plan....80
Appendix F: Biohazardous Risk Assessment for Lawson Research Areas....90

Chapter 1: Introduction to Lawson's Biosafety Program and Manual

The Lawson Health Research Institute (Lawson) is committed to providing a safe and productive laboratory environment for its faculty, staff, students, and volunteers to work in. Lawson's biosafety manual and associated policies and Safe Work Practices (SWPs) are developed in association with The University of Western Ontario (Western), St. Joseph's Health Care (St. Joseph's), and London Health Sciences Centre (LHSC). The purpose of this manual is to guide lab personnel on the safe use of biological materials that have the potential to cause harm to people and animals. The goal is to prevent any incidents or accidents that could result in physical harm or in a laboratory-acquired infection (LAI) of lab workers, and to protect the general public and environment from a biohazardous exposure.

Lawson will comply with all applicable Federal, Provincial, and Municipal legislation with respect to health and safety, and will keep abreast of any changes in such legislation and communicate them to the necessary individuals. Legislated standards in health and safety are accepted by Lawson as the minimum acceptable standards, and Lawson reserves the right to establish and enforce more stringent standards as may be appropriate. Such policies are considered as binding upon all faculty, staff, students, and volunteers. All laboratories are to take full responsibility for any visitors entering the laboratory and must ensure they are briefed on lab specific safety and policies prior to entering the laboratory.

The Biosafety Manual will provide information, guidelines, policies and safe work practices (SWPs) to be used in conjunction with Western's biosafety manual, relevant hospital policies, and other related regulations and guidelines including:

Canada

Canadian Biosafety Standard (CBS)
Canadian Biosafety Handbook (CBH)
Human Pathogens and Toxins Act (HPTA)
Human Pathogens and Toxic Regulation (HPTR)
Hazardous Products Act (HPA)
Canadian Environmental Protection Act (EPA)
Containment Standards for Facilities Handling Plant Pests
Containment Standards for Facilities Handling Aquatic Animal Pathogens

Ontario

Ontario Occupational Health and Safety Act and Regulations (OHSA)

Some of the content of this manual is taken directly from the Canadian Biosafety Standard (CBS) and is inserted throughout this manual for your convenience. It is still recommended that the CBS be reviewed as it the primary resource document for Canadian standards in biosafety laboratories.

All work conducted by Lawson personnel with potentially hazardous biological material on London Health Sciences Centre (LHSC) or St. Joseph's Health Care (St. Joseph's) hospital premises or under the control of the hospitals is to be performed in accordance with the requirements of this manual.

Any questions regarding the application or interpretation of this manual should be directed to the Lawson Biosafety Officer:

- Charis Johnson-Antaran, extension 61456
- Email: charis.johnsonantaran@lawsonresearch.com

1.1 Definitions

Biological hazard (Biohazard) refers to any biological material that can pose a threat to the health of living organisms, primarily that of humans, or to the environment.

Biological material refers to pathogenic and non-pathogenic microorganisms, proteins, and nucleic acids, as well as any biological matter than may contain microorganisms, proteins, and nucleic acids (or any parts thereof). This includes, but is not limited to, bacteria, viruses, fungi, prions, parasites, toxins, genetically modified organisms, nucleic acids, tissue samples, live vaccines, and isolates of a pathogen.

Biosafety is defined as the containment principles, technologies, and practices that are implemented to prevent unintentional exposure to infectious material and toxins, or their accidental release. The objective of (bio) containment is to confine biohazards through physical lab design and operational practices to protect personnel, the immediate work environment, and the community from an exposure to a biological material.

Human pathogen is a micro-organism, nucleic acid or protein capable of causing disease or infection in humans that (*a*) is listed in the HPTA in any of Schedules 2 to 4 or in Part 2 of Schedule 5; or (*b*) is not listed in any of the Schedules but falls into Risk Group 2, Risk Group 3 or Risk Group 4.

(Microbial) Toxin is a poisonous substance that is produced or derived from a microorganism and can lead to adverse health effects in humans or animals. A human pathogen or toxin includes:

- A substance that contains a human pathogen or toxin; and
- Any synthetic form of the human pathogen or toxin. Human toxins are listed in the HPTA in Schedule 1 or in Part 1 of Schedule 5.

Security Sensitive Biological Agents (SSBAs) are biological agents that have been determined to pose an increased biosecurity risk due to their potential for use as a biological weapon. SSBAs are identified as prescribed human pathogens and toxins by Section 10 of the *Human Pathogens and Toxins Regulations*.

Universal Precautions refers to the practice, in medicine (lab medicine and research), of avoiding contact with patients' bodily fluids, by means of the wearing of nonporous articles such as medical gloves, goggles, face shields and laboratory coats.

1.2 Abbreviations

ACC	Animal Care Committee	**ICAO**	International Civil Aviation Organization
ACVS	Animal Care and Veterinary Services	**LAI**	Laboratory-acquired Infection
ACRC	Agriculture & Agri-Food Canada Research Station	**LBAPP**	Lawson Biohazardous Agent Permit Process
ATCC	American Type Culture Collection	**LBOC**	Lawson Biosafety Oversight Committee
BAPA	Biological Agent Permission Application	**LBSC**	Lawson Biohazards Sub-Committee
BBP	Blood Borne Pathogens	**LHSC**	London Health Sciences Centre
BSO	Biological Safety Officer	**LRA**	Local Risk Assessment
BTWC	Biological and Toxin Weapons Convention	**LRCP**	London Regional Cancer Program
CBS	Canadian Biosafety Standards	**NIOSH**	National Institute for Occupational Safety and Health
CCAC	Canadian Council on Animal Care	**OHSA**	Occupational Health and Safety Act
CFIA	Canadian Food Inspection Agency	**OHSS**	Occupational Health and Safety Services
CL	Containment Level	**PHAC**	Public Health Agency of Canada
DGR	Dangerous Goods Regulation	**PPE**	Personal Protective Equipment
DFATD	Department of Foreign Affairs, Trade and Development	**PSDS**	Pathogen Safety Data Sheets
DNA	Deoxyribonucleic acid	**rDNA**	Recombinant DNA
ECD	Export Controls Division	**RNA**	Ribonucleic Acid
ECL	Export Controls List	**RNAi**	RNA interference
EPA	Environmental Protection Act	**RG**	Risk Group
ERP	Emergency Response Plan	**SEMD**	Safety-engineered medical device
HAA	Health of Animals Act	**St. Joseph's**	St Joseph's Health Care
HAR	Health of Animal Regulations	**SOP**	Standard Operating Procedure
HEPA	High Efficiency Particulate Air	**SSBA**	Security Sensitive Biological Agents
HPIR	Human Pathogen Import Regulation	**SWP**	Safe Work Practise
HPTA	Human Pathogen and Toxin Act	**TDG**	Transportation of Dangerous Goods
HPTR	Human Pathogen and Toxin Regulation	**UHSC**	University Health & Safety Committee
HVAC	Heating, ventilation, and air conditioning	**UWO**	University of Western Ontario
IATA	International Air Transport Association	**VRL**	Victoria Research Laboratories

1.3 Regulation of Biological Material within Lawson

The Lawson Biosafety Program is overseen by the Lawson Biological Safety Officer (BSO) in conjunction with Lawson's biosafety program. The BSO administers Lawson's biosafety program on a day-to-day basis and provides technical advice on safety procedures, equipment and relevant regulations as they apply to Lawson. Lawson works in conjunction with Western to ensure that all Western-affiliated staff and students are working safely with biohazardous materials. However, the ultimate responsibility of biological safety resides with the Principal Investigator (PI) and their staff.

All Lawson scientists who wish to work with any biological materials must obtain authorization through Lawson's approval process by completing a detailed Lawson Biohazardous Agent Permit Process form (LBAPP). The Lawson Biohazards Sub-Committee (LBSC; a sub-committee to the Lawson Biosafety Oversight Committee (LBOC)) will review and approve all LBAPP submissions and make recommendations to the PIs on all matters related to the use of potentially biohazardous materials within Lawson. The sub-committee will ensure all research conducted with biohazardous agents and human pathogens conforms to the most current Canadian Biosafety Standard (CBS) published by the Public Health Agency of Canada (PHAC). More information on Lawson's biosafety program can be found in in this manual as well as the Lawson Health and Safety Intranet.

1.4 Responsibilities

Lawson's Biological Safety Officer, the Principal Investigators, and all laboratory personnel need to work together to ensure the health and safety of everyone working with or near biohazardous materials. Additional responsibilities are outlined below.

<u>Biological Safety Officer</u>

- Complete and submit all required licence applications, verifying accuracy along with all required sections of the Administrative Oversight Plan required by Public Health Agency of Canada (PHAC);
- Facilitate compliance with all relevant federal/provincial/municipal regulatory requirements, including animal pathogen import permit applications and transfer applications;
- Develop and maintain the Lawson Biosafety Manual;
- Act as a resource for standard operating procedure (SOP) and safe work practise (SWP) development;
- Help facilitate compliance with the Biosafety Manual and SOPs by working closely with the PIs informing the licence holder and animal pathogen import permit holder, as applicable, in writing of any non-compliance by a person working with human or animal pathogens, toxins or other regulated infectious material that is not being corrected by the person after they have been made aware of it;

- Review and assist in the completion of all LBAPPs;
- Send completed LBAPPs to the LBSC for approval;
- Communicating with the PHAC and the Canadian Food Inspection Agency (CFIA) on behalf of the licence holder and animal pathogen import holder;
- Perform visits, inspections, and audits to ensure compliance;
- Conducting biosafety / biosecurity risk assessments;
- Provide support, advice, and consultation on biosafety issues;
- Provide or coordinate and document employee biosafety training where required;
- Investigate incidents related to laboratory biosafety and biosecurity; and
- Report to PHAC any loss of hazardous biological agents, accidental acquisition of hazardous biological agents and any known exposures to risk group 2, 3 or 4 pathogens and toxic agents.

The Biosafety Officer may require any person who conducts controlled activities as described… under the licence to provide them with any records that are necessary to assist them in carrying out their functions.

The Principal Investigators are primarily responsible for the safety of their lab personnel and for ensuring a safe working environment in their lab(s).

<u>Principal Investigator</u>

- Complete all LBAPP forms where required;
- Send all LBAPP forms to the Biological Safety Officer for approval;
- Ensure all conditions of the permit are followed;
- Ensure that the appropriate containment cabinets are used and are functioning properly by confirming they are tested annually as per Appendix A: Lawson Biological Safety Cabinet Safe Work Practice;
- Participate in biosafety training and any other relevant training programs as required by the Human Pathogen and Toxin Act (HPTA) and Human Pathogen and Toxin Regulation (HPTR);
- Contact the BSO before importing or exporting any controlled biohazardous materials;
- Contact the BSO in the event of the loss of or change in risk group to a controlled biohazardous material;
- Ensure that all persons working under their control have had appropriate training in working safely with potential biohazardous materials;
- Provide appropriate personal protective equipment (PPE), standard operating procedures(SOPs) and safe work practises (SWP);
- Ensure that all persons working under their control follow applicable Lawson / Hospital safety manuals, procedures and policies;
- Ensure that all laboratory personnel are using containment engineering controls such as Biological Safety Cabinets (BSC), fume-hoods and laminar flow hoods correctly;

- Ensure that all laboratory personnel are wearing effective personal protective equipment (PPE) in all CL2 and CL2+ laboratories as well as while using BSCs;
- Ensure that Laboratory Risk Assessment forms are up-to-date;
- Keep an up-to-date inventory of all biological agents, toxins and cell lines that must be handled in a minimum Containment Level 2 Laboratory
- Ensure that all laboratory personnel have all the required immunizations necessary for the agents they are handling;
- Regularly inspect their biohazard containment areas for (potentially) hazardous conditions;
- Ensure all incidents (including near-misses) are reported and investigated; and
- Ensure that all biological materials listed on the BAPA(s) are up-to-date; and
- Have any changes in use of biological material approved by a Lawson Biological Safety Officer / Lawson Biosafety Sub-committee through the LBAPP modification process.

Laboratory personnel must follow the policies and procedures outlined in this manual, and by their supervisor, and with all applicable legislation.

<u>Laboratory Personnel</u>

- Follow the policies and safe work practices outlined in all applicable Lawson / Hospital safety manuals, policies and procedures;
- Participate in all training courses as directed by their supervisor;
- Wear personal protective equipment appropriate to the hazards present;
- Ensure full understanding of the potential risks associated with the biohazardous materials used / stored in the laboratory and any activities involving biohazards;
- Participate in medical surveillance programs as deemed necessary by Occupation Health and Safety Services (OHSS) and / or Workplace Health (Western staff / students);
- Report hazards, incidents, laboratory-acquired infections, and unsafe conditions to their supervisor immediately; and
- When required, seek information from their supervisor or other resources, including the Biological Safety Officer.

When to notify your BSO:

- You believe that a pathogen or toxin has been released;
- An unauthorized person comes into possession of the pathogen or toxin;
- You believe that an incident caused or may cause disease;
- A pathogen or toxin has been stolen;
- (SSBA has not been received with 24hrs of its expected arrival;

- Someone with security clearance has been convicted of a criminal offence;
- Changes have been made to the physical structure of the facility in which the pathogen or toxin is used or stored;
- Changes have been made to your equipment or SOPs

1.5 Criminal Code of Canada

In 2004, workplace safety legislation was passed which establishes, for the first time in Canadian history, a duty to ensure workplace health and safety under the Criminal Code. These changes apply to all Canadian workplaces including the administrative, teaching and research areas at all Lawson sites.

In 2004, the Criminal Code of Canada imposed a legal duty which applies to everyone who undertakes, or has the authority, to direct how work is performed.

If you are diligently following applicable Occupational Health and Safety (OHS) regulations and best practices in your workplace, and are monitoring compliance, then these legislative changes will serve to reinforce the importance of your efforts.

Chapter 2: Regulatory Agencies, Guidelines and Standards

Activities involving the use of biological agents and laboratory animals, the production and disposal of waste, and the use of certain equipment are governed by various legislation, guidelines and standards. Adherence to the requirements of this manual will ensure that work is performed safely and in compliance with the requirements of external agencies and regulatory bodies.

2.1 Public Health Agency of Canada

In 2004, the Government of Canada established The Public Health Agency of Canada (PHAC). PHAC was confirmed as a legal entity in 2006 by the Public Health Agency of Canada Act. PHAC exists to promote and protect the health of Canadians through leadership, partnership, innovation and action in public health. PHAC also serves as the national authority for the biosafety and biosecurity of human pathogens and toxins in Canada.

PHAC's Centre for Laboratory Biosafety and Biosecurity is responsible for administering and enforcing the Human Pathogens and Toxins Act (HPTA) and the Human Pathogens and Toxins Regulations (HPTR). The HPTA/HPTR came into force on December 1, 2015. As of this date, the Human Pathogens Importation Regulations (HPIR) has been repealed, and a licence under the HPTA/HPTR is required to conduct any controlled activities (i.e. possession, usage, storage, importing, exporting, etc.) with human pathogens or certain biological toxins in Canada. The HPTR establishes national licensing and security clearance requirements and enables the Government of Canada to harmonize the requirements for all domestic use of human pathogens and toxins.

The HPTA licence number replaces the need to register under the HPTA and obtain compliance letters and import permits for controlled activities. To import or export a controlled human pathogen or toxin, you must notify the Lawson Biosafety Officer.

PHAC provides several tools for those who design, operate or work in laboratories that contain human pathogens and toxins. PHAC's Pathogen Safety Data Sheets (PSDS) are technical documents that describe the hazardous properties of various human pathogens and provide detailed information and descriptions of these hazards.

PHAC, along with the Canadian Food Inspection Agency (CFIA) has developed a joint Canadian Biosafety Standard (CBS) and a Canadian Biosafety Handbook (CBH) that pertain to human and terrestrial animal pathogens and toxins. These standards and guidelines are used by laboratory researchers who work in facilities that handle, store, or use such biohazardous agents. The CBS is used by PHAC and the CFIA to verify regulatory compliance of facilities that handle and store biohazardous materials, and provides the guidelines for certifying containment zones. The CBS 2nd edition has replaced and streamlined the following documents:

- Canadian Biosafety Standards and Guidelines (CBSG), 1st edition;
- Laboratory Biosafety Guidelines 3rd Edition, 2004 (PHAC);
- Containment Standards for Veterinary Facilities 1st Edition, 1996 (CFIA); and

- Containment Standards for Laboratories, Animal Facilities and Post Mortem Rooms Handling Prion Disease Agents, 2005 (CFIA)

2.1.1 The Human Pathogens and Toxins Act/Regulation (HPTA/HPTR)

The Human Pathogens & Toxins Act received royal assent on June 23, 2009. The purpose of the HPTA is to establish a safety and security regime to protect the health and safety of the public against the risks posed by human pathogens and toxins. The HPTA applies to any Lawson researcher who conducts specified activities with human pathogens and toxins whether they are imported or domestically acquired. This includes: production, storage, release, handling, possession, transfer, import/export, use, disposal, access to, and abandonment of human pathogens and toxins.

The HPTA is the enabling act of the Human Pathogens and Toxins Regulation, which came into effect on December 1st, 2015. The HPTR contains detailed information on licensing requirements, BSO duties and responsibilities, Security-Sensitive Biological Agents (SSBAs) and their exemption quantities and exemptions to licensing requirements.

A licence under the HPTA must be held in order to conduct controlled research activities in Canada. Lawson holds two licences: one for research conducted within St. Joseph's Healthcare, and one for London Health Sciences Centre (Victoria Hospital and University Hospital).

2.1.2 Importing Human Pathogens

The Human Pathogens Importation Regulations (HPIR) was the regulatory authority for facilities wishing to import human pathogens into and transfer specimens within Canada. The HPIR was repealed on December 1, 2015 and import permits are no longer issued. Any researcher wishing to import a human pathogen in Risk Group 2, 3, or 4, or a toxin, must notify the Lawson BSO before they can import a human pathogen or toxin. The researcher must be approved to import and safely store/use the pathogen or toxin as assessed by the Lawson BSO. This assessment will ensure that the appropriate containment is in place as per the Canadian Biosafety Standard (CBS) and the Canadian Biosafety Handbook (CBH) in all facilities dealing with imported human pathogens and toxins.

Some Canadian suppliers of biological materials have import procedures in place for biohazardous materials requiring CL2. For example, Cedarlane supplies some ATCC cell lines, and will require proof that the laboratory to which the materials are being shipped has a valid HPTA licence before the materials will be shipped.

Risk Group 1 pathogens or those pathogens that require containment level 1 facilities are not regulated by PHAC (nor the HPTR), and therefore a licence is not required for their importation.

Researchers wishing to import pathogens that require containment level 2 must be inspected and certified by the BSO. This ensures that the facility meets the CBS requirements for containment. A PHAC inspector can also visit the premises of any containment level 2 (or

higher) facility at any time. Please note that licences are issued in the name of the institute-wide licence holder who is legally responsible for the imported material.

2.1.3 Importing Animal Pathogens

Many human pathogens are animal pathogens as well. Animal pathogens are regulated by the Canadian Food Inspection Agency (CFIA) (see section 2.2). If you import pure cultures of terrestrial animal pathogens and toxins, with the exception of non-indigenous animal pathogens and pathogens causing emerging animal disease, you will need to apply to PHAC for an importation permit under the authority of the Health of Animals Regulations (HAR). If you do not have the appropriate importation permit you will be considered non-compliant and subject to enforcement actions.

For the purpose of this program the term "terrestrial animal" includes avian and amphibian animals but does not include aquatic animals, bees and invertebrates.

The authority under the HAA/HAR to issue import permits for animals, animal products and by-products (e.g., tissue samples, serum), aquatic animal pathogens, bee pathogens, pathogens that cause foreign animal diseases and pathogens that cause emerging animal diseases remains with the CFIA.

2.1.4 Importing Plant Pathogens

Please refer to the Containment Standards for Facilities Handling Plant Pests, 1st Edition for information on working with plant pathogens. The CFIA issues permits under the Plant Protection Act and Regulations. The CFIA is responsible for plant protection import control and enforcement issues and provides advice regarding the Plant Protection Act and Regulations. Please refer to the CFIA webpage on Plants for more information and for import permit applications:

http://www.inspection.gc.ca/plants/plant-pests-invasive-species/imports/eng/1324569244509/1324569331710

2.1.5 Export Requirements for Biological Materials

Canada is a State Party to the 1972 Biological and Toxin Weapons Convention (BTWC). This international Convention stresses the goal of non-proliferation of biological and toxin weapons through the prohibition of the development, production, stockpiling or acquisition of microbiological (biological) and toxin weapons and their destruction. In Canada, the Export controls have been implemented within Group 7 of the Export Control List (ECL). The Export Controls Division (ECD) of Global Affairs Canada is responsible for the administration of export controls under the authority of the Export and Import Permits Act. Any Lawson personnel wishing to export any biological materials included on the ECL to a country that is included on the Area Control List must first receive a Permit to Export from Global Affairs Canada. The permit application form can be found here:

http://www.international.gc.ca/controls-controles/report-rapports/list_liste/forms-formulaires.aspx?lang=eng

Please note that when shipping regulated materials to another country, it is the shipper's responsibility to ensure all necessary documentation accompanies the shipment. This includes any importation documents required by the recipient country.

2.1.6 Transportation of Human Pathogens

When moving or transporting biohazardous material outside of a containment zone (e.g. moving out of the laboratory to an autoclave, or to another laboratory) the material must be labelled, contained within a closed, leak-proof and shatterproof secondary container, and must be surface-disinfected. A cart should be used whenever possible.

When biohazardous materials are transported off Lawson premises, or are being packaged for transport, you must comply with the Transportation of Dangerous Goods Regulation (TDG) administered by Transport Canada. Transport Canada defines the labelling, packaging and documentation requirements necessary for shipping infectious substances, including diagnostic specimens, within Canada. Their regulation also requires that any individual transporting an infectious substance be trained in the transportation of dangerous goods (infectious substances). In addition, shippers of risk group 4 materials are required to have an emergency response assistance plan to respond to any shipping emergency occurring anywhere in Canada.

The air transportation of infectious substances internationally is regulated by the International Civil Aviation Organization (ICAO). As the majority of carriers (both passenger and courier/cargo) around the world are members of this organization, anyone shipping infectious substances internationally is likely subject to ICAO regulations. The ICAO regulations define the labelling, packaging and documentation requirements necessary for international shipping of infectious substances by air. It also requires that any individual transporting an infectious substance be trained in the transportation of dangerous goods (infectious substances). The ICAO requirements are based upon the United Nations Recommendations on the Transportation of Dangerous Goods.

Shipping infectious substances by air also falls under the *Dangerous Goods Regulations* (DGR) of the International Air Transport Association (IATA). These regulations set out all the ICAO mandates and the airline industry's universal rules on how to safely package and transport infectious substances.

2.2 Canadian Food Inspection Agency

The Canadian Food Inspection Agency (CFIA) is dedicated to safeguarding food, animals and plants to enhance the health and well-being of Canadians, the environment and the economy. The CFIA works with PHAC scientists and technical experts to establish the biocontainment levels, procedures and protocols that are needed to work safely with animal and zoonotic pathogens, chemical hazards, and plant pests of quarantine significance, and to protect laboratory staff, the Canadian public, and the environment.

As mentioned in section 2.1.3, in accordance with the Health of Animals Act (HAA) and its regulations (HAR), CFIA continues to issue permits for non-indigenous animal pathogens (pathogens that cause foreign and/or emerging animal diseases), aquatic and plant pathogens as well as for animals, animal products and by-products, tissue, sera and blood that are infected with animal pathogens. Animal disease fact sheets for reportable diseases, immediately notifiable and annually notifiable diseased are prepared by the CFIA and can be found here:

http://www.inspection.gc.ca/animals/biohazard-containment-and-safety/pathogen-imports/disease-agents/eng/1312495508549/1312497560331

The CFIA and PHAC have joined forces to update and consolidate the three existing Canadian biosafety standards and guidelines by creating the Canadian Biosafety Standard (CBS). Further information on this consolidation and the CBS can be found in section 2.1.

The CFIA does not regulate export of pathogens and toxins from Canada. The Export Controls section of Global Affairs Canada is responsible for administering the Export and Import Permits Act. Global Affairs Canada produces an Export Control List with Group 7 Chemical and Biological Weapons, Non-Proliferation List containing the list of human, animal and plant pathogen and toxins that require an export permit.

2.3 Laboratory Animals

In addition to this manual, all animals in research and the operational procedures for the care and maintenance of animals must satisfy the following guides and agencies at a minimum:

- The Guidelines for the Care and Use of Experimental Animals (Canadian Council on Animal Care);
- The Animal Care and Veterinary Services (ACVS) of the University of Western Ontario (Western); and
- Western's Animal Care Committee (ACC).

This is to ensure that not only are laboratory personnel and the environment protected, but that every care is taken to avoid causing the animals' unnecessary pain or suffering and to provide the animals with the highest quality care. Under the Ontario Animals for Research Act and its Regulations, it is a requirement that all Principal Investigators who intend to conduct research, testing or teaching projects at Lawson that involve the use of animals, must obtain the approval of Western's ACC before starting the research.

To obtain an approval, the Principal Investigator must submit an Animal Use Protocol Form to the ACC. The forms can be found at Western's Animal Care and Veterinary Services web site.

http://www.uwo.ca/animal-research/esirius/index.html

The Animal Use Protocol Form contains sections that address occupational health and safety issues (including biosafety) and is reviewed by the institutional BSO. All PI's completing animal

protocols must provide MSDS and chemical, drug or biological questionnaire sheets for the BSO's reference.

The completed protocol form must be signed by the Principal Investigator and is then submitted to the ACC for review, approval and signature. Please refer to the practices and procedures in Section 4.4 Working with Laboratory Animals.

2.4 Waste Management

The Lawson laboratories generate many different kinds of hazardous and non-hazardous waste. The handling, packaging, transport and disposal of hazardous wastes in Ontario are governed by municipal, provincial and federal government legislation. All Lawson laboratories must comply with the relevant regulations regarding biohazardous waste. Both institutional Waste Management Manuals detail the proper disposal methods for infectious waste, including human and animal anatomical waste and cytotoxic waste.

St. Joseph's (note that many links can only be accessed through LHSC or St. Joseph's intranet)

- Waste Management Manual: (https://intra.sjhc.london.on.ca/sites/default/files/pdfs/occ_hs_biomanual.pdf)
- Safe Handling of Biomedical Waste: (https://intra.sjhc.london.on.ca/sites/default/files/occ_hs_safe_handling_of_biomedical_waste.pdf)
- Chemical Waste Disposal Procedures: (https://intra.sjhc.london.on.ca/sites/default/files/OCC%20hs%20c%20chemwaste.pdf)
- Handling Precautions for Hazardous Drugs (including Cytotoxic Drugs) (https://apps.sjhc.london.on.ca/sj_files/pharmacy/mag/hazardous_drugs_handling_precautions.pdf)

LHSC

- Waste Management videos https://intra.lhsc.on.ca/priv/ohss/training/waste/index.htm

- Waste Management Policy https://policy.lhsc.on.ca/policy/waste-management.htm)

Chemical Waste Control Procedure
(https://intra.lhsc.on.ca/priv/waste/chemical/index.htm

Chemical Waste disposal form
https://appserver.lhsc.on.ca/waste_management/form.php

2.5 Autoclaves

An autoclave is a piece of equipment found in many laboratory-associated facilities within Lawson. The purpose of an autoclave is to render treated material sterile (i.e. free of any living organisms). This is useful when sterilizing tools and equipment and for treating biohazardous waste. See Appendix B for Lawson's general Autoclave Safe Work Practice.

The Lawson Intranet maintains a list indicating the locations of autoclaves. Annual safety inspections are performed automatically, according to this list. If you have received a new autoclave, have moved an autoclave or are using one that has not been inspected during the last 12 months, please notify Lawson facilities. You must provide the information necessary to have this equipment added to the equipment list so that the required inspections are scheduled and performed in the future.

Lawson autoclaves must be operated as per the Safe Work Practice for Autoclaving (Appendix B). This procedure must be posted near all Lawson autoclaves. Prior to using an autoclave, personnel must be trained on its safe and proper use. For training and autoclave information, contact your supervisor or a Lawson safety officer.

2.6 Biological Safety Cabinets

Biological Safety Cabinets, or BSCs, when used correctly in research and teaching activities involving the manipulation of hazardous biological agents, are effective in containing and controlling exposure to the agents. BSCs complement good laboratory best practices and procedures. All BSCs used in laboratory activities at Lawson must be inspected, tested and approved for use annually as required by NSF/ANSI 49-2012, and only by trained service personnel to ensure that the cabinet is functioning as intended by the manufacturer (see Appendix A).

A biological safety cabinet (BSC) is a ventilated cabinet that uses HEPA filtration and laminar air flow to provide protection from particulates or aerosols of biohazardous materials. This protection can be to personnel, products, and the environment. BSCs must be used when handling Risk Group 2 or higher biohazardous materials that are exposed in open containers, when there is an increased risk of airborne infection and when there is a high probability of generating aerosols. A BSC is distinguished from a chemical fume hood by the presence of a HEPA filter and by the laminar air flow involved.

HEPA filters have a minimum efficiency of 99.97% removal of particles 0.3 microns in diameter, and are more efficient at trapping particles of a greater size (99.99% efficiency). This ensures all known infectious microbes are trapped by the HEPA filter. There are three classes of BSCs, which differ in the type of protection provided, as outlined in Table 1.

Type of Protection	Appropriate BSC
Personnel protection from RG 1-3 biohazards	Class I, Class II, Class III
Personnel protection from RG4 biohazards	Class III

Product Protection	Class II, Class III (if laminar flow included)
Volatile radionuclide/chemical protection (small quantities only)	Class IIA2, Class IIB1, Class IIB2 (if vented to the outside)
Volatile radionuclide/chemical protection	Class I if hard-ducted, Class IIB2, Class III

Table 1 - Biological Safety Cabinet Selection

2.6.1 Class I BSC

Class I cabinets have non-recirculated airflow directed away from the user that is discharged through a HEPA filter. Class I cabinets provide protection to personnel and the environment, but offer no protection to the experimental product.

2.6.2 Class II BSC

Class II cabinets provide a high degree of protection to personnel, the experimental product and the environment. These cabinets are suitable for work at Containment Level 1, 2 and 3, and are divided into two types (A and B) based on their construction type, airflow velocities and patterns of exhaust systems. These cabinets differ from Class I BSCs in that they only allow HEPA filtered air to flow over the work surface.

Class II, Type Al

Cabinet air may be recirculated back into the laboratory or ducted out of the building. It is able to maintain a minimum average face velocity of 0.38 m/s and may have a positive pressure contaminated plenum. This cabinet is not suitable for work with low levels of volatile chemicals or radioisotopes. If there was a structural integrity failure of the plenum, the environment could become contaminated, since the pre-HEPA filtered, positively pressurized air would leak out of the plenum.

Class II, Type A2

Cabinet air may be recirculated back into the laboratory or ducted out of the building by a thimble connection. It is able to maintain a minimum average face velocity of 0.5 m/s and has a plenum that is under negative pressure. This cabinet is not suitable for work with low levels of volatile chemicals or radioisotopes unless it is exhausted through a canopy (then minute amounts may be used).

This is the most common type of BSC found within Lawson labs. The negative pressure of the plenum provides an added layer of containment, since if there was a structural integrity failure of the plenum, the contaminated air would not be released into the environment.

Class II, Type B1

This cabinet is hard-ducted through a dedicated duct exhausted to the atmosphere, after passing through a HEPA filter. It contains negative pressure air flow and 30% of the air is recirculated within the cabinet. It can maintain a minimum average face velocity of 0.5 m/s. This cabinet type is suitable for work with low levels of volatile chemicals and trace amounts of radioisotopes.

Class II, Type B2

This cabinet is hard-ducted through a dedicated duct exhausted to the atmosphere to which 100% of the cabinet air exhausts after passing through a HEPA filter. This cabinet contains negative pressure air flow and maintains a minimum average face velocity of 0.5 m/s. It is suitable for work with volatile chemicals and radioisotopes in combination with high-risk biohazards.

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

2.6.3 Class III BSC

These cabinets are completely enclosed, gas-tight cabinets with HEPA-filtered supply and exhaust air. The cabinet is kept under negative pressure of at least 120 Pa, and airflow is maintained by an exterior exhaust system. Operation within the work surface is accessed through rubber glove ports or sealed air locks. These cabinets provide a completely contained area to protect the worker, the experimental product and the environment, and are the only BSC suitable for work at containment level 4. Material can only be removed from this cabinet through a dunk tank, double door autoclave or an air-lock pass-through for decontamination.

Chapter 3: Program Organization and Administration

The Lawson Biosafety Manual describes the requirements and procedures established by Lawson for work involving potentially hazardous biological agents. It is based on the Public Health Agency of Canada's Canadian Biosafety Standard, 2nd edition (CBS) and reflects current best practices. All work conducted by Lawson personnel with potentially hazardous biological agents on hospital premises or under the control of the hospital is to be performed in accordance with the requirements of this manual.

The organization of the Biosafety Program at Lawson includes the following:

- Lawson Biosafety Oversight Committee;
- Lawson Biohazards Sub-Committee;
- Lawson Safety Task Forces;
- Lawson Biosafety Officer;
- Principal Investigators (and/or Project Directors); and
- Persons using biological materials

3.1 The Lawson Biosafety Oversight Committee

Lawson Biosafety Oversight Committee Terms of Reference

Purpose

The Lawson Biosafety Oversight Committee (LBOC) helps to ensure that all research activities involving controlled biohazardous materials are conducted in a safe manner and conform to all legislated and other relevant standards. The LBOC reviews any specific biosafety problems, concerns or policy/protocol improvements that are presented by the Biosafety Officer (BSO) or another committee member. The LBOC may also assist the BSO with risk assessments, biosecurity plans and procedures, biosafety protocol and safe work practice reviews/approvals, and any disputes regarding biosafety matters or concerns.

As per the Canadian Human Pathogens and Toxins Act (HPTA), the Lawson Health Research Institute (Lawson) holds two licences (one for Lawson at St. Joseph's, and one for Lawson at LHSC) that allow for controlled biohazardous research activities to be conducted. The LBOC makes recommendations to the licence holder on all matters pertaining to biosafety within those Lawson laboratories that conduct controlled biohazardous research, as defined in the HPTA. The LBOC fulfills the need for an institutional biosafety committee (as per the Canadian Biosafety Handbook, 2nd edition, section 5.1.5), and verifies that all research involving biohazardous materials is conducted in accordance with all applicable legislation, regulations, and the policies and procedures of Lawson, London Health Sciences Centre and St. Joseph's Health Care, London.

LBOC committee members are not personally liable for their committee work so long as the members do not break the law or act negligently in their assigned duties. Lawson affirms that

the primary responsibility for the safety of staff, students, volunteers, and the public lies with the principal investigator responsible for conducting controlled biohazardous research.

Mandate

1. To oversee the Biosafety Program, and provide direction and recommend changes.
2. To promote and monitor compliance with all applicable policies, procedures, and legislation as outlined by PHAC and Lawson.
3. To develop and recommend policies and procedures to ensure compliance with all applicable legislation, policies, procedures, standards, guidelines as they apply to teaching and research activities.
4. To review the use and procurement of any human source samples, tissues, blood and other body fluids, and to recommend and monitor the appropriate safety precautions and procedures for this work.
5. In collaboration with the Lawson Biosafety Officer, review, recommend, and evaluate the biosafety program at Lawson; to act as an expert resource to the Lawson Biosafety Officer on such matters.
6. To recommend to Lawson and the hospitals any additional required safety programs, policies, and procedures be established as necessary to maintain compliance as per the above terms.
7. To establish sub-committees as necessary to carry out specific tasks as mandated by the LBOC.
8. To collaborate with the University of Western Ontario's Biosafety Committee as needed to maintain consistency between the two organizations.
9. To approve protocols involving the use of potentially biohazardous agents including genetically modified organisms and animals potentially carrying infectious zoonotic agents and to confirm the appropriate containment level for the work, to verify that the appropriate facilities and procedures are in use and to ensure that appropriate procedures for the use, storage and disoposal of the named agents are followed.

Definitions

Consensus Decision-making – is a group decision-making process in which committee members develop and support decisions that are in the best interests of all stakeholders. It is a process by which the committee members seek to generate mutual levels of participation and agreement.

Controlled Research – possessing, handling, importing, exporting, producing, storing, transferring, releasing, abandoning, and/or permitting any person access to biohazardous materials

Quorum – the minimum number of voting members who must be present at a meeting in order to conduct business in the name of the Committee

Responsibilities

Chair:

- To schedule meetings and arrange meeting locations*
- To create meeting agendas and distribute to the committee at least 48 hours ahead of a meeting*
- In the absence of the Chair, the voting members in attendance will select a member as acting Chairperson

*or to delegate these tasks to the Lawson Health and Safety team assistant

Membership

The LBOC is composed of the Chair, Researcher members, and Additional members as set out below. When deemed necessary, *ad hoc* consultants may be brought in based on their specific expertise.

The Biosafety Committee members are appointed by the Integrated Vice President, Research in consultation with the Biosafety Officer. Each member of the committee shall have an alternate approved by the Chair.

- Voting members:
    - Chair (to be nominated and elected by the committee)
    - Lawson Vivarium Facility manager or delegate
    - 1 laboratory technical representative knowledgeable in the use of biological hazards
    - At least 3 scientists* conducting controlled research within Lawson (one of which must be involved in clinical research)
        - One of the scientist members must be a member of the Lawson Biohazards Sub-committee
    - A senior leader from Pathology and Laboratory Medicine

- Non-voting members:
    - VP Research or delegate (ex-officio)
    - Occupational Health and Safety representation from LHSC and St. Joseph's
    - The Lawson Biological Safety Officer (ex-officio)

*Definition of scientist includes adjunct, associated and full scientists

Membership Term

A maximum appointment of 3 years,renewable for voting members.

Meetings

Meetings will be held quarterly, or at the call of the Chair. Any member may place items on the agenda for discussion. Items for inclusion on the agenda must be received by Lawson Health and Safety at least 7 days prior to the next scheduled meeting. An agenda will be distributed at least 48 hours prior to the meeting.

Quorum and Voting

Quorum is fifty percent (50%) plus one, of the members eligible to vote. Decisions will primarily be made by consensus. If consensus cannot be reached, then the decision shall be made by majority vote.

Minutes

Minutes shall be recorded by the Lawson Health and Safety administrative assistant or their delegate, and distributed to the committee members.

Records

Records of the Committee meetings will be maintained by the Lawson Biosafety Officer.

3.2 Lawson Biohazards Sub-Committee (for the Review of Biohazardous Agent and Gene Therapy Protocols)

Lawson Biohazards Sub-Committee Terms of Reference

Purpose

The Lawson Biohazards Sub-Committee (LBSC) is a sub-committee of the Lawson Biosafety Oversight Committee (LBOC), and makes recommendations to the LBOC on all matters pertaining to biosafety within Lawson laboratories. The LBSC reviews all protocols involving controlled research with potentially biohazardous materials in Lawson laboratories, and ensures that the use of these materials is in compliance with all legislated requirements as determined by the Public Health Agency of Canada (PHAC), Health Canada, and the policies and procedures of Lawson, London Health Sciences Centre and St. Joseph's Health Care, London.

Lawson affirms that the primary responsibility for the safety of staff, students, volunteers, and the public lies with the principal investigator that is conducting controlled biohazardous research.

Mandate

1.To review and approve protocols related to the use of potentially biohazardous materials (including but not limited to: genetically modified organisms, zoonotic infectious agents, recombinant DNA technologies, etc.).
2.To assess and confirm which containment levels are appropriate for any proposed biohazardous work and ensure all appropriate procedures related to the containment levels are followed.
3.To review the LBAPP form annually to ensure the form meets the current needs of the Lawson Biosafety Program and to recommend changes, as appropriate, to the LBOC.
4.To generate an Annual Report to be forwarded to the LBOC by May 1st of each year.

Definitions and Abbreviations

Conflict of Interest – a situation in which someone in a position of trust has competing professional and/or personal interests.

Controlled Research – possessing, handling, importing, exporting, producing, storing, transferring, releasing, abandoning, and/or permitting any person access to biohazardous materials used in research.

Non-compliance – any breach of applicable regulations, legislation, guidelines, policies, or safety standards that has occurred or is occurring.

Quorum – the minimum number of voting members who must be present at a meeting in order to conduct business in the name of the Committee.

LBAPP – Lawson Biohazardous Agent Permit Process

LBOC – Lawson Biosafety Oversight Committee

Responsibilities

Chair:

- To schedule meetings and arrange meeting locations*
- To create meeting agendas and distribute to the committee at least 7 days ahead of a meeting*
- To sign all approved LBAPP submissions
- To investigate issues of non-compliance, and suspend or withdraw permits related to any work that is deemed to pose a biosafety risk, and to require the immediate cessation of that work; for the purpose of an investigation, to enter any laboratory or other research space under the jurisdiction of Lawson to examine the equipment, operations, materials, personnel, and any other processes therein
- In the absence of the Chair, the voting members in attendance will select a member as acting Chairperson

*or to delegate these tasks to the Lawson Health and Safety team assistant

Members:

- To read, understand, and adhere to the mandate of the committee
- To review all LBAPP submissions in a fair and impartial manner
- To declare any conflicts of interest as applicable

Membership

The LBSC is composed of the Chair, Researcher members, Resource members, and Additional members as set out below:

- Voting Members:
  - Committee Chair (to be nominated and elected by the committee)
  - 3 microbiologist members (are researchers who hold active LBAPPs and who are appointed by the chair; at least one member must have virology expertise)
    - One of the above members to also sit on Lawson Biosafety Oversight Committee
  - One Clinician (to attend on an as-needed basis, whenever clinical LBAPPs are being reviewed)
  - The Western Animal Research Safety Consultant

- Non-voting:
  - Lawson Biosafety Officer (ex-officio member)

Membership Term

A maximum appointment of 3 years,renewable.

Meetings

Meetings will be held monthly, or at the call of the Chair. Any member may place items on the agenda for discussion. Items for inclusion on the agenda must be received by Lawson Health and Safety at least 7 days prior to the next scheduled meeting. An agenda will be distributed at least 5 days prior to the meeting.

Quorum and Voting

Quorum is fifty percent (50%) plus one, of the members eligible to vote. Proxy voting is allowed by means of a committee member submitting their comments and decisions to another member who will be present at the meeting. Voting is through consensus decision making with unanimous agreement to finalize the decision.

Minutes

Minutes shall be recorded by the Lawson Health and Safety administrative assistant or their delegate, and distributed to the committee members.

Records

Records of the Committee meetings will be maintained by the Lawson Biosafety Officer.

Conflict of Interest

Should a committee member feel they have an actual, potential, or perceived conflict of interest regarding an LBAPP submission, the member must declare a conflict of interest prior to or at the beginning of the meeting. The Chair will excuse the member during discussion of such an item.

Decisions

Once the committee is satisfied with an LBAPP submission, the Biosafety Officer and the Chair of the committee will sign the LBAPP document. An LBAPP number will then be issued to the PI, and will be sent to any relevant administrative groups.

3.3 Lawson Biohazardous Agent Permit Process

A Lawson Biohazardous Agent Permit Process form (LBAPP) is required for all Lawson laboratory activities (research and teaching) which involve the use or manipulation of potentially hazardous biological agents, and materials containing such agents (including viruses, bacteria, fungi, parasites, recombinant DNA, prions, other microorganisms/genetic systems, and human and animal tissues, cells, blood and body fluids.

All such activities are to be conducted and performed in accordance with the Lawson Biosafety manual, the Lawson Biosafety Policy and any relevant guidelines or legislation. A Lawson LBAPP must be filled out for research being conducted on a hospital site (i.e., the physical location of the research); a Western BAPA must be filled out for research being conducted on Western grounds.

All activities involving potentially hazardous biological agents and meeting any of the above criteria must be identified on the LBAPP. The release of grants and supporting funds by Western and Lawson is dependent on a completed signed University or Lawson permit.

After completion, the form is sent to a Lawson or Western Biosafety Officer for review, and then is submitted to the appropriate committee for final approval (please see the Western Biosafety manual for more information).

The Lawson LBAPP form is reviewed by the Lawson Biohazards Sub-committee, voted upon and approved by the Chair of the Biohazards Sub-committee and the Lawson Biosafety Officer. Upon approval the permit is valid for a maximum of 3 years.

The submission of an application for an LBAPP implies willingness to allow the Lawson Biosafety Officer to visit the laboratory sites used by the Principal Investigator in order to determine compliance with Lawson's Biosafety Policies and Procedures Manual.

For research requiring containment levels 2 or 2+, the Lawson Biosafety Officer will inspect the worksite annually to ensure that it meets the operational and physical requirements as per the current Public Health Agency of Canada's CBS and CBH. If importation of biohazardous materials is required (for importing human pathogens or toxins in Risk Group 2), then Lawson's Biosafety Officer will need to approve the request to import.

After this period, the Principal Investigator must submit a new application form every three years even if the activities involving biological agents have not been altered or modified since the previous submission. If the activities involving biological agents have been altered or modified (for example request of a new level 2 pathogen or toxin) since the previous submission, an LBAPP modification form must be completed. This form can be obtained from Lawson's Biosafety Officer.

Chapter 4: Laboratory Biosafety

Individuals who work in any laboratory that handles infectious substances are at risk of exposure to the substances and agents present in the laboratory. According to the Public Health Agency of Canada, laboratory-acquired infections (LAIs) are not uncommon; reported LAIs in the United States between 1979 and 2004 were 2,156 infections, with 17 confirmed deaths. Reported LAI in North America between 2000 and 2009 were 81 exposures with 34 confirmed LAIs with 3 fatalities. These figures are believed to be significantly low due to underreporting. Additionally, only about 20% of LAIs can be attributed to any known, single exposure event.

There are a number of ways in which infectious substances can enter the body and cause infection. These include ingestion, inhalation, or contact with mucous membranes, including conjunctivae (transfer of microorganisms to the eyes by contaminated hands), or with non-intact skin.
The types of events that can lead to an infection include: exposure to infectious aerosols; spills and splashes; accidental needle stick injuries; cuts from sharp objects and broken glass; bites and scratches from animals or ectoparasites; oral pipetting (which is prohibited); centrifuge accidents.
Exposure to aerosols may be the greates biohazard facing laboratory workers. Aerosols can present a risk in terms of inhalation, ingestion, mucous membrane contact etc. Operational practices and techniques must be used to minimize the creation of aerosols associated with common lab procedures.

Anyone who works with biological materials must be trained prior to beginning work. Supervisors are responsible for ensuring that all personnel in their laboratories are properly trained.

See Appendix A: Lawson Biological Safety Cabinet Safe Work Practice?????

WHMIS Training

WHMIS regulations require that all people working with or likely to be exposed to biohazards must be educated and trained on biohazards. Workers must be educated in general information such as the classes and symbols of controlled products. Training refers to instruction in site – specific information such as standard operating procedures and emergency procedures. Both education and training are important parts of understanding the risks that may be present at your workplace.

4.1 Biosafety Training

Prior to beginning any work with biohazardous materials (which can include but is not limited to microorganisms, cell cultures, human blood and body fluids) all new workers must participate in a biosafety training program. This includes reading the Lawson Health Research Institute's Biosafety Manual as well as the Public Health Agency of Canada's Canadian Biosafety Standards. All new workers must also complete Lawson's Biosafety Training course and receive

a passing grade on the biosafety exam. As well, the Principal Investigators/Supervisors are responsible for training their workers in all laboratory-specific hazards and procedures as per their own Standard Operating Procedures (SOPs). Recommended topics for laboratory-specific biosafety training may include:

- Use of safety equipment
- Health/physical hazards
- Safe work procedures
- Emergency procedures
- Spill clean-up procedures
- Access/security controls
- Biohazardous inventory

Other Training

WHMIS
XRay Safety
Radiation Safety
Laser Safety
Supervisor Training
Health and Safety Awareness
Laboratory Safety
Transportation of Dangerous Goods

Safety Data Sheets (SDS)

WHMIS regs require that workers have access to information on all hazards in the workplace, including biohazards. SDS for infectious microorganisms (biological agents) have been prepared byt the Office of Laboratory Security, PHAC. SDS contain health hazard information, recommended precautions, safet handling methods, decontamination methods and other information that is relavent to the lab setting. In the absence of a PHAC SDS, all attempts to get Health and Safety information on a biohazard must be made. This includes contacting the supplier, distributor, or other source of the biohazard.

4.2 Personal Protective Equipment

Personal Protective Equipment (PPE) is protective equipment and/or clothing that is designed to minimize the risk of exposure to various hazards. PPE can include respirators, hand and foot protection, head and eye protection, and full-body protection. PPE should always be the last form of control in reducing exposure to hazards.
The guiding principles on using Personal Protective Equipment (PPE) include:

- Using PPE properly to provide protection;
- Understanding that PPE only protects yourself and not your fellow lab workers; and

- Knowing that PPE is used to build redundancies in protection and should not be the sole source of protection from biohazards (i.e. using engineering controls such as biological safety cabinets or fume hoods to minimize exposure).

PPE provides protection for skin, mucous membranes, the respiratory tract, and the gastrointestinal tract by reducing the portals of entry for pathogens. By covering up exposed skin, for example, there is a reduced risk of a dermal exposure to a pathogen. It is important to consider using the proper PPE for the procedure being performed. PPE can be made from different types of materials and can be rated for resistance to various risks (e.g. flame retardant or water repellent). You must ensure you are using the correct PPE for the hazards you are working with.

The minimum PPE standard for working in Containment Level 1 and 2 laboratories at Lawson include:

- A properly fastened lab coat - this protects the worker's clothing from contamination
- Enclosed footwear with low or no heels; closed toe and heel is mandatory, and the shoes must cover the entire foot. The material should be non-absorbent (i.e., leather not canvas)
- Eye and face protection as is appropriate to the hazards encountered including eye glasses/goggles and respirators
- Clothing must provide continuous coverage of the skin from the shoulders to the feet; this means no short skirts, capris, or shorts are permitted
- Gloves appropriate to the hazards being worked with

Appropriate gloves must be worn for all procedures that may involve direct or accidental contact with biohazardous materials. It is important to check gloves for integrity before donning (check for any damage or holes). The integrity of a glove decreases with prolonged use especially when repeatedly decontaminating gloves with alcohol or other chemicals. As such, gloves should be changed frequently and must never be reused after doffing. Glove materials differ in their resistance to permeation by different chemicals.

When working with biohazardous materials, gloves must be wrapped over the cuff of the lab coat to ensure the wrists are protected (see Figure 1). Double-gloving is recommended for higher risk procedures including using nitrile gloves while working with cytotoxic agents and lentiviral vectors. Open wounds or cuts must be covered with a water-proof bandage before donning gloves. If a laboratory worker has severely compromised skin (such as weeping dermatitis) they should not be allowed to work with any biological materials.

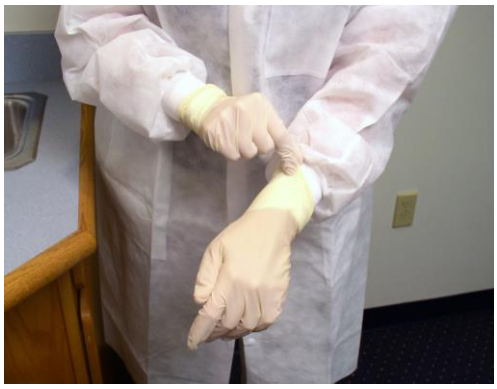


Figure 1 - Glove over cuff technique

In addition to the minimum protection required for working in a Lawson laboratory, additional PPE may be required when working with infectious agents. This may depend on the type of work being done (i.e. using a centrifuge or vortex) and the engineering controls that are available. Chapter 9 of the CBS details the use of PPE in Containment Level 2 laboratories.

Visitors

All biosafety laboratory visitors must dress appropriately, as required by the Laboratory Supervisor. They must wear the PPE required to be worn in the lab.
All visitors must be accompanied by the Laboratory Supervisor or designate who is responsible for them in case of an emergency.
All lab visitors must follow the rules and procedures of the lab.
Anyone not complying with the above will not be allowed entry into the lab or will be asked to leave the lab.

4.2.1 Glove Removal (Doffing)

It is important to change gloves as soon as is possible after contamination or compromise occurs. This can include contact with an infectious agent, tears or rips, and chemical exposures that compromise glove integrity. Gloves must also be removed when work with the biohazardous materials is complete and before leaving the containment zone.

The procedure for properly doffing gloves must be employed (see Figure 2):

1. With both hands gloved, grasp one glove from the palm and carefully peel it off and hold it with the gloved hand.
2. Grasp the inside cuff of the second glove with the exposed hand and peel it off, keeping the first glove tucked inside the second.
3. Dispose of the gloves immediately in an appropriate biohazardous waste container.
4. Take care to never touch the outside of a glove with bare skin.
5. Wash hands as soon as possible using proper hand washing technique.

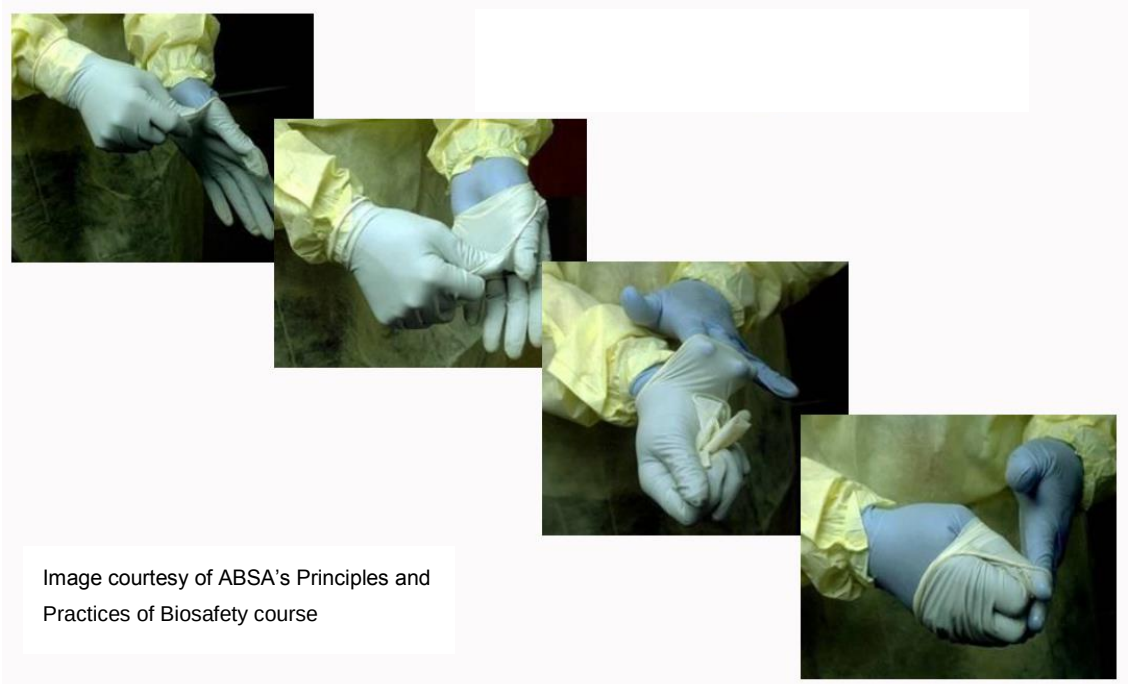


Figure 2 - Proper glove removal technique

4.2.2 Hand Washing

Hands must be washed with soap and water under clean running water, for a minimum of 20 seconds, before leaving the work area/containment zone and after doffing gloves. Ensure hands are thoroughly washed, including under fingernails, backs of hands and between fingers. Soap should be rinsed off thoroughly and hands dried completely. Hand sanitizers can be used as long as they are effective against the pathogen or toxin handled.

4.3 General Laboratory Safe Work Practices

The following general practices are required by the Public Health Agency of Canada and Lawson for all laboratories handling infectious substances.

1. A documented procedural (safety) manual must be available for all staff, and its requirements followed; it must be reviewed and updated regularly.
2. Personnel must receive training on the potential hazards associated with the work involved and the necessary precautions to prevent exposure to infectious agents and release of contained material; personnel must show evidence that they understood the training provided; training must be documented and signed by both the employee and supervisor; retraining programs should also be implemented.
3. Eating, drinking, smoking, storing of food, personal belongings, or utensils, applying cosmetics, and inserting or removing contact lenses are not permitted in any laboratory; the wearing of contact lenses is permitted only when other forms of corrective eyewear are not suitable; wearing jewellery is not recommended in the laboratory (S.32 of

O.Reg. 67/93).

4. Oral pipetting of any substance is prohibited in any laboratory.
5. Long hair is to be tied back or restrained so that it cannot come into contact with hands, specimens, containers or equipment.
6. Access to laboratory and support areas is limited to authorized personnel.
7. Doors to laboratories must not be left open (this does not apply to an open area within a laboratory).
8. Open wounds, cuts, scratches and grazes should be covered with waterproof dressings.
9. Laboratories are to be kept clean and tidy. Storage of materials that are not pertinent to the work and cannot be easily decontaminated (e.g., journals, books, correspondence) should be minimized; paperwork and report writing should be kept separate from such biohazardous materials work areas.
10. Protective laboratory clothing, properly fastened, must be worn by all personnel, including visitors, trainees and others entering or working in the laboratory; suitable footwear with closed toes and heels must be worn in all laboratory areas.
11. Where there is a known or potential risk of exposure to splashes or flying objects, whether during routine operations or under unusual circumstances (e.g., accidents), eye and face protection must be used. Careful consideration should be given to the identification of procedures requiring eye and face protection, and selection should be appropriate to the hazard.
12. Gloves (e.g., nitrile, vinyl, co-polymer) must be worn for all procedures that might involve direct skin contact with biohazardous material or infected animals; gloves are to be removed when leaving the laboratory and decontaminated with other laboratory wastes before disposal; metal mesh gloves can be worn underneath the glove.
13. Protective laboratory clothing must not be worn in non-laboratory areas; laboratory clothing must not be stored in contact with street clothing.
14. Rolling up of lab coat sleeves is prohibited. There must be no exposed skin from shoulders down to feet.
15. If a known or suspected exposure occurs, contaminated clothing must be decontaminated before laundering (unless laundering facilities are within the containment laboratory and have been proven to be effective in decontamination).
16. The use of needles, syringes and other sharp objects should be strictly limited; needles and syringes should be used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles; caution should be used when handling needles and syringes* to avoid auto-inoculation and the generation of aerosols during use and disposal; where appropriate, procedures should be performed in a BSC; needles should not be bent, sheared, recapped or removed from the syringe; they should be promptly placed in a puncture-resistant sharps container (in accordance with Canadian Standards Association [CSA] standard Z316.6-95(R2000)) before disposal.
17. Hands must be washed after gloves have been removed, before leaving the laboratory and at any time after handling materials known or suspected to be contaminated.
18. Work surfaces must be cleaned and decontaminated with a suitable disinfectant at the end of the day and after any spill of potentially biohazardous material; work surfaces that have become permeable (i.e., cracked, chipped, loose) to biohazardous material must be replaced or repaired.
19. Contaminated materials and equipment leaving the laboratory for servicing or disposal

must be appropriately decontaminated and labelled or tagged out as such.

20. Efficacy monitoring of autoclaves used for decontamination with biological indicators must be done regularly (i.e., consider weekly, depending on the frequency of use of the autoclave), and the records of these results and cycle logs (i.e., time, temperature and pressure) must also be kept on file.**
21. All contaminated materials, solid or liquid, must be decontaminated before disposal or reuse; the material must be contained in such a way as to prevent the release of the contaminated contents during removal; centralized autoclaving facilities are to follow the applicable containment level 2 requirements.
22. Disinfectants effective against the agents in use must be available at all times within the areas where the biohazardous material is handled or stored.
23. Leak-proof containers are to be used for the transport of infectious materials within facilities (e.g., between laboratories in the same facility).
24. Spills, accidents or exposures to infectious materials and losses of containment must be reported immediately to the laboratory supervisor; written records of such incidents must be maintained, and the results of incident investigations should be used for continuing education.
25. An effective rodent and insect control program must be maintained.

There are acceptable laboratory procedures that require alternative uses of needles. These procedures must be assessed and a specific safe work procedure put into place for such work.

****This is only required when using Containment Level 2+ or higher (e.g. lentivirus work). Waste produced in CL2 and lower facilities can be removed as biohazardous waste.***

4.4 Working with Laboratory Animals

Animals can harbour infectious organisms, which are acquired naturally. Some infectious agents can give rise to a chronic carrier state, or an agent might be shed intermittently. If the possibility that such an agent may be excreted, secreted, exhaled or shed by an animal during the course of an experiment cannot be excluded, then all those animals should be kept at the containment level appropriate to the risk. Animals may also be intentionally inoculated with viruses or other organisms in any of the four Risk Groups or with viable materials (e.g., transformed cells) that are suspected of containing these agents. Under these circumstances, the animals should be kept at the containment level appropriate to the risk of the agent. In some cases, *in vivo* work may increase that risk. Naturally occurring or experimentally induced infections in laboratory animals may be transmitted to other laboratory animals, invertebrates and laboratory workers. Laboratory animals and insects may scratch or bite or may be the source of aerosols.

Besides the risk from an infection that the animal or insect may be harbouring, there is also a risk that some of the material being injected may adhere to the fur or exoskeleton and remain as a potential hazard. In all situations, it is the responsibility of the principal investigator, Lawson Biosafety Officer, Western University's Animal Research Safety Consultant and Lawson's Biohazards Sub-committee in consultation with Government agencies and the animal care authorities, to determine the risk levels inherent in the proposed activity.

The requirements for the maintenance of animals may differ in scale and degree, but the basic principles for microbiological safety will be similar to those outlined in Section 4.3 and should include the following precautions.

1. Infected animals and insects should be segregated from uninfected animals wherever possible, and it is preferable to separate any handling area from the holding area.
2. Animals or insects in use in an experiment must be maintained at a level of containment that is at least equivalent to the containment level for the biological agent with which it has been infected or treated.
3. Provision must be made to ensure that inoculated animals or insects cannot escape.
4. Dead animals or insects and the refuse from the animal room and cages (e.g. bedding, feces and food) must be placed in a leak-proof container and autoclaved or incinerated, if potentially infected.
5. All cages must be properly labelled, and procedures in the holding area must minimize the dispersal of dander and dust from the animals and cage refuse.
6. Gloves and safety glasses should be worn by animal care providers while feeding and watering animals or cleaning cages.
7. Gloves, boots, floors, walls and cage racks should be disinfected frequently.
8. All aspects of the proposed use of animals in research must meet the current veterinary standards and regulations for the care and maintenance of experimental animals as described by the Canadian Council on Animal Care, relevant provincial legislation, Western University and the Animal Care Committee.
9. The appropriate species must be selected for the animal experiments.
10. The investigator and/or person(s) responsible for the animal experiment must ensure that all those having contact with the animals and waste materials are familiar with and aware of any special precautions and procedures that may be required. Where possible, personnel should be protected by immunization with appropriate vaccines.
11. All incidents, including animal bites and scratches or cuts from cages or other equipment must be documented and the employee should report to OHSS for medical assessment and follow-up.
12. All Animal Care and Veterinary Services (ACVS) procedures and protocols must be followed with respect to the proper handling and care of animals. All staff members that work with animals must have training as required by ACVS.
13. There are animal facilities within Lawson (at LHSC, St. Joseph's, and Western University) that require specific personal protective equipment and operating procedures. Use of these animal facilities requires strict adherence to these procedures.

4.5 Human Pathogens

Some microorganisms (viruses, bacteria, fungi, etc.) are species-specific, selectively infecting and causing disease in a limited number of, or only one, host species. Unrelated and distantly related species may not be similarly affected by the same infectious microorganism due to differences in physiology, metabolism, biochemistry, and other factors. In general, the risk to a laboratory technician working with a virus that only infects and causes disease in rodents is lower than the risk to a laboratory technician working with tissues and cells from humans or other primates. If the human material contains a viable pathogen, it will likely be a human pathogen, with the potential to infect and cause disease in another human. Although a single

mode of transmission may predominate, disease-causing micro-organisms can be spread or transmitted from one host to the next, directly or indirectly, by a number of methods. Transmission methods include aerosol generation and inhalation, ingestion of contaminated food and water, skin and mucous membrane contact with contaminated surfaces, contact contamination of an open wound or lesion, autoinoculation via a cut, and laceration or puncture with a contaminated instrument.

4.5.1 Human Bloodborne Pathogens

Human blood is recognized as a potential source of pathogenic microorganisms that may present a risk to workers who are exposed during the performance of their duties. Although the hepatitis B virus (HBV) and the human immunodeficiency virus (HIV) are often cited as examples, a “bloodborne pathogen” is any pathogenic microorganism that is present in human blood or other potentially infectious materials and that can infect and cause disease in persons who are exposed to blood containing this pathogen. “Other potentially infectious materials” means materials that have the potential to transmit bloodborne pathogens. This includes infected human tissues and the following body fluids: semen, vaginal secretions, cerebrospinal fluid, synovial fluid, pleural fluid, peritoneal fluid, pericardial fluid, amniotic fluid, saliva in dental procedures, and any other body fluid that is visibly contaminated with blood.

In 1988, the Centers for Disease Control published a series of recommendations and precautions for the protection of workers who have, or are likely to have, contact with human blood and certain body fluids and may be at risk of exposure to bloodborne pathogens such as hepatitis B virus (HBV) and human immunodeficiency virus (HIV). These recommendations became known as “Universal Blood and Body Fluid Precautions” or simply, “Universal Precautions”.

4.5.2 Universal Blood and Body Fluid Precautions

The possibility of undiagnosed infection combined with the increasing prevalence of HBV and HIV led the Center for Disease Control (Atlanta, Georgia) to recommend that blood and other body fluids from all humans be considered potentially infectious and that precautions be taken to minimize the risk of exposure. This approach, called "Universal Precautions", is a method of infection control, intended to prevent parenteral, mucous membrane, and non-intact skin exposure of workers to bloodborne pathogens.

All human blood, human body fluids, and other materials are considered potentially infectious for hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus (HIV), and other bloodborne pathogens. Precautions must be consistently used. Body fluids to which universal precautions apply include blood, body fluids containing visible blood, semen, vaginal secretions, cerebrospinal fluid, synovial fluid, pleural fluid, peritoneal fluid, pericardial fluid, and amniotic fluid.

It is prudent to minimize non-intact skin and mucous membrane contact with these materials. Hepatitis B immunization is highly recommended as an adjunct to universal precautions for workers who have occupational exposure to human blood or other potentially infectious materials. Western University Workplace Health provides this immunization to employees at

risk, free of charge. OHSS at LHSC and St. Joseph's also offers Hepatitis B vaccinations (free of charge) to hospital employees.

Personal Protective Equipment

All workers must wear PPE appropriate to the hazards encountered. This includes at a minimum: Lawson-approved lab coat, long pants, and close-toe/heel shoes. PPE required may also include: gloves, safety glasses, safety goggles, surgical masks, respirators (e.g., N95), bonnets, shoe covers, and disposable gowns (see Section 4.2 for more information).

General Precautions

1. All workers should routinely use appropriate barrier precautions to prevent skin and mucous membrane exposure when contact with human blood or other body fluids is anticipated.
2. Eating, drinking, smoking, applying cosmetics or lip balm, and handling contact lenses are prohibited (S.32 of O.Reg. 67/93).
3. Gloves should be worn when touching blood and body fluids, mucous membranes, or non-intact skin, for handling items or surfaces soiled with blood or body fluids, and for performing venipuncture and other vascular access procedures. If a glove is torn or damaged during use, it should be removed and a new glove should be used as promptly as safety permits. Disposable gloves should not be washed or disinfected for reuse. Washing with surfactants may enhance penetration of liquids through undetected holes in the glove. Disinfecting agents may cause deterioration of the glove material.
4. Masks and protective eyewear or face shields should be worn during procedures that are likely to generate droplets of blood or other body fluids to prevent exposure of mucous membranes of the mouth, nose, and eyes.
5. Gowns or aprons should be worn during procedures that are likely to generate splashes of blood or other body fluids. Protective clothing should be removed before leaving the area.
6. Hands and other skin surfaces must be washed immediately and thoroughly if contaminated with blood or other body fluids. Hands should be washed immediately after gloves are removed since no barrier is 100% effective.
7. Workers should take precautions to prevent injuries caused by needles, scalpels, and other sharp instruments or devices during procedures, when cleaning used instruments, during disposal of used needles, and when handling sharp instruments after procedures. Where appropriate, the use of SEMDs is recommended. Needles and syringes should be used only in those situations when there is no alternative. To prevent needlestick injuries, needles should not be recapped, purposely bent or broken by hand, removed from disposable syringes, or otherwise manipulated by hand. After they are used, disposable syringes and needles, scalpel blades, and other sharp items should be placed in puncture-resistant containers for disposal. The puncture-resistant container should be located as close to the use area as practical. Contaminated reusable pointed and sharp objects such as large bore needles and scalpels should be placed in a puncture resistant container for transport to the reprocessing area.
8. Mouthpieces, resuscitation bags, or other ventilation devices should be available for use in areas in which the need for resuscitation is predictable.

9. Workers who have exudative lesions, weeping dermatitis, cuts, open wounds or other breaks in the skin should either refrain from all direct contact with blood and other body fluids until the condition resolves, or utilize protective barriers to reduce the risk of exposure.
10. Pregnant workers should be especially familiar with and strictly adhere to precautions to minimize the risk of prenatal transmission of bloodborne pathogens.

4.6 Medical Surveillance and Immunoprophylaxis

The basic purpose of a medical surveillance program is to help prevent and detect illnesses related to the exposure of personnel to infectious material or toxins. The focus of the program is primarily preventative, although it also includes a response plan through which a potential laboratory-acquired infection (LAI) can be identified, assessed, and treated before serious injury or disease occurs.

All Lawson laboratory personnel should be protected against LAIs by appropriate immunization with relevant, licensed vaccines unless they already have documented protective levels of pre-existing immunity.

Hepatitis B immunization is strongly recommended for all workers who routinely handle or have occupational exposure to human blood, body fluids, organs or tissues. Western University offers and provides hepatitis B immunization free of additional cost to at-risk Western employees through Workplace Health. OHSS at both LHSC and St. Joseph's offers Hepatitis B vaccinations (free of charge) to hospital employees. Vaccinations for tetanus and rabies are also available through the OHSS departments.

Post-exposure treatments are available for exposures to Hepatitis B/C, HIV, and Tuberculosis. Please contact OHSS as soon as possible after a possible exposure has occurred.

St. Joseph's Blood-Borne Pathogen (BBP) Exposures in Staff and Affiliates

St. Joseph's Post-Exposure Drug List

LHSC Post Blood / Body Fluid Exposure

Centers for Disease Control and Prevention - Exposure to Blood: What Healthcare Personnel Need to Know

4.7 Medical Procedures and Incident Reporting

The following emergency response procedures shall be followed when a worker has been potentially exposed to a biohazardous agent via a needlestick, cut, animal bite or scratch, via mucous membrane contact, or via non-intact skin contact.

Worker:

1. The exposed site must be washed immediately.

a) In case of a needlestick, cut, animal bite or scratch, wash with soap and water after allowing the wound to bleed freely. Do not squeeze the wound to encourage bleeding.
b) If mucous (eyes, nose, mouth) membrane or non-intact (cuts, rash, eczema or dermatitis) skin contact, flush with water at the nearest faucet or eye wash station for a minimum of ten minutes.

2. The worker must immediately inform the Supervisor/Principal Investigator of the exposure incident.
3. The worker must seek prompt medical attention at OHSS (during the hours of operation), the nearest hospital emergency department or an urgent care centre, or a Medical Practitioner of their choosing. Any information including the Material or Pathogen Safety Data Sheet or equivalent for the biohazardous agent must also be taken to the care provider.
4. The worker must provide information for an Accident/Incident Report (obtained from her/his Supervisor/Principal Investigator), describing the incident in detail, including the route of exposure and the emergency actions taken, and a description of the worker's duties as they relate to the exposure incident.

Supervisor / Principal Investigator:

1. Supervisors/Principal Investigators must complete and sign a workplace occurrence report (St. Joseph's) or an AEMS report (LHSC).
2. The supervisor must then ensure that exposure incidents are reported within 24 hours to Western University's Human Resources, fax (519) 661-2079, if Western staff or students are involved. The form can be found at:

http://uwo.ca/hr/form_doc/health_safety/form/aiir.pdf

3. The supervisor must refer the affected worker(s) to their site's OHSS department, the nearest hospital emergency department or an urgent care centre.

Occupational Health & Safety Services:

1. Occupational Health and Safety Services (OHSS) and the Lawson BSO, will investigate accidents/incidents as appropriate.
2. Accidents/incidents may be used as training tools for faculty, staff and students as long as confidential information is omitted.

Workplace Health (Western staff and students):

1. Workplace Health Services shall confer with the affected individual(s) and/or attending physician(s)/caregiver(s).
2. Counselling regarding potential exposure and infection, immunoprophylaxis and follow-up testing shall be offered to any worker if her/his exposure is determined to be of a nature that may transmit biohazardous agents.

Important Emergency Contact Numbers

Western University:

Workplace Health: ext. 82047
Campus Community Police Services: 911 from any campus phone or 519-661-3300 from a cellular or off-campus phone

St. Joseph's Health Care:

OHSS Department: ext.64332
Urgent Care Centre: ext.67021
Security (for after-hours emergencies): ext.44555

London Health Sciences Centre:

University Hospital OHSS Department: ext.33201
Victoria Hospital OHSS Department: ext.52286; after hours OHN pager #13522
Security: ext.44555

4.8 Spills

Emergency response plans required at Containment Levels 2 and 2+ must include procedures for dealing with spills or other laboratory incidents that could be expected to result in the release of biological agents. Since the capacity of most commonly used laboratory culture containers is small, it is anticipated that most spills within the laboratory will be limited in size and therefore, of a minor nature. Although the specific response will depend on the type and nature of the incident, decontamination and clean-up procedures incorporating the steps outlined below are recommended. If the spill is large or of a nature that cannot be handled by laboratory personnel, call security at ext.55555 and report a Code Brown.

Effective disinfectants must be available in the laboratory at all times and for immediate use. The recommendation is to have a universal spill kit present in each laboratory (to order spill kit: HMMS item #74747). These spill kits contain absorbent material, Virox disinfectant, and a red biohazard bag for a biohazard spill clean-up. In the event of a spill or container breakage resulting in the unintentional release of a biological agent that is *within your control* to clean up:

1. Evacuate personnel from the lab, and don appropriate PPE before attempting to clean the spill
2. Place paper towel or absorbent pads from the spill kit on the liquid
3. Flood the spill by carefully pouring a strong disinfectant solution (i.e. 10% bleach, Virox) around, but not on the spill, and mix the disinfectant with the spilled material cautiously, working from the outside of the spill to the inside;
4. Evacuate the laboratory for a time expected to be sufficient for decontamination of the mixed material, normally 20 minutes;
5. Carefully place paper into a biohazard bag for disposal; and
6. Decontaminate all surfaces exposed to the spill with the disinfectant.

If aerosols may have been created in the spill or unintentional release, evacuate the laboratory for a time sufficient for most aerosols to settle, be dispersed, or removed by the ventilation system, usually 20-30 minutes. The use of respiratory protection should be considered for re-entry. Then proceed with items (1)-(6) above.

For more information on how to effectively decontaminate certain biohazardous agents, please refer to the relevant Pathogen Safety Data Sheet(s), if applicable. For pathogens not in this list, 10% bleach (must be 0.5% hypochlorite by volume) or Virox will be sufficient.

During an emergency, the first priority is the protection of the health and safety of personnel, followed by the environment (i.e. sewer drains), followed by equipment or property.

4.9 Access/Security Controls

The CBS requires that the international biohazard warning symbol be displayed if any biohazardous materials (including body fluids, unfixed cell or organ cultures, viruses, bacteria, fungi, parasites, or toxins) that require containment at CL2 or higher are present. The containment level of the laboratory must also be indicated. Biohazard warning signage must also include the name and telephone numbers of a contact person, and entry requirements. The sign can be further supplemented with additional requirements for entry, a list of relevant processes and primary containment equipment used in large scale production areas, or information on other hazards (e.g., fire, chemical) present in the containment zone (see Figure 2).

Laboratory doors must be kept locked when the laboratory is unoccupied, and only authorized personnel are permitted to enter laboratory work areas. Any area outside of the containment zone where biohazardous materials are stored must have a biohazard warning sign and be kept locked at all times (e.g., a freezer in a common equipment room that contains risk group 2 pathogens).

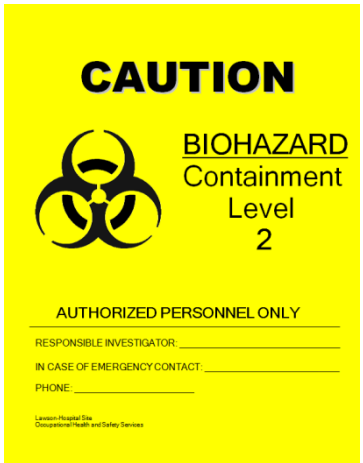


Figure 2 - Representative biohazard warning signage

4.10 Containment Level 2 Requirements

See Appendix C: Safe Work Practice - Containment Level 2 Labs / Tissue Culture Lab / BSC work

In addition to the general precautions listed in Section 4.3, the following list describes the minimum operational procedures required for Containment Level 2 (CL2) laboratories.

1. Biohazard signage (see Figure 2) must be posted on the entry doors to all Containment Level 2 rooms. The containment level of the laboratory must be posted on all points of entry.
2. Non-laboratory visitors to the CL2 laboratory area must be provided with guest lab coats that are to be worn at all times when in the containment zone.
3. Biological Safety Cabinets (BSCs) must be used for any procedures that may produce aerosols or that involve work with high concentrations and/or volumes of the biological agent. Animal handling and necropsies must be performed in a BSC as well.
4. All surfaces to be constructed from non-absorbent materials and able to be cleaned.
5. Any potential Dual-Use Agent (soon to be SSBAs) must be stored in a secured and locked area when an authorized worker is not present in the immediate area of the agent.
6. Lawson provides lab coats for all laboratory workers, and provides specific lab coats for working in a CL2 laboratory. These CL2 lab coats are to be worn only in level 2 containment areas and must be doffed before leaving the CL2 zone.

4.11 Containment Level 2+ Requirements

In addition to the CL2 requirements listed above, there are additional precautions that must be taken when working with certain biohazardous materials. The need for CL2+ will be determined based on a risk assessment performed by the Principal Investigator, the Lawson BSO, and by the Lawson Biohazards Sub-Committee. For example, all work with lentiviral vectors must be conducted in a CL2+ laboratory work area (see the Lawson safe work practice for working with lentivirus and lentivirus-based vectors).

1. All surfaces must be impervious to water.
2. Must have special ‘sharps precautions’ in place.
3. Autoclave must be available in the containment zone - this is required to decontaminate solid waste.
4. There must be an inward airflow into the containment zone; this must be checked prior to entry into the CL2+ area (using a smoke pencil, or a tissue held near the doorway).

5. Must have dedicated CL2+ PPE that remains in the containment zone. This includes: safety glasses, booties or dedicated footwear, an additional layer of protective clothing such as a solid-front level 2 gown with tight-fitting wrists (or a Tyvek suit), double nitrile gloves, and a fit-tested, NIOSH-approved N95 respirator to be worn when handling infectious materials outside of a BSC.
6. Infectious materials must be stored in a leak-proof container in a restricted area.
7. Where possible, all activities should be conducted in a BSC.
8. If using a centrifuge, closed containers with sealed safety cups must be used, or the rotor must be unloaded in the BSC. Must wait at least 5 minutes after centrifugation stops before opening the lid of the centrifuge.
9. Mark the floor (with tape) to indicate dirty and clean sides
10. Housekeeping and facilities maintenance workers must not enter the room unless accompanied by a qualified laboratory worker.
11. Only lab personnel trained in CL2+ requirements may enter the CL2+ area.
12. In the event of an emergency, remove all PPE if possible, upon exiting the lab. If this is not possible, remove as much PPE as possible; remove gloves if at all possible. Report the incident to the Lawson BSO as soon as possible.

REMEMBER: In an emergency, your safety is <u>more important</u> than maintaining biocontainment. If you must exit the containment zone to receive medical care, then do so.

Chapter 5: Biological Material

5.1 Classification of Biological Agents

Biological materials are pathogenic and non-pathogenic microorganisms, proteins, and nucleic acids, as well as any biological matter than may contain microorganisms, proteins, and nucleic acids (or any parts thereof). This includes, but is not limited to, bacteria, viruses, fungi, parasites, prions, toxins, genetically modified organisms, nucleic acids, tissue samples, live vaccines, and isolates of a pathogen.

Biohazardous materials are any biological materials that can pose a threat to the health of living organisms, primarily that of humans, or to the environment. The risk of biohazardous materials can be through direct infection or indirect through damage to the environment or economy. Biohazardous agents can be classified into the following groups:

- Microorganisms (bacteria, viruses, fungi, protozoa)
- Parasites
- Toxins (those produced by bacteria, animals or plants)
- Prions
- rDNA
- RNAi
- Animals
- Viral Vectors

5.2 Prions

Prions are proteinaceous infectious particles, which are abnormally folded isoforms of a normal cellular protein that are generally accepted to be the cause of a group of progressive neurodegenerative diseases in humans and animals known as Transmissible Spongiform Encephalopathies (TSEs). Examples of such disease are: Creutzfeldt Jakob Disease (CJD) in humans; Scrapie in sheep and goats; Bovine spongiform encephalopathy (BSE) in cows; and chronic wasting disease (CWD) in deer and elk.

Prions are highly resistant to destruction by chemical and physical procedures that would normally inactivate other infectious agents, including autoclaving.

When working with any neurological tissues (fixed or unfixed) there is a possibility that prion proteins could be present and appropriate precautions should be taken. This includes:

- Handling as RG 2 or higher (see PSDSs for more information);
- Handling formalin-fixed neurological tissues as infectious; and
- Follow the most up-to-date disinfection protocols available for these pathogens

5.3 Recombinant DNA and Interfering RNA

Recombinant DNA (rDNA) often involves inserting a gene from one organism into the genome of a different organism, generally of a different species. For the purposes of this manual, rDNA includes:

- DNA molecules that are produced outside of living cells by joining natural or synthetic DNA segments to DNA molecules capable of replication in living cells;
- DNA molecules that are produced in living cells by joining enriched or natural segments to intracellular DNA; and
- DNA molecules that are the result of the replication of such recombinant molecules

Guidance in assessing potential risks in recombinant DNA research can only be very general; each case requires individual assessment. It is unrealistic to define all of the genetically engineered organisms that might be created or used in the laboratory. The majority of this research involves only a very low possibility of creating a hazard because the source of the DNA being transferred, the vector and the host are all innocuous or have low risk characteristics. However, some genetic manipulation does raise a significant possibility of risk. Recombinant DNA can be a concern, because it raises the possibility of modifying a host or vector to impart new properties that were not considered in the original risk group classification. The use of rDNA requires a risk assessment based on:

- The effects of the gene(s) being transferred;
- Modifications to genes that are already present in the organism;
- Gene expression in the recombinant organism;
- The vector system used; and
- The consequences of the end product including possible virulence factors.

In addition to using rDNA to modify or add expression of certain genes, technologies exist to create the loss of gene expression in an organism. This is typically referred to as interfering RNA (RNAi). RNAi is used to knock-down expression of a cellular protein. Safety considerations for the use of RNAi include:

- Is the effect of the knocked-down protein local?
- Or is it disseminated (e.g., are you eliminating a protein that regulates growth control)?

In general, containment levels for activities involving rDNA or RNAi will be assigned according to the following criteria and considerations:

1. If none of the components of the genetic manipulation (DNA, vector, host) present any known hazard and none can be reasonably foreseen in their combination, then no restrictions beyond the requirements of Containment Level 1 are necessary.
2. If one of the components used in the procedure is hazardous, then, in general, determination of the containment level required will begin at the level appropriate to the known hazard. The level of containment may be increased or decreased depending on the particular gene transferred, the expression of the gene in the recombinant organism, the envisaged interactions between the transferred gene and the host-vector system, and other relevant factors.
3. In any activity involving genes coding for hazardous products, host-vector systems with limited ability to survive outside of the laboratory (affording biological containment) should be used. Their use may reduce the level of physical containment required.
4. The containment level may be reduced if it is known that the DNA or vectors are mutant and defective in their disease-causing or replication characteristics.
5. In the case of animal virus vectors, including retroviruses, one must consider the nature of the helper cells and the likelihood that replication-competent viruses may be produced.

5.4 Animals

Animals that are used in a research setting or those found in the field can pose numerous risks to personnel, including physical injury (from bites, scratches, or kicks), allergies and other adverse reactions, or zoonotic diseases. All work involving animals should be considered a biohazard risk since animals can harbour infectious organisms that can be transmitted to humans.

Any applicable Animal Care Facility (site-specific), Lawson and Western University protocols must be followed.

5.4.1 Animal Tissues

The biological hazards of animal cells, tissues, blood and body fluids arise from the possibility that they might contain or transmit infectious agents. It is prudent to consider all cell lines to be potentially infectious. Cells known or suspected to contain such agents, or primary cultures from animals and humans known or reasonably suspected to be infected, should be assigned to the risk group for the suspected agent.

Primate cell lines, all samples of human tissues and fluids, all primate tissues, and all cell lines new to the laboratory should be handled at Containment Level 2. When handling items such as human blood and body fluids, workers must be aware that these samples may contain pathogens such as influenza, HIV and hepatitis.

Factors such as the particular source of the material, the volume and concentration of the agent, the extent of culturing and incubation, the types of manipulations to be conducted, and the use of additional precautions could influence the containment level required.

5.4.2 Cultured Animal Cells

1) Primary cell cultures and animal tissues
The following containment requirements apply to primary cell cultures and tissues from human, non-human primate and non-primate animal sources when handled in the laboratory. Cells and tissues known or suspected to be contaminated or infected with biohazardous agents must be handled at the containment level appropriate to those agents.

2) Established cell lines
Human or other animal cell lines known to not be contaminated or infected with biohazardous agents may be handled at Containment Level 1. Cultures known or suspected to be contaminated or infected with any biohazardous agents must be handled at the containment level appropriate to those agents.

5.5 Blood and Body Fluids

The need for precautionary measures extends also to situations in which human blood, saliva, urine and other body fluids or feces must be handled. The precautions required may be more stringent when the specimens are used for culturing purposes, but initially, their handling should be consistent with CL 2. Reduction of the containment level may be acceptable if potential hazards associated with the material are expected to be diminished because of dilution, use of chemical or other treatments or additional protective measures and practices.

1) Culturing of specimens in research laboratory
Blood or blood fractions and other body fluid specimens of human or animal origin that are known or suspected to contain any biohazardous agents must be handled at the containment level appropriate to those agents when these specimens are cultured in volumes greater than that which is necessary for routine diagnostic work.

2) Clinical diagnostic work in laboratory
For clinical diagnostic work with specimens of human blood, serum and other body fluids (urine, cerebrospinal fluid, etc.) from the general population, CL 2 and Universal Precautions apply. For routine clinical diagnostic work with specimens that are known to be from infected individuals, the containment level appropriate to the agent must be maintained.

5.5.1 Fixed Tissues and Tissue Sections

Tissues and tissue sections from human and animal sources are routinely fixed by treatment with chemical agents, such as formaldehyde to preserve structures for later examination and study. Generally, these chemical treatments inhibit all biological activity. Most human blood and tissue specimens are exempt under PHAC's HPTR, as long as a pathogen is not directly extracted, manipulated, or cultured.

In general, fixed tissues and tissue specimens should be handled under at least CL 1 conditions. A higher level of containment may be required depending on the source of the material, the nature of the agent and whether or not it is inactivated. Contact the Lawson BSO

for information and clarification if needed. For completing a Lawson LBAPP form, the laboratory PI must provide documentation to the LBSC which supports a request for a lower level of containment.

5.6 Viral Vectors

There are inherent risks present when working with viral vectors. The following information applies when working with adenoviral and adeno-associated viral vectors, retroviral vectors and lentiviral vectors.

Viral vectors are usually designed to enter human or mammalian cells and deliver genes of interest (known as 'transduction'). These vectors are usually made to be replication-deficient which enhances the biosafety of using these vectors. However, there are still several biosafety concerns that may arise, including:

- Pathogenicity of the parent virus;
- Cytopathogenicity of the vector - effects on the host cells caused by the viral vector;
- Tropism (host range) - is the viral vector ecotropic (narrow host range) or made to be amphotropic (wide host range that can infect many different cell types);
- Reconstitution of Replication Competent Virus (RCV) - this is a rare event that occurs when the viral vector gains back the deleted genes required for replication through a process known as recombination;
- Requirements for specialized facilities - some viral vectors must be handled in a CL2 facility using CL3 practices; and
- Enhanced training requirements for personnel-must ensure that all personnel handling (or working in close proximity to) the viral vectors have specific biosafety training.

Work with viral vectors should be performed at a minimum of containment level 2. For work with certain viral vectors, use of containment level 3 practices may also need to be followed (known as CL2+). Any laboratory where viral vectors are handled must meet the requirements of the CBS, and in the case of lentivirus, must adhere to the Lawson Safe Work Practice for using Lentiviral Vectors (Appendix D). For additional information on working with viral vectors, please refer to the NIH document Biosafety Considerations for Research with Lentiviral Vectors.
General Laboratory Practices for Working with Viral Vectors:

1. Laboratory doors must remain closed.
2. All handling of viral vectors must be performed in a certified BSC.
3. When centrifuging liquids containing viral vectors/virus, safety-sealed rotor cups should be used, or the entire rotor must have a sealed lid.
4. For aspiration of virus-containing liquid, use a double vacuum flask trap with a collector flask and an overflow flask. There must also be an in-line HEPA filter placed between the overflow flask and the vacuum line. The tubing must be non-collapsible and capable of withstanding disinfection. (Figure 4).
5. Lawson's Biological Safety Cabinet Safe Work Practice must be followed (Appendix A).

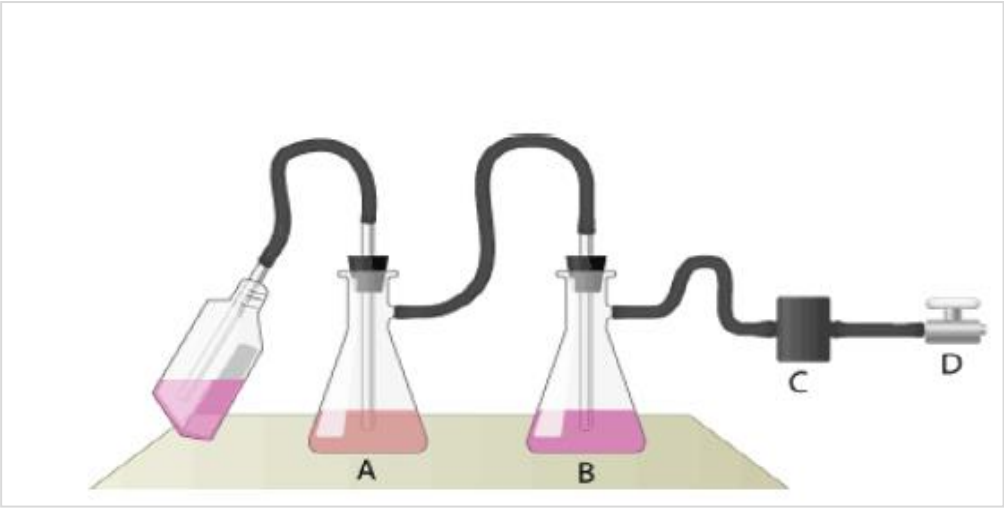


Figure 4 - Correct vacuum system set-up. A: collection flask; B: overflow flask; C: in-line HEPA filter; D: vacuum line. (image courtesy of Dalhousie University).

Chapter 6: Biological Agent Risk Groups and Containment Levels

6.1 Risk Factors

Many of the biological agents used in research laboratories are pathogenic to humans, animals, or plants. The use of these agents poses a risk, which is dependent on the agent, how it is manipulated or altered, and how it is used. A useful tool available for performing risk assessments on pathogens is the Public Health Agency of Canada's Pathogen Safety Data Sheets.

Referencing a known pathogen's risk group may not be sufficient, and many other factors can influence the precautions needed to work with a given biological agent.
The factors used to determine into which risk group an organism falls is based upon the particular characteristics of the organism, such as:

- Pathogenicity/Virulence - does the pathogen infect and cause disease in animals or humans? What is the severity of the disease in individuals?
- Infectious dose - how much of the pathogen is required to cause an infection in the host (number of organisms required to cause infection)?
- Mode of transmission (host range) - how does the pathogen travel to the host (e.g., direct or indirect contact, aerosols, airborne, or vectors);
- Host Range - what are the hosts of the pathogen? Can the pathogen cause infection in many species or only a few?
- Transmission/Communicability - how can the pathogen be transmitted from person to person, animal to animal, human to animal, or animal to human (e.g., direct or indirect contact, airborne)?
- Stability - how long does the pathogen survive outside of a host (i.e. survival on fomites)?
- Incubation Period - how long between the infection of an individual by a pathogen and the manifestation of symptoms of disease?
- Availability of effective preventive measures (availability of effective treatment).

6.2 Risk Groups

Classification of organisms according to risk group has traditionally been used to categorize the relative hazards of infective organisms. These classifications presume ordinary circumstances in the research laboratory or growth in small volumes for diagnostic and experimental purposes. Four levels of risk have been defined by the Public Health Agency of Canada as follows.

6.2.1 Risk Group 1 (low individual and community risk)

Any biological agent that is a) not capable of causing human or animal disease; or b) unlikely to cause disease in healthy workers or animals. Risk Group 1 agents also pose a low risk to public health, livestock, or poultry. RG1 Pathogens can be opportunistic and may pose a threat to immunocompromised individuals. Many biohazardous materials used at Lawson fall

into this category, such as *Escherichia coli* DH5α competent bacteria which are widely used in molecular biology experiments.

6.2.2 Risk Group 2 (moderate individual risk, low community risk)

Any pathogen that can cause human disease but under normal circumstances is unlikely to be a serious hazard to laboratory workers, the community, livestock or the environment. Laboratory exposures rarely cause infection leading to serious disease; effective treatment and preventive measures are available, and the risk of spread is limited. Risk Group 2 agents include most viral vectors and certain cell lines (e.g., HeLa cells, HEK293 cells). More examples of RG2 human pathogens can be found in Schedule 2 of the HPTA.

6.2.3 Risk Group 3 (high individual risk, low community risk)

Any pathogen that usually causes serious human disease or can result in serious economic consequences but does not ordinarily spread by casual contact from one individual to another, or that causes diseases treatable by antimicrobial or antiparasitic agents. Depending on the pathogen, the risk of spread to livestock or poultry can range from low to high. Risk Group 3 agents include certain viral vectors, bacteria such as *Bacillus anthracis* and *Mycobacterium tuberculosis*, and the Creutzfeldt-Jakob prion. More examples of RG3 pathogens can be found in Schedule 3 of the HPTA.

6.2.4 Risk Group 4 (high individual risk, high community risk)

Any pathogen that usually produces very serious human disease that is often untreatable, and may be readily transmitted from one individual to another, or from animal to human (or vice-versa), directly or indirectly, or by casual contact. Depending on the pathogen, the risk of spread to livestock or poultry can range from low to high. Risk Group 4 agents include Ebola Virus and Lassa Virus. More examples of RG4 pathogens can be found in Schedule 4 of the HPTA.

NOTE: There are no RG3 or RG4 pathogens currently approved for use in any Lawson labs; any shipments including such agents must not be accepted. Contact the Lawson BSO if you anticipate or contemplate their use, or if you accidently receive a sample that contains a RG3/4 pathogen. Any experiment that results in a gain-of-function (increase in Risk Group) of a pathogen must be reported to the BSO immediately.

As a general precaution, agents should be elevated to the next risk group when manipulation may result in the production of infectious droplets and aerosols.

6.3 Containment of Biological Hazards

Bacteria, viruses, fungi and parasites are used in a variety of laboratory settings, in many cases because of their significance as etiological agents, but also because a better understanding of their nature is important to many areas of biology. In addition, there is growing interest in the

use of this information and the agents themselves in industrial applications. Hazards may not always be readily apparent.

Risks posed by biological agents and other potentially pathogenic materials will vary with the agent or material, and the circumstances under which it is used. Risks can be minimized to acceptable levels by controlling or reducing the hazards, but they may not be entirely eliminated. Some laboratory procedures and processes are more likely than others to contribute to the dissemination of hazardous agents. Among factors that can contribute to the risk involved, the following are generally viewed as particularly significant.

6.3.1 Aerosols

Because of their insidious nature, aerosols pose special problems in that the laboratory worker may be unwittingly exposed to the material handled. Procedures which can produce aerosols include grinding, blending, sonicating, resuspending packed cells or viruses, inserting a hot loop into a culture, centrifugation, flaming an inoculation loop so that the material sputters, forceful ejection of fluid from a pipette or syringe, and opening a tube within which the air pressure may differ from that of the room. This may occur when the tube is opened at a temperature different from that at which it was sealed. Formation and dispersal of aerosols can be controlled by the use of proper techniques or special equipment.

For example, both screw-capped safety cups and sealed centrifuge heads permit use of a centrifuge in an open laboratory with minimum risk of aerosol dispersal, provided that the cup or head is opened inside a suitable biological safety cabinet. However, while the use of available safety devices is recommended, their use is not a substitute for good technique. Once formed, aerosols can be captured by HEPA filters or removed from the laboratory by local and room ventilation methods. A BSC provides some operator protection against airborne materials, including aerosols.

6.3.2 Large Volumes and High Concentrations

The risks to laboratory personnel or the environment may increase as the volume or concentration of the biological agent increases. The procedures described in this manual relate primarily to small scales of operation normally encountered in research laboratories.

6.3.2 Effluents and Waste

Effluents are a major potential means for dissemination of agents to the environment outside of the laboratory. These include air exhausted or escaping to the outside, liquid and solid wastes, and contaminated glassware.

Air

The purpose of an air exhaust system is to remove contaminated air from a work area, to convey it through a decontaminating system if necessary, and to discharge it to the outside. Its design should provide adequate air exchanges, a negative pressure differential between the room and the air source to ensure that contaminated air departs only through the exhaust

system, and airflow patterns through the room so that all parts of the room are swept by the airflow. The influence of opening and closing doors on these airflow patterns is of particular importance.

Decontamination of air is best achieved with a HEPA filter. HEPA filters are ineffective unless properly installed. Testing of these filters *in situ* with an aerosol at the time of installation and at regular intervals is essential to ensure the integrity of the barrier. Normally, HEPA filters will require replacement only when they offer excessive resistance to airflow due to loading or when irreparable leaks are detected. Vacuum lines also serve as a conduit through which air may leave the laboratory and must also be protected.

Liquids

Some liquid wastes, particularly those in which agents have been cultured, will require sterilization or disinfection to inactivate the agent before disposal to the sewage system. Hazardous chemical and radioactive liquid wastes may require an additional procedure to inactivate viable biological agents before removal from the laboratory. It is dangerous and illegal to dispose of hazardous chemicals and radioactive materials into drains and the sewage system. Autoclaving (steam sterilization) is generally the best method of inactivating biological agents and should be used whenever possible.

Liquid waste containers designed to withstand autoclaving temperatures must be used. Containers of liquid waste must be placed into a tray or pan of sufficient capacity to contain all liquid in the event of vessel failure or breakage inside the autoclave chamber. Although some chemical disinfectants can be used for the inactivation of many biological agents, others may be less effective against particular microorganisms, or may be suitable only for some of the types of disinfection required in the laboratory (disinfection of work surfaces or instruments, clean up after spills or accidents, and disinfection of liquid wastes). Before adoption, it is recommended that a disinfectant be tested against the biological agent to determine the concentration and contact time required to achieve the objective under the conditions employed.

Solids

Reusable items such as glassware should be sterilized by autoclaving whenever possible. Otherwise, a specific chemical disinfection procedure, proven to be effective against the particular biological agent, must be used.

Disposable items which are contaminated with biological agents only should be disposed of in biohazard bins, which are removed for disposal by Stericycle.

Disposable sharp waste must be carefully collected in a puncture-resistant waste container and put out for waste pick-up by sealing the sharps container lid properly and placing in a biohazard bin. Intact and broken glassware for disposal must be collected in puncture-resistant containers and properly labelled.

Disposable non-sharp items (gloves, empty plastic culture dishes, flasks and tubes, absorbent tissue, etc.) which are contaminated with biological agents must be collected in autoclavable biohazard bags and placed into biohazard bins.

Hazardous chemicals and radioactive solid wastes have unique procedures to inactivate viable biological agents which may be present before removal from the laboratory. Autoclaving is generally not recommended in all situations involving such wastes, since the high temperature, steam and pressure may contribute to potentially hazardous reactions. It is dangerous and illegal to dispose of hazardous chemicals and radioactive materials in the regular garbage going to landfill.

6.3.3 Pipetting

Mouth (oral) pipetting is prohibited in any laboratory. Using commercially available pipetting devices can reduce pipetting accidents. However, delivery of fluids should be slow, as forceful ejection produces bubbles and spraying which can generate an aerosol. Pipettes, especially glass, must be inserted into pipetting devices carefully and without excessive force, to avoid breakage and potential injuries. Using filtered pipette tips is recommended when pipetting liquids that contain biohazardous agents. This will help to prevent the contamination of the pipettor and help to reduce aerosol formation.

6.4 Biosafety Containment Levels

Four levels of containment (1 - 4), appropriate to the four risk groups for potentially hazardous biological agents, are defined. These levels of containment are regarded as adequate for most laboratory uses of the listed agents. It remains the responsibility of the Principal Investigator and Lawson to require a higher level of containment for specific manipulations, if these appreciably increase the possibility of infection. Containment Level Two laboratories are inspected at least annually by the Lawson BSO.

Classification of organisms according to risk group is not meant to establish the actual handling of biological hazards in the laboratory setting. For example, the risk group system does not take into account the procedures that are to be employed during the manipulation of a particular organism. Containment levels are selected to provide the end-user with a description of the minimum containment required for handling the organism safely in a laboratory setting.

In addition to the inherent characteristics of each organism as described in section 6.2, the containment system includes the engineering, operational, technical and physical requirements for manipulating a particular pathogen. These containment levels are applicable to facilities such as diagnostic, research, clinical, teaching and production facilities that are working at a laboratory scale. Requirements for containment levels 1, 2 and 2+ are described as follows.

6.4.1 Containment Level 1 (CL1)

This applies to the basic laboratory that handles agents requiring containment level 1. CL1 requires no special design features beyond those suitable for a well-designed and functional laboratory. Biological safety cabinets (BSCs) are not required. Work may be done on an open

bench top, and containment is achieved through the use of practices normally employed in a basic microbiology laboratory.

The legislation administered by the PHAC and the CFIA does not apply to RG1 human and animal pathogens. The CBS does not specify the requirements for activities with RG1 material, however universal precautions and good microbiological laboratory practices should still be followed since RG1 biological material does pose a low risk to the health of individuals or animals.

Physical Containment Recommendations

1. Separated from public areas by door.
2. Size of door openings to allow passage of all anticipated equipment.
3. Surfaces to be scratch, stain, moisture, chemical and heat resistant in accordance with laboratory function (recommended).
4. Surfaces to provide impact resistance in accordance with laboratory function (recommended).
5. Interior coatings to be gas and chemical resistant in accordance with laboratory function (e.g., will withstand chemical disinfection, fumigation) (recommended).
6. Bench tops to have no open seams (recommended).
7. Bench tops to contain spills of materials (e.g., with marine edges and drip stops) (recommended).
8. Benches, doors, drawers, door handles, etc. to have rounded rims and corners (recommended).
9. Backsplashes, if installed tight to wall, to be sealed at wall-bench junction (recommended).
10. Reagent shelving to be equipped with lip edges (recommended).
11. Drawers to be equipped with catches, i.e., to prevent the drawer from being pulled out of the cabinet (recommended).
12. Cabinet doors not to be self-closing (recommended).
13. Autoclave or other acceptable means of waste treatment/disposal to be provided (recommended).
14. Windows, if they can be opened, to be protected by fly screens.
15. Hooks to be provided for laboratory coats at laboratory exit; street and laboratory clothing areas to be separated.
16. Hand washing sinks to be located near the point of exit from the laboratory or in anteroom.

Operational Recommendations

Basic laboratory safety practices must be followed. Where chemical disinfection procedures are employed, effective concentrations and contact times must be used. Chemical disinfectants used to decontaminate materials to be removed from the laboratory must be replaced regularly.

6.4.2 Containment Level 2 (CL2)

Containment Level 2 is suitable for work with agents in Risk Group 1 or 2. The primary exposure hazards associated with organisms requiring CL2 are through the ingestion, inoculation and mucous membrane route. Agents requiring CL2 facilities are not generally transmitted by airborne routes, but care must be taken to avoid the generation of aerosols (aerosols can settle on bench tops and become an ingestion hazard through contamination of the hands) or splashes. Primary containment devices such as BSCs and centrifuges with sealed rotors or safety cups are to be used as well as appropriate personal protective equipment (i.e., gloves, laboratory coats, protective eyewear). As well, environmental contamination must be minimized by the use of hand-washing sinks and decontamination facilities (autoclaves). The legislation administered by the PHAC and the CFIA does apply to CL2 facilities as outlined below.

Physical Containment Requirements

1. Access limited to authorized personnel.
2. Laboratory room doors to have appropriate signage (e.g., biohazard sign, containment level, contact information, entry requirements).
3. Where unique hazards exist, project-specific signage to be posted at animal room, animal cubicle, and post mortem room points of entry.
4. Doors to the containment laboratory are lockable (this does not apply to areas within the containment laboratory).
5. Office areas to be located outside of the containment laboratory. Paperwork stations for data collection can be within the containment laboratory provided they are located away from laboratory work areas.
6. Doors, frames, casework and bench tops are to be non-absorptive (i.e. the use of organic materials should be avoided).
7. Working surfaces of bench-tops are to be non-absorptive.
8. Surfaces and interior coatings (including walls, ceilings, floors, furniture, and benchtops) to be cleanable, non-absorbent, and resistant to scratches, stains, moisture, chemicals, heat, impact, repeated decontamination, and high pressure washing.
9. Floors must be slip-resistant in accordance with function.
10. Autoclave or other acceptable means of waste treatment/disposal is to be provided.
11. Windows, if they can be opened, are to be protected by fly screens.
12. Hooks are to be provided for laboratory coats at laboratory exit; street and laboratory clothing areas are to be separated.
13. Hand-washing sinks are to be located near the point of exit from the laboratory or in anteroom.
14. Hand-washing sinks are to be provided with "hands-free" capability (recommended).
15. Decontamination technologies to be provided within the containment zone or SOPs must be in place to transport waste out of the containment zone to a designated decontamination area.
16. Decontamination technologies to be provided with monitoring and recording devices that capture operational parameters.
17. An autoclave must be capable of operating at the appropriate temperature for

decontamination, as determined by validation.
18. Emergency eyewash and shower equipment to be provided in accordance with activities.
19. Biological Safety Cabinets (BSCs) and other primary containment devices are to be provided. Examples for use include procedures with the potential for producing aerosols and those involving high concentrations, large volumes or particular types of agents.
20. Class II B2 BSCs (where present) must be set-up to eliminate reversal of airflow from the face of the BSC (i.e., puff-back) during a failure of the HVAC system or BSC exhaust fan; if not possible, the risk associated with puff-back to be mitigated through physical and operational means.
21. BSCs must be located as far as possible from high traffic areas, doors, openable windows, and air supply/exhaust diffusers.
22. Process equipment, closed systems, and other primary containment devices must be designed to prevent the release of infectious materials/toxins.
23. Vacuum systems must be equipped with a mechanism to prevent internal contamination.
24. Two-way communication systems must be provided inside the containment zone to allow communication between the inside of the containment barrier to the outside of the containment zone, in accordance with function.

Operational Requirements

In addition to the general practices required for all laboratories handling infectious substances, the following describe the minimum operational practices required for containment level 2, as outlined in the CBS.

1. A biosafety program must be in place for the oversight of safety and containment practices.
2. A biosafety representative (i.e., designated BSO) must be designated for the oversight of biosafety and biosecurity practices.
3. Contact information provided to the PHAC and the CFIA, as applicable, to be kept up-to-date.
4. Program intent must be documented and kept up-to-date.
5. An overarching risk assessment must be conducted and documented to identify the hazards present and appropriate mitigation strategies for the proposed activities involving biohazards.
6. A biosecurity risk assessment must be conducted and documented.
7. A biosecurity plan based on point 6, must be developed, implemented, evaluated and improved as necessary, and kept up-to-date.
8. A local risk assessment (LRA) must be conducted to examine each task involving biohazards to ensure the risks are identified and safe work practices are developed and documented.
9. A training needs assessment must be conducted.
10. A biosafety manual must be developed, implemented, kept up-to-date, made available to all appropriate personnel, and contain institutional biosafety policies, programs, and plans, based on points 5-8 above.

11. A medical surveillance program must be developed, implemented, and kept up-to-date, based on points 5-8 above.
12. Emergency medical contact cards to be issued to personnel handling non-human primates or a biohazard identified in a LRA.
13. A respiratory protection program (i.e. fit-testing) to be in place where respirators are used.
14. A training program based on points 5-8 above, to be implemented, evaluated, and improved as necessary, and kept up-to-date.
15. SOPs specific to the work being conducted in the containment zone to be developed and documented (including PPE, entry/exit procedures, containment equipment use, decontamination, transportation).
16. An ERP, based on points 5-8 above, to be developed, implemented, and kept up-to-date.
17. Containment zone personnel must immediately notify appropriate personnel if an incident occurs that could result in an exposure to a biohazard.
18. Good microbiological laboratory practices intended to avoid the release of infectious agents are to be employed.
19. BSCs must be used for procedures that may produce infectious aerosols and that involve high concentrations or large volumes of biohazardous material. Laboratory supervisors, in consultation with the Biological Safety Officer/Institutional Biosafety Committee, should perform a risk assessment to determine which procedures and what concentrations and volumes necessitate the use of a BSC.
20. Appropriate signage indicating the nature of the hazard being used (e.g., biohazard sign, containment level) must be posted outside each laboratory; if infectious agents used in the laboratory require special provisions for entry, the relevant information must be included on the sign; the contact information of the laboratory supervisor or other responsible person(s) must also be listed.
21. Entry must be restricted to laboratory staff, animal handlers, maintenance staff and others on official business.
22. All people working in the containment area must be trained in and follow the operational protocols for the project in process. Trainees must be accompanied by a trained staff member. Visitors, maintenance staff, janitorial staff and others, as deemed appropriate, must also be provided with training and/or supervision commensurate with their anticipated activities in the containment area.
23. Emergency procedures for spill clean-up, BSC failure, fire, animal escape and other emergencies must be written, easily accessible and followed. A record must be made of other people entering the facility during an emergency.

6.4.3 Additional Requirements for Containment Level 2 Plus Work

Some work involving RG 2 agents must be done in CL2 facilities using some CL3 practices. A Lawson Biosafety Officer will help to perform a risk assessment on biohazardous material that may require this containment level. The follow measures, in addition to CL2 requirements, are to be followed.

1. There must be a program in place (with appropriate authority to oversee safety and containment practices) for the management of biological safety issues.

2. General operational protocols must be supplemented with protocols similar to each project in progress.
3. Personnel must have demonstrated proficiency in microbiological practices and techniques.
4. Infectious agents should be stored inside the containment laboratory; agents stored outside of the containment laboratory must be in leak proof containers in a restricted area; emergency response procedures must take into account the existence of infectious agents that are stored outside of the containment area.
5. Personnel entering the containment laboratory must remove street clothing and change into dedicated laboratory clothing and shoes. Dedicated laboratory clothing and shoes must be removed before leaving the containment laboratory in a manner that minimizes any contamination of the skin with the potentially contaminated dedicated laboratory clothing. The use of full coverage protective clothing (i.e., completely covering all street clothing) is an acceptable alternative. When a known or suspected exposure may have occurred, all clothing, including street clothing, requires appropriate decontamination.
6. If an additional layer of protective clothing (e.g. solid-front gowns with tight fitting wrists, gloves, respiratory protection) is worn over laboratory clothing when handling infectious materials, it should be removed after completion of work (e.g. dedicated for use at the BSC).
7. Centrifugation of infectious materials must be carried out in closed containers placed in sealed safety cups or rotors that are unloaded in a BSC.
8. It is recommended that all activities with infectious materials are conducted in a BSC. If this is not possible, other primary containment devices in combination with personal protective clothing and equipment must be used.
9. In the event of an emergency, exit protocols must be established whereby routine procedures might be bypassed; a reporting area must be identified where further steps must be taken (e.g. disinfecting footwear, changing, showering).

6.5 Animal Biohazard Containment Facilities

Due to their unpredictable behaviour, in vivo work with pathogens involving live animals increases the risk associated with any given procedure. In addition, large volumes of contaminated waste can be generated in animal containment zones. Laboratory facilities must provide containment for laboratory animals exposed to or harbouring infectious agents that is appropriate to the risk level of the infectious agents involved. In addition to the physical requirements identified in Section 6.4, special equipment (e.g., filter cages, isolation caging systems) appropriate to the animal species as well as to the level of risk must be used. Operational procedures for the care and maintenance of the infected animals must satisfy the Guidelines for the Care and Use of Experimental Animals of the Canadian Council on Animal Care and the Western University Animal Care Committee. This ensures not only protection for laboratory personnel and the environment, but to ensure that every care is taken to avoid causing the animals' unnecessary pain or suffering and to provide the animals with the highest quality care.

6.5.1 Animal Escape

Rodents kept in micro isolators rarely escape from the biological safety cabinets. If they do escape, they can easily be corralled into a corner. Use the proper personal protective equipment and retrieve the animal with tongs or other suitable equipment.

References

(1) Canadian Biosafety Standards, 2nd Edition, 2015
(2) Canadian Biosafety Handbook, 1st Edition, 2015
(3) University of Western Ontario Biosafety Manual, 2015
(4) American Biological Safety Association: Principles and Practices of Biosafety, 2015
(5) Public Health Agency of Canada website, 2015

Appendix A: Lawson Biological Safety Cabinet Safe Work Practice

1. PURPOSE/background

This SWP will provide details and instruction on the safe operation of biological safety cabinets (BSC) in Lawson laboratories. Proper containment of biological materials will also be detailed.

2. SCOPE

BSCs provide effective primary containment for work involving potentially infectious materials or toxins, when they are properly maintained and used correctly. There are various types and classes of BSCs that all operate under the same principle: a continuous laminar flow of inward air prevents aerosols from escaping through the front opening of the cabinet. The air is exhausted either back into the containment zone or into the environment after passing through a HEPA filter. Some cabinets also protect the experimental product by pushing HEPA-filtered air into the working space of the cabinet, which flushes out contaminated air and prevents unfiltered air from entering the workspace.

The most common type of BSC used in Lawson labs is the Class II, type A2 cabinet. These cabinets protect personnel, the environment, and the experimental product. These BSCs are primarily used for sterile manipulations of cell cultures. The culturing/handling of all risk group 2 (RG2) and greater cell lines must be performed in a certified BSC, in a CL2 laboratory.

3. RESPONSIBILITIES

This SWP applies to all trainees, staff, and researchers who use a BSC for any experimental work. The Principal Investigator (PI) is responsible for ensuring all personnel under their supervision are properly trained in the safe and effective use of BSCs. Personnel should also have a basic understanding of the different classes/types of BSCs and how they operate.

Lawson ensures the BSCs are re-certified annually to NSF/ANSI STD.49, by H.E.P.A. Filter Services Inc. If the BSC you are using has not been certified in the past year, please contact Lawson's Facility Manager, Paul Coakwell, and do not use the BSC.

If you purchase or acquire a new BSC, or move an existing BSC, it must be certified before it can be used. If the acquired BSC is coming from another facility, it must be professionally decontaminated before it can be moved. Please contact your site's Biosafety Officer/Safety Analyst if you are planning to move a BSC to or from your facility.

4. DEFINITIONS

BSC: biological safety cabinet
HEPA: High Efficiency Particulate Air
UV: ultraviolet
CL2: containment level 2 (biohazard containment level)
RG2: risk group 2
CBS: Canadian Biosafety Standard

5. PROCEDURE

Start-up Procedure:

1) Turn off UV lights[1] if in use, and ensure that the sash is opened to the proper height (some BSCs indicate the correct height and have an alarm if the sash is opened too far)
2) Turn on the fluorescent light and the cabinet blower, if it is off. Most modern blowers will turn on when the sash is lifted, but you must double-check this.
3) Confirm inward airflow by holding a tissue under the open sash and ensuring that it is being drawn in.
4) Disinfect the interior surfaces with a suitable disinfectant[2]
5) Load all required materials and equipment into the cabinet, taking care not to obstruct the air grilles. Place any aerosol-generating equipment (vortex, centrifuge) towards the back of the cabinet, without blocking the air grilles. This is a good time to properly prepare your work space, including segregating “clean” from “contaminated” areas/items.
6) Wait 5 minutes to allow airborne contaminants to be purged from the working area.

Working in the BSC:

1) Begin by donning the appropriate PPE. It is recommended that no skin be exposed inside the cabinet, as any sloughed skin can contaminate your materials. Note that any jewellery that can interfere with the PPE (rings, watches, bracelets) must not be worn inside the BSC.
2) Seat yourself so that your underarms are at the same height as the bottom of the open sash window. Perform all procedures as far to the rear of the work area as is possible.
3) Avoid movement of materials or excessive movement of hands and arms through the front sash during use. Excessive movement disrupts the laminar air flow and can compromise the sterility of the work area. If you need to move your arms in and out of the cabinet, try to do so from straight on, and wait a few seconds to allow the air flow to stabilize before resuming work.
4) Keep discarded, contaminated material to the rear of the cabinet; do not discard materials in containers outside of the cabinet. If using a liquid waste flask/container, try to keep it inside the cabinet. If you must store the waste outside the cabinet, place it on the floor in a secondary container. For work with

RG2 materials, the waste flask must be double-trapped and have a hydrophobic filter placed between the overflow flask and the vacuum port.

5) Do not work with open flames inside the cabinet[3].
6) If a spill occurs, decontaminate the surfaces of all objects in the cabinet with an appropriate disinfectant while the cabinet is still in operation (do not turn the cabinet off while cleaning).

Completion of 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 disinfect any objects that were in contact with contaminated material before removal from the cabinet.
4) Remove contaminated gloves, and dispose of as is appropriate; wash hands.
5) Don clean gloves and place all contaminated materials into biohazard bags within the cabinet. Place biohazard waste in appropriate waste container.
6) Use a suitable disinfectant (e.g., 70% ethanol, 10% bleach, Virox) to disinfect the interior surfaces of the cabinet[4].
7) Turn off the fluorescent light and cabinet blower when appropriate (some cabinet blowers are left on at all times, or there may be someone who is using the cabinet next).
8) Turn on the UV light if appropriate. Do not turn the UV light on if people are working close by. It is recommended that you place a 'UV in use' warning sign near the entrance to the BSC-containing room.

Notes:

[1] The use of UV lights in a BSC must be carefully considered, and is in fact, strongly discouraged. UV germicidal lamps have very limited effectiveness as a surface disinfectant inside the cabinet. UV irradiation will not penetrate dust or dirt, so the lamp must be wiped down before each use. If any microorganisms are covered in dirt/dust/organic matter then the UV will be ineffective. UV irradiation does not penetrate into cracks or into the grilles of the cabinet. UV irradiation can cause the deterioration of certain materials over time (including certain plastics and tubing).

For a UV lamp to be effective, it must be routinely tested with a UV metre to ensure the proper intensity is being emitted from the bulb, at an appropriate wavelength, into the centre of the cabinet working area.

[2] Be sure to choose an appropriate disinfectant for the materials being used. Not all disinfectants are effective against all organisms. 70% ethanol is a good choice and will be effective most of the time. If using a corrosive disinfectant (such as bleach), wipe down the surface with 70% ethanol after disinfection (corrosives can damage the stainless steel surface in the cabinet). An accelerated hydrogen peroxide product, such as Virox is highly effective as a disinfectant against most pathogens and is an excellent surface disinfectant.

[3] Open flames in the BSC create air turbulence which disrupts laminar airflow patterns and can also damage the HEPA filter. Sustained open flames in BSCs are prohibited, and on-demand open flames are to be avoided (CBS R4.6.28). Touch-plate micro-burners that have a pilot light to provide an on-demand flame may be used if there are no other alternatives.

Alcohol burners may be used, with caution, inside the cabinet, but the volume of alcohol must be kept to a minimum, and should be contained in a metal (not glass) container. Alternatives should be considered, including disposable Pasteur pipettes and bacterial loops.

[4] The BSC should undergo a periodic thorough cleaning. This may include cleaning underneath the cabinet surface (including the catch pan), and wiping down the exterior surfaces of the BSC. Never put your head inside the BSC when cleaning it!

6. REFERENCES

Canadian Biosafety Handbook, 1st edition, 2015

Canadian Biosafety Standard, 2st edition, 2015

The Center for Food Security & Public Health website: a resource for disinfectant selection: http://www.cfsph.iastate.edu/Disinfection/index.php

http://www.bakerco.com/introduction-biological-safety-cabinets

Appendix B: Lawson Autoclave Safe Work Practice

1. PURPOSE/background

This SWP is intended to instruct staff and trainees on the detailed safe and proper use of both institutional autoclaves. There are simplified SWPs for each autoclave attached to this document for quick reference.

2. SCOPE

This SWP covers safe usage of autoclaves. These autoclaves use high pressure-saturated steam (heated to either 121 or 132^{o}C) to sterilize liquids and dry materials. This document also covers proper usage of autoclaves to achieve complete sterilization of samples/instruments/consumables.

3. RESPONSIBILITIES

This SWP applies to all trainees, staff, and researchers who use the institutional autoclaves.

4. DEFINITIONS

PPE = personal protective equipment
PP = polypropylene
PS = polystyrene
PC = polycarbonate
PE = polyethylene

5. PROCEDURE

5.1. Don appropriate PPE (lab coat, protective eyewear, apron, heat-resistant gloves, and closed-toe shoes). If autoclave door is closed (it should always be left with the door closed), press foot pedal to open door. Take a few steps back while door is opening, allowing steam to dissipate, while minimizing contact with steam vapour.

WARNING: BURN HAZARD: The sterilizer, racks, and door may be hot-use care when touching these items

5.2. Remove lower rack in autoclave. Remove the drain strainer (at the bottom front of the chamber, just inside the door) and ensure it is free of any debris (if the strainer is clogged, the cycle will not run properly).

5.3. Replace drain strainer and lower rack, then load your items into the autoclave (see reference section for more information on proper loading). DO NOT operate autoclave without the drain strainer in place!!!

5.4. Select the appropriate cycle type from the on-screen menu: Liquid or dry goods? (See reference section for more information on items that can be autoclave sterilized). Liquids must be run using a liquid cycle, which allows for slow-venting and prevents boiling of liquids. Do not process dry goods with liquids.

- For liquids: ensure any lids/caps on liquid containers are loosened. Place all liquid containers on a heat-resistant tray
- For solids: do not over-fill autoclave, and do not stack containers. This will prevent steam from penetrating all items which will result in poor sterilization

5.5. Close autoclave door and press the cycle start button. An approximate time remaining will be displayed.

- Once cycle is complete, press foot pedal to open door (follow procedure in 5.1). Carefully remove items from autoclave: WARNING: BURN HAZARD: The sterilizer, racks, and door will be hot-use care when touching these items
- Be extra cautious when removing liquids-take care not to jolt liquid containers as this can cause hot bottle explosions
- Once items are removed from autoclave, close the door

6. REFERENCES

Items that CAN be autoclaved include:

- Laboratory consumables, such as pipette tips, pipettes, glass bottles, surgical instruments, equipment (some equipment may not be autoclavable-check first)
- Liquids: water, buffers, aqueous solutions, cell growth media

Items that **CAN NOT** be autoclaved include:

- Materials containing: solvents, volatile, or chlorinated compounds (HCL, bleach) or corrosive chemicals (phenol, trichloroacetic acid, ether, chloroform)
- Flammable liquids (ethanol)
- Material contaminated with chemotherapeutic agents
- Radioactive material
- Some plastics (if you are unsure of the material you want to autoclave, please ask the Biosafety Officer)

Tips for Successful Autoclaving:

1. Packaging

   As the success of the sterilization is dependent upon the penetration of heat, how material is initially prepared will greatly affect the outcome. Consideration must be given to the primary container (containing your liquids, or solids), volume of liquid, amounts of material, and any secondary containers (containing the primary container-e.g., placing several pipette tip boxes in one larger plastic bin).

   The primary container is the container which comes into direct contact with the material or fluid. This may include such items as: flasks or vials holding liquids (either media or infectious material), wrapping paper or muslin protecting instruments, metal canisters, pipette tip boxes, and biohazard bags containing waste.

   This packaging must permit heat (steam) penetration, and ensure pressure differentials are not created as this could result in breakage. (No sealed containers must be placed in an autoclave!) This may be accomplished by using techniques such as:

   - Loosening screw caps/lids, or using vented caps
   - Using aluminum foil to cap open containers
   - Opening plastic bags (slightly) before loading into autoclave
     - Note that autoclave bags are usually made of PP and do not have good steam permeability-adding some water to the bag before autoclaving will facilitate steam production inside of the bag (I recommend filling a small ziplock bag with water, sealing it, and placing it in the bag)

   The structural integrity of the container is an important consideration. Not all containers withstand the demands placed on them during the autoclave process. Desirable characteristics are heat resistance, good thermal conductivity, puncture resistance and imperviousness to water.

2. Good choices for containers

   - borosilicate glass is very resistant to temperature fluctuations, so less likely to break when subjected to removal from the autoclave
   - polypropylene (PP) and polycarbonate (PC) are heat resistant plastics
   - stainless steel is a good heat conductor and thus facilitates sterilization

3. Poor choices for containers

    - Polystyrene (PS), polyethylene (PE) and high density polyethylene (HDPE) do not resist heat well.
    - If there is a risk of material melting ensure it is placed in a secondary container which is resistant to heat.

4. Secondary Containers

    - Liquids should always be autoclaved in a secondary container, to prevent spillage into autoclave cavity. If using a metal pan, liquid-containing bottles can be placed directly into pan. If using a PP plastic tub, place 1-2" of water in the container. This facilitates even sterilization of liquids and may also help prevent bottle breakage.
    - If placing solids in a secondary container, do not overfill container. All solids need direct exposure to steam to properly sterilize.

5. Volume Limits and Loading Capacity

    - It is important to not fill a container with more than 75% of its volume with liquid. This will allow for liquid expansion and prevent overflow.
    - For solid materials, do not fill a container beyond 75% of its holding capacity. This will allow for proper steam penetration to all materials

6. Other tips and techniques

    - When autoclaving large items (such as cylinders and large glass flasks), place the item on its side in the autoclave-this will allow for better steam penetration during cycle. If an item is placed upright, the steam may never reach the bottom of the container to displace the air there. If an item must be upright, add some water in the bottom of the item. This will facilitate steam production and assist with sterilization.
    - If autoclaving pouches/bags of items (e.g., steripouches filled with surgical instruments), try to keep the bags on their sides by propping up against other items in the autoclave (such as bottles or other flasks). This will allow more surface area for steam to penetrate.

Main Contacts:

Charis Johnson-Antaran Biosafety Officer Phone: 61456 Email: charis.johnsonantaran@lawsonresearch.com	Melissa Reid Health and Safety Analyst Phone: 75103 Email: MelissaK.Reid@LawsonResearch.com

Appendix C: SWP for working in Containment Level 2 Labs / Tissue Culture Labs / BSC work

BACKGROUND - Best Microbiological Practices

Laboratory workers are the first line of defense for protecting themselves, their coworkers / fellow researchers, and the public from exposure to hazardous biological agents. Protection depends on the conscientious and proficient use of good microbiological practices and the correct use of safety equipment such as BSC (biological safety cabinets) and PPE (personal protective equipment). A risk assessment should identify any potential deficiencies in the practices of the laboratory workers.

Careless procedures and practices are the most serious concern, because it can compromise any safeguards in the laboratory and increase the risk to everyone. In order to reduce the inherent risks while working with biohazardous agents, employees require:

- laboratory and biosafety training;
- knowledge of the agent
- experience in agent handling;
- knowledge of the hazards associated with the procedures being used (aliquoting, centrifuging, etc…);
- knowledge of safe laboratory habits; and
- concern for their health, as well as all the other laboratory staff.

New laboratory employees will require supervision, training and mentoring to acquire these important skills. Laboratory directors or principal investigators should train and retrain new staff to the point where aseptic (sterile) techniques and safety precautions become second nature.

SAFE WORK PRACTICE - Working in BSCs, CL2 and Tissue Culture Laboratories

1.) Enter the tissue culture / CL2 lab with clean hands (no gloves). Best microbiological safe practice include: **a.** Legs **must** be completely covered when human or zoonotic pathogens are handled; **b.** Shoes **must** completely cover the feet (metatarsals); **c.** Jewellery (rings) that elevate the danger of exposure **should** be removed.	REFERENCES: **Canadian Biosafety Standard (2nd edition - 2015)** 1a - section 4.4.5 1b - section 4.6.3 (biological exposure) & OHSS Policy Number OHS021 → Safety footwear (in revision state) 1c - section 4.6.4 **Biosafety in Microbiological and Biomedical Laboratories - CDC (5th edition - 2009)** 1 - Section III, Principles of Biosafety; pages 22 to 29. 1 – Section IV, Laboratory Biosafety Level Criteria, pages 33 to 38.	
2.) Long hair must be pulled back and not able to enter the contaminated area. 3.) Don gloves in the Containment Zone.	REFERENCES: Canadian Biosafety Standard (2nd edition - 2015) 2 – section 4.6.2 3 - sections 4.4.1 and 4.4.4 **Biosafety in Microbiological and Biomedical Laboratories - CDC (5th edition - 2009)** 2 / 3 - Laboratory Biosafety Level Criteria, pages 33 to 38.	

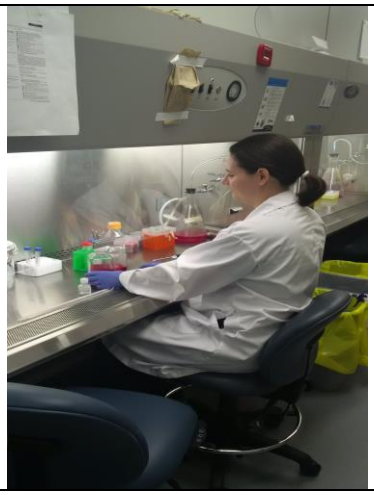
<table>
<tr>
<td>4.) Gloves must be worn at all times inside the BSC while handling any RG2 or higher toxin or pathogen.

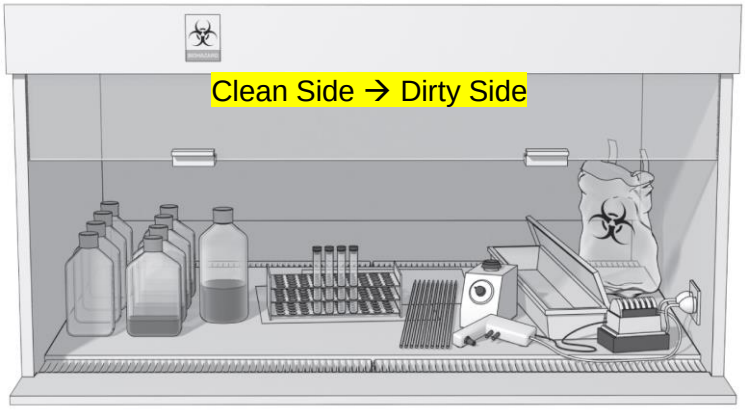
5.) Personal items and electronics <u>are not to be taken in or used</u> in CL2 laboratories or while working in BSC.</td>
<td><u>REFERENCES:</u>

Canadian Biosafety Standard
(2nd edition - 2015)

4 - sections 4.4.1 and 4.4.4
5 - section 4.5.11 and 4.6.35

(iphones, ipods, ear buds, cell phones and other electronic items cannot be easily decontaminated. This <u>excludes</u> items required for personal health such as medication pumps, hearing aids and heart monitoring equipment.)

  </td>
</tr>
<tr>
<td>6.) Set up all the BSC with a “Clean to Dirty” side to keep the employee, work area and agents safe from contamination.</td>
<td><u>REFERENCES:</u>

Canadian Biosafety Standard
(2nd edition - 2015)

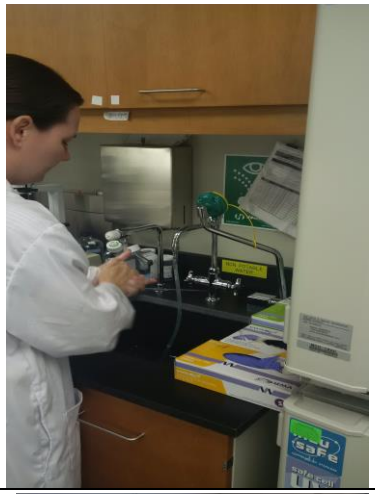
6 - sections 4.6.11 and 4.6.36; Lawson Biological Safety Cabinet Safe Work Practice; PaLM Safety Manual - Decontamination Methods.

7 - section 4.5.14, 4.5.15 and 4.6.26; WHO - Laboratory Biosafety Manual, Basic laboratories → Biosafety Levels 1 and 2, page 10.</td>
</tr>
</table>

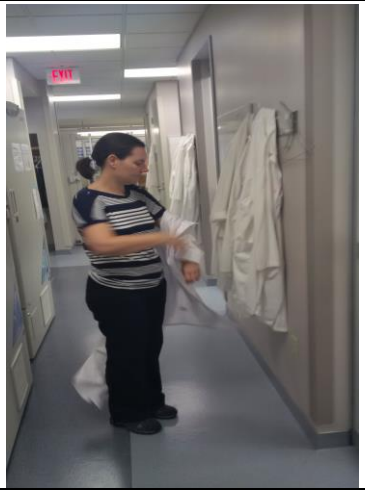
<table>
<tr>
<td>7.) Place used gloves (and pipet waste tips) in a waste container in the BSC.

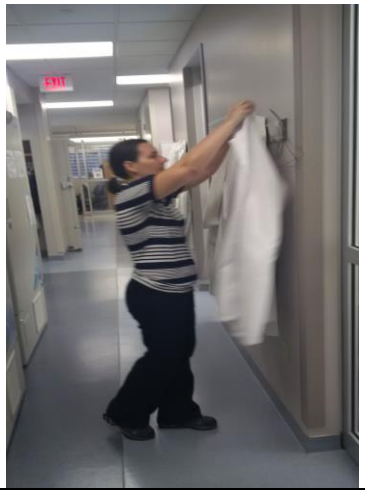
All gloves must be doffed (removed) before taking hands out of the BSC at the end of the experiment.</td>
<td></td>
<td> 
</td>
</tr>
<tr>
<td>8.) Before leaving the containment zone, the employee must perform effective hand hygiene with soap and water.</td>
<td>REFERENCES:

Canadian Biosafety Standard (2nd edition - 2015)

9 - sections 4.5.14, 4.5.15, 4.6.26 and 4.6.27
Note section 3.6.1 states “Sinks to be provided and located to facilitate hand-washing upon exit from the containment zone.”</td>
<td>  </td>
</tr>
<tr>
<td>9.) Doff all PPE in the appropriate area inside the containment area if possible (or as close to as is possible).</td>
<td>REFERENCES:

Canadian Biosafety Standard (2nd edition - 2015)

10 - sections 4.4.1 and 4.5.14</td>
<td>  </td>
</tr>
</table>

11.) Hang the lab coat up in a designated area that will ensure against cross contamination of personal clothes, chairs, paperwork, etc.		

REGULATORY REQUIREMENTS - CBS and HPTR

The Government of Canada's Canadian Biosafety Standard (CBS), 2nd Edition, 2015, is a harmonized national standard for the handling or storing of human and terrestrial animal pathogens and toxins in Canada. Activities in Canada involving human and animal pathogens and toxins are regulated by the Public Health Agency of Canada (PHAC) and the Canadian Food Inspection Agency (CFIA) in accordance with the Human Pathogens and Toxins Act (HPTA), the Human Pathogens and Toxins Regulations (HPTR), the Health of Animals Act, and the Health of Animals Regulations.

The standards outlined in this safe work practise will be enforced by the PHAC are the minimum standards required for all Containment Level 2 and higher laboratories. It is the responsibility of the Laboratory Directors and Principal Investigators to ensure these practises are followed in all CL2 labs, tissue culture labs and any work completed in Biological Safety Cabinets.

Appendix D: Lawson SWP for Persons Working with Lentivirus-based Vectors *in vitro*

1. PURPOSE/background

I. Introduction

This SWP will provide details and instruction on the safe production, use and handling of all human pathogenic lentivirus-based vectors. The containment level required will be based on a risk assessment conducted by the Biosafety Officer in consultation with the Principal Investigator. The major risk from working with human pathogenic lentivirus-based vectors is the generation of replication-competent lentivirus (RCL), and the potential for oncogenesis from random host chromosomal integration. The transgene being introduced by the viral vector must also be considered when assessing the risk from exposure to the virus.

II. Lentivirus

Lentiviruses are a subset of enveloped retroviruses that include the following human pathogens: Human Immunodeficiency Virus (HIV), Simian Immunodeficiency Virus (SIV), and Human T-lymphotropic Virus. These viruses have the ability to integrate into host chromosomes and can infect non-dividing and terminally differentiated cells and have high rates of mutation. Infection can occur by exposure through the mucous membranes, by inoculation (injections or punctures), and through scratches, cuts or skin abrasions. Lentiviruses are not airborne pathogens; however aerosols containing viral particles can result in exposure and infection.

Lentiviral vectors are used in research to deliver genetic material into a cell. These vectors contain recombinant transgene sequences and viral packaging and regulatory sequences flanked by long terminal repeats (LTRs). These vectors have been designed to be replication incompetent and non-pathogenic through various modifications. There are 3 generations of replication-incompetent lentivirus systems; each subsequent generation is safer than the previous one.

Generally, the transgene-containing virus is produced by transfecting a packaging cell line (usually HEK 293T cells) with 3 different plasmids, each one containing genes needed for viral production: the packaging plasmid contains the HIV genes ‘gag’ and ‘pol’. The second plasmid is the envelope vector, which often contains the VSV-G gene (for a broad host range, including humans). The third plasmid contains the transgene. The only way to obtain viral particles is to transfect the packaging cells with all three plasmids, and harvest the supernatant from the cells (this is where the virus will be). Although the virus collected will technically be incapable of self-replication, there is a very small risk that the virus will become replication-competent through homologous recombination.

2. SCOPE

This SWP describes the work practices and biosafety considerations when working with human pathogenic lentiviruses or lentivirus vector systems. Section 5 of this SWP covers the requirements for working with lentivirus in any Lawson laboratory.

3. RESPONSIBILITIES

This SWP applies to all trainees, staff, and researchers who work with or may come into contact with lentivirus. It is expected that anyone who will be working in a CL2+ area will be trained on the safe use and handling of lentivirus.

4. DEFINITIONS

BSC: biological safety cabinet
HEPA: High Efficiency Particulate Air
UV: ultraviolet
CL2: containment level 2 (biohazard containment level)
CL2+: CL2 facility using CL3 practices
VSV-G: vesicular stomatitis virus G-protein

5. PROCEDURE

I. Physical Containment/Equipment Requirements
In addition to complying with CL2 requirements, lentivirus work requires CL2+ practices are implemented, which requires the following:

- All handling of lentivirus must be done in a Class II type A2 BSC (see BSC SWP); all cultures containing lentivirus must be kept in a dedicated incubator with signage containing contact information for the person(s) culturing the lentivirus-containing cells.
- Vacuum lines used for aspiration must have a double-flask trap, with both a hydrophobic and a HEPA filter. The flasks must contain 10% bleach (approximately 5cm of bleach per 1L flask). The vacuum must be generated by a portable vacuum system (ideally) or vacuum pump system-NO house vacuum is to be used with lentiviral work.
- The room must have a closing door (preferably one that locks; door must have a lock if it opens into a public hallway) and ideally would be a negative pressure room.
- If centrifugation of virus is performed, there are certain requirements that will help prevent the generation of aerosols, including: properly balancing rotors, use appropriate plastic tubes with exterior thread screw caps (must have g-rating that is at least equal to the required spin speed), using sealed centrifuge cups/rotors and unloading the cups/rotors ONLY in the BSC

- Hand washing sink must be located as close as possible to the point of exit of the containment zone, and would preferably have a 'hands-free' capability
- All decontamination must occur within the containment zone, and may include the presence of a portable autoclave

II. Personal Protective Equipment

- Full body PPE coverage is required when in the CL2+ zone; this PPE must be dedicated to the lentiviral work and be kept inside the containment zone at all times (must doff PPE before exiting area)
- PPE must be decontaminated prior to disposal or laundering
- A lab coat or solid-front gown (preferably disposable) is to be worn at all times when in the CL2+ zone; elasticized cuffs are best, but sleeves can be taped as well to prevent skin exposure
- Hands must be double-gloved when handling lentivirus; DO NOT spray nitrile gloves with 70% ethanol as this can increase the permeation of the gloves, compromising barrier integrity
    - Vinyl gloves are recommended for lentivirus work
- NO shorts, or open-toed/heel shoes are to be worn in the containment zone; long hair must be tied back; all jewellery must be removed before entering the containment zone

III. Handling of Lentivirus

- All handling involving open vessels of lentivirus is to be conducted in an approved BSC
- Do not touch anything outside of the BSC without first doffing the outer pair of gloves
- Whenever possible, work with lentivirus during normal working hours to enable the best response possible to an adverse event.

  For Transduction and Handling of Cell Cultures:

    - Ideally, all transduced cells will be cultured in flasks with filtered, vented caps; if cells must be grown in dishes, keep in sealable plastic containers and lift the lid to permit air exchange only once the cells are in the incubator
    - Transduced cells should not be removed from the CL2+ zone until 3 days post-infection (the theoretical life span of the virus in solution); after 3 days, wash the cells 4-5x with PBS or medium to remove any unintegrated virus (and be sure to decontaminate ALL washes). NOTE: the cells must still be disposed of as if infectious!

- For Live-Cell Imaging:
    - Do not remove cells from CL2+ zone for 3 days post-infection; remove and wash the cell culture growth medium as above
    - For flasks, seal lid with parafilm and wipe the outside of the flask with Virox; for dishes, seal lid with parafilm, place in a sealable plastic container and wipe the outside of the container with Virox
    - After imaging, put plates back into container for transport back to CL2+ zone; disinfect the microscope area with Virox. NOTE: Do not use Virox on or near any sensitive microscope components.

- Any containers of infectious materials that are stored or transported outside of the CL2+ zone must be labelled, leak proof, and impact-resistant
- All centrifugation of lentivirus-containing material is to be carried out in sealed safety cups (or rotors) and must be loaded and unloaded in a BSC

- For Decontamination/Waste Disposal:
    - Please note that housekeeping will not be allowed in the CL2+ area, so room cleaning procedures must be put into place
    - To help reduce contamination, take care not to create aerosols or splashes; never touch anything outside of the BSC without first doffing outer pair of gloves
    - All waste generated during work must be decontaminated in the BSC: Place all solid waste into biohazard bags, seal the bag, and disinfect the outside of the bag with 70% ethanol before removal from the BSC; autoclave the waste the same day as it was generated
    - Note: Do not autoclave anything containing bleach!!!!
    - Decontaminate the BSC using Virox; let the surface stay wet for at least 30 seconds, then wipe up or air dry.
    - Note: Virox is viable for 30 days from the preparation date; be sure to label the prep date on the Virox container, and when the Virox has expired, dispose of it and make a fresh solution. NEVER mix old, expired solutions with fresh solutions!
    - For vacuum flasks, run 25-50ml of a 10% bleach solution through the vacuum line and allow to sit for 30 minutes. Prior to pouring the liquid waste down the sink, ensure proper PPE is worn (safety goggles, gloves, lab coat).
    - Note: UV has no effect on lentivirus and is not an effective method of decontamination

IV. Spill Procedure

- If a spill occurs in or outside of the CL2+ zone, in an incubator, a centrifuge, or any other equipment, follow the Lawson SWP for spill clean-up. Below is a summary of what to do:

- For small spills:

    - Ensure you are wearing proper PPE
    - Allow any aerosols time to settle
    - Gently cover the spill area with paper towel (or other inert, absorbent material) to cover the entire spill area
    - Gently flood the spill area (working from the outside of the spill, in) with Virox-DO NOT spray Virox on the spill.
    - Let sit for at least 30 seconds
    - Carefully dispose of the paper towels into a biohazard bag and dry the remaining liquid
    - Apply more Virox, let stand for 5 minutes, and dry with paper towel
    - Rinse the spill area with 70% ethanol
    - Place all waste and outer gloves into the biohazard bag
    - Don clean gloves and autoclave the waste as soon as possible

- For major spills, initiate a CODE BROWN (call x55555) and evacuate the area

6. REFERENCES

Public Health Agency of Canada, Canadian Biosafety Standard, 2015

http://en.wikipedia.org/wiki/Lentivirus

Appendix E: Lawson Biosecurity Plan

1.0 Purpose

The Human Pathogens and Toxins Act (HPTA) and Human Pathogens and Toxins Regulation (HPTR) have established a number of rules and regulations that govern the use of certain biological agents and toxins. Lawson, as located in St. Joseph's Health Care London (St. Joseph's) and as located in London Health Sciences Centre has a licence for each hospital for all research using controlled biohazardous materials. Section 4.1.11 of the Canadian Biosafety Standard (CBS) requires that facilities using biological agents have a biosecurity plan in place.

Lawson has two levels of Biosafety, consisting of a Biosafety Oversight Committee and a Biohazards Sub-Committee. Both committees consist of personnel from Lawson and a member of the University of Western Ontario's (UWO) Animal Care Committee / Veterinarian Services. These committees meet to discuss the safe use, handling and storage of biological agents and determine the physical, personnel and pathogen controls required. Additionally, the committees will communicate both internally (to the scientific community) and externally (the hospitals as a whole) issues related to biosecurity and biosafety.

Research groups must have a lawful purpose to possess, use and transport hazardous biological agents, and procedures to identify and characterize the agents held at Lawson labs within St. Joseph's and LHSC.

The Lawson Biosecurity Plan and the Public Health Agency of Canada's (PHAC) CBS will specify the physical and administrative security requirements for laboratories using biological agents. Hospital administration for both St. Joseph's and LHSC, require that all users of biological agents adopt the requirements outlined by PHAC and any additional measures as required in this Biosecurity Plan.

2.0 Definitions

Biosafety is defined as the containment principles, technologies, and practices that are implemented to prevent unintentional exposure to infectious material and toxins, or their accidental release. The objective of (bio)containment is to confine biohazards through physical lab design and operational practices to protect personnel, the immediate work environment, and the community from an exposure to a biological material. [1]

Biosecurity refers to the institutional and personal security measures designed to prevent the loss, theft, misuse, diversion, or intentional release of pathogens, toxins, and other related assets (e.g., personnel, equipment, non-infectious material, and animals).
Dual-Use Agents[2] include organisms and toxins derived from biological agents that pose a risk to biosecurity because they:

[1] Canadian Biosafety Standard, Second Edition, 2015
[2] NSABB: *Enhancing Responsible Science – Considerations for the Development and Dissemination of Codes of Conduct for Dual Use Research*, 2010

- can be easily grown, processed, and weaponized;
- can be moderately to easily disseminated or transmitted from person to person while maintaining virulence and/or toxicity;
- result in or have the potential for low to high mortality/morbidity rates;
- have the potential for major public health impact;
- might cause public panic and social disruption;
- require special action for public health preparedness; and
- may require specific enhancements for diagnostic capacity and enhanced disease surveillance.

The use of these agents is also valuable for many legitimate applications (including research, medical, and commercial).

Human pathogen means a micro-organism, nucleic acid or protein capable of causing disease or infection in humans that (*a*) is listed in the HPTA in any of Schedules 2 to 4 or in Part 2 of Schedule 5; or (*b*) is not listed in any of the Schedules but falls into Risk Group 2, Risk Group 3 or Risk Group 4.

(Microbial) Toxin means a poisonous substance that is produced or derived from a microorganism and can lead to adverse health effects in humans or animals. A human pathogen or toxin includes (*a*) a substance that contains a human pathogen or toxin; and (*b*) any synthetic form of the human pathogen or toxin. Human toxins are listed in the HPTA in Schedule 1 or in Part 1 of Schedule 5.

Risk Group is the classification of biological material based on its inherent characteristics, including pathogenicity, virulence, risk of spread, and availability of effective prophylactic or therapeutic treatments, that describes the risk to the health of individuals and the public as well as the health of animals and the animal population.

Risk Group 1 means a category of human pathogens that pose a low individual and community risk and are unlikely to cause disease in healthy workers or animals.

Risk Group 2 means a category of human pathogens that pose a moderate risk to the health of individuals and a low risk to public health and includes the human pathogens listed in the HPTA in Schedule 2. They are able to cause serious disease in a human but are unlikely to do so. Effective treatment and preventive measures are available and the risk of spread of disease caused by those pathogens is low.

Risk Group 3 means a category of human pathogens that pose a high risk to the health of individuals and a low risk to public health and includes the human pathogens listed in the HPTA in Schedule 3. They are likely to cause serious disease in a human. Effective treatment and preventive measures are usually available and the risk of spread of disease caused by those pathogens is low.

Risk Group 4 means a category of human pathogens that pose a high risk to the health of individuals and a high risk to public health and includes the human pathogens listed in the

HPTA in Schedule 4. They are likely to cause serious disease in a human. Effective treatment and preventive measures are not usually available and the risk of spread of disease caused by those pathogens is high.

Security sensitive biological agents (SSBAs) refer to a subset of human pathogens and toxins that have been determined to pose an increased biosecurity risk due to their dual-use potential. SSBAs are listed in Section 10 of the HPTR, as well as all toxins listed in Schedule 1 of the HPTA when in a quantity greater than that prescribed in Section 10(2) of the HPTR.

Trigger Quantity refers to the minimum quantity above which a toxin regulated by the HPTA is considered a 'prescribed toxin' and, therefore, an SSBA (as described by the above definition).

3.0 Identification of Biosecurity Agents

All principal investigators (PI) using biological agents must apply for a permit to use biological agents using the Lawson Biohazardous Agent Permit Process form (LBAPP). This form may be obtained by contacting the Lawson Biosafety Officer.

The LBAPP is reviewed and signed off by the Lawson Biosafety Officer and submitted to the Lawson Biohazards Sub-committee. The Lawson Biohazards Sub-committee reviews the information to determine whether the proposed handling of biological materials conforms to the policies and procedures for Lawson and the Canadian Biosafety Standard, and to confirm the required biosafety containment level. A biosafety permit is then issued to the PI. These permits are generally valid for three years and are specific to the protocols and biological agents listed on the application.

When deemed a possible biosecurity risk, protocols may be referred to the Lawson Biosafety Oversight Committee or the Biohazards Sub-committee for additional review. Because of the nature of biosecurity, each situation will be dealt with on a case by case basis. The Biosafety Oversight Committee and Biohazards Sub-committee have the right to recommend restricting or prohibiting the use and storage of biological agents at Lawson labs (located at either LHSC or St. Joseph's).

Where human pathogens and/or SSBAs are handled or stored, the PI may need to meet some or all of the following conditions:

1. Completion of a detailed risk assessment (using Section 5 as a guideline).
2. A safety, security and emergency response plan (see Section 5.4).
3. Restriction and one-on-one escorting of individuals without security clearance to access SSBAs.
4. A process to immediately report any theft, loss or release of dual-use agents (see Section 6.4)
5. Detailed records of information necessary to give a complete accounting of all activities related to dual-use agents.
6. Training, including the safe storage and use of the dual-use agents.
7. Physical security measures such as locked facilities, fridges and/or freezers.

4.0 Designation of a Licence Holder

The Scientific Director for the Lawson Health Research Institute (primarily located at St. Joseph's) is the Licence Holder on Lawson's Risk Group 2 Pathogen Licences.

The Licence Holder, St. Joseph's/LHSC Security, Emergency Management Program and the Lawson Biosafety Officer (BSO) are responsible for the development, training and implementation of biosecurity and emergency response plans. The BSO must be contacted as soon as possible in the event of any theft, loss or release of human pathogens. The BSO is involved in the risk assessment process and the biosecurity measures taken such as inventory control, background checks and transfers of biological agents.

5.0 Risk Assessment for Dual-use agents and SSBAs

When recognizing a possible biosecurity risk, Lawson will use a method compatible to that set out by PHAC's Office of Laboratory Biosafety and Biosecurity, and use a graded implementation approach to the level of risk and necessary control measures (see Figure 2).

The basic process is to identify the assets with respect to potential risk factors, identify the potential threats to the asset, review how a person could potentially access the asset and lastly, understand how we could mitigate or control identified gaps in the asset security.

5.1 Asset Identification

Risk group classifications provide a starting point for understanding risk factors inherent to the biological agent. Risk group 1 agents have a low risk to individuals and to public health. Risk group 2 agents have a moderate risk to individuals and a low risk to public health. Risk group 3 agents have a high risk to individuals and a low risk to public health. Risk group 4 agents have a high risk to individuals and a high risk to public health.

The Public Health Agency of Canada maintains a list of Security Sensitive Biological Agents (SSBAs) that require additional laboratory security checks and balances.

The U.S. Centers for Disease Control also maintains lists of biological agents of concern arranged by category of potential bioterrorism impact (Category A, B, or C).

5.2 Threat Identification

Once the biological agent is identified and its inherent characteristics quantified, then one would:

- work to understand particular threat scenarios,
- define the characteristics, motivations and capabilities of adversaries, and
- evaluate the probability and consequences of scenarios.

5.3 Understanding Vulnerabilities

The third step in a risk assessment would be working through how a perpetrator would circumvent normal procedures or exploit system vulnerabilities based on the scenarios developed under threat identification. A good starting point for understanding where the system is vulnerable is in the current security and physical design of the laboratory and pathogen storage areas, personnel monitoring and access control features, record keeping and pathogen accountability processes and emergency response procedures.

5.4 Threat Mitigation Strategies

The final step would be to address each of the system vulnerabilities with potential mitigation or control strategies. Engineering and administrative controls would probably have a higher importance with respect to security of pathogens from theft and misuse than personal protective equipment or worker specific controls.

Since only Risk Group 1 and 2 agents are currently approved for use at St. Joseph's and LHSC it is expected that any risk assessment (see Figure 1) would only involve low, or medium threat level human pathogens or toxins; however some labs may use SSBAs if the trigger quantity of an agent is met. With the physical and administrative security controls in place for all laboratories at Lawson's Health Research Institute, this would be expected to result in a lower likelihood and impact, and put the overall risk as "low". High consequence risk agents (Risk Group (RG) 3 and 4) are not procured, used or stored at St. Joseph's London or LHSC.

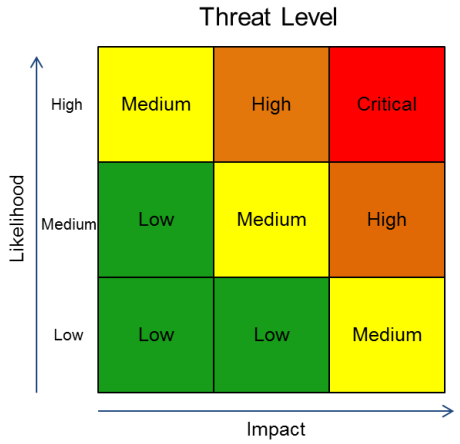


Figure 1: Risk Assessment Matrix[3]

[3] ABSA Principles and Practices of Biosafety, 2015

6.0 Biosecurity Risk Mitigation Strategies

6.1 Physical Protection and Security

Lawson, at St. Joseph's and LHSC implements graded protection based on the biosecurity risk of materials. Methods include:

- St. Joseph's -Closed circuit television cameras (CCTV);
- St. Joseph's, LHSC-Facility security such as security guards, with regular patrols;
- St. Joseph's, LHSC-Building security such as restricting access after-hours and electronic card access;
- St. Joseph's, LHSC-Laboratory security such as key and swipe card access and locking of laboratories, fridges and freezers;
- St. Joseph's, LHSC-Agent specific security including locking of storage areas and freezers; and
- St. Joseph's, LHSC-Data security.

6.2 Personnel Suitability/Reliability

Through the LBAPP and approval process, staff working with biohazardous agents are required to have training on the safe handling of these agents. Training is also required on the biosecurity plan. Training records may be validated during inspections and document reviews.

Personnel access to laboratories is restricted to laboratory staff, maintenance/janitorial personnel and visitors accompanied by suitable escorts. Further restrictions will be in place to certain areas where SSBAs are used, stored or otherwise present. Approval will be required to have access to the area or agent of concern. Approval may require:

- Personnel qualifications and training;
- Background checks and valid HPTA security clearances where needed;
- Periodic investigations and inspections;
- Escorts and badges for non-approved personnel;
- Photo identification badges; and
- Visitor sign in logs.

6.3 Pathogen Accountability/Inventory

Good record keeping is required of all biological agents used in research. An inventory of all biohazardous material must be maintained from time of acquisition to disposal. These records should include:

- Detailed inventory including location, agent, sample type and quantity;
- Record of transfers within and outside Lawson labs (at St. Joseph's or LHSC);
- Tracking of internal access;

- Inactivation and disposal of cultures and records including date and decontamination method;
- Sample labeling;
- Notification of BSO if there is a loss, theft, misuse, inadvertent release or production, or gain-of-function of a pathogen with a biosecurity risk;
- Notification of BSO when an SSBA is not received within 24 hours of the date and time it was expected to be received; and
- Notification of BSO when importing or exporting a human pathogen/toxin (either from a commercial source or from another facility).

6.4 Biosecurity Incident and Emergency Response

All researchers working with biohazardous materials must report all security incidents to the BSO and Hospital Security as soon as possible. Security incidents include, but are not limited to, breach of containment, unauthorized removal of pathogens, and unauthorized personnel in restricted areas. Security can be contacted through calling extension 55555 for emergencies and 44555 for non-emergent situations.

Please refer to the St. Joseph's or LHSC Disaster and Emergency Response Manuals for information on biohazard spill response procedures and other emergency procedures (e.g., fire, evacuation).

The St. Joseph's Workplace Occurrence Reporting policy and form can also be used to document these incidents, where there are staff injuries or near misses at a Lawson-St. Joseph's lab. If a more detailed reporting plan is required under the requirements of Section 3, this should be developed by consulting with the Lawson BSO, St. Joseph's London Security, and Risk Management, as required, with additional resources including the Western Biosafety Committee and/or the Western Biohazards Subcommittee.

The LHSC Adverse Event Reporting System (AEMS) is used to document the same incidents as listed above when they occur in a Lawson-LHSC lab.

In general, biosecurity requirements for Risk group 1 and 2 containment laboratories are incorporated into the containment level requirements under PHAC and CFIA, and validated during inspections required for the LBAPP and/or importation permits. These requirements should be sufficient for most biological agents handled at Lawson labs.

Lawson acknowledges the St. Joseph's Health Care London and University of Western Ontario Biosecurity Plans in the development of this document.

LAWSON
HEALTH RESEARCH INSTITUTE

Score and Prioritise Matrix					
	2. How likely is it to be that bad? [Probability of event]				3. Prioritize the action needed
1. How severely could it hurt someone or how ill could it make someone? [Severity of event]	***Very likely*** Could happen at any time	***Likely*** Could happen sometime	***Unlikely*** Could happen, but very rarely	***Very unlikely*** Could happen, but probably never will	***Action Priority***
Kill or cause permanent disability or ill health	**1**	**1**	**2**	**3**	***1 = Urgent*** → **Act now; Shutdown area or task. Notify Manager / Coordinator immediately. Area leadership to notify H&S immediately. Action immediately.**
Long term illness or serious injury	**1**	**2**	**3**	**4**	***2 = High Priority*** → **Isolate affected. Notify Manager / Coordinator immediately. Action within the week (7 days)**
Medical attention and several days off work	**2**	**3**	**4**	**5**	***3 = Medium Priority*** → **Action required within 30 days.**
First aid needed	**3**	**4**	**5**	**6**	***4 = Low Priority*** → **Action required within 90 days.**
Insignificant	**4**	**5**	**6**	**7**	***5/6/7 = Monitor Risk*** → **If hazard increases in risk, take action. Action required within 12 months**

Figure 2. Graded implementation approach to the level of risk and necessary control measures

Appendix 1 - Dual-use Agents

In addition to the Risk Group classification and the CDC bioterrorism categories, the following information may be useful in terms of asset identification in the risk assessment process.

Biological agents that are biosecurity agents of LOWEST RISK include:

- cell lines from plant, animal or human origins;
- biological agents that must be ingested to cause pathogenicity or other harm;
- rodents or other animals not known to be infectious;
- level 1 microorganisms;
- other level 1 biological agents;
- other biological agents to be identified as lowest biosecurity risk; and
- human and animal source materials such as tissues and blood.

Human pathogens/toxins that could be deemed to be possible biosecurity threats:

- toxins of biological origin;
- animals which may be infectious;
- any SSBAs (stored or used above the trigger quantities);
- other Risk Group 2 or higher organisms or biological agents;
- New or existing pathogens that are created, re-created or modified (see Figure 2); and
- other biological agents to be identified as low or medium biosecurity risk.

Agents of high biosecurity risk are not to be used or stored in Lawson labs without consultation with the Biosafety Officer.

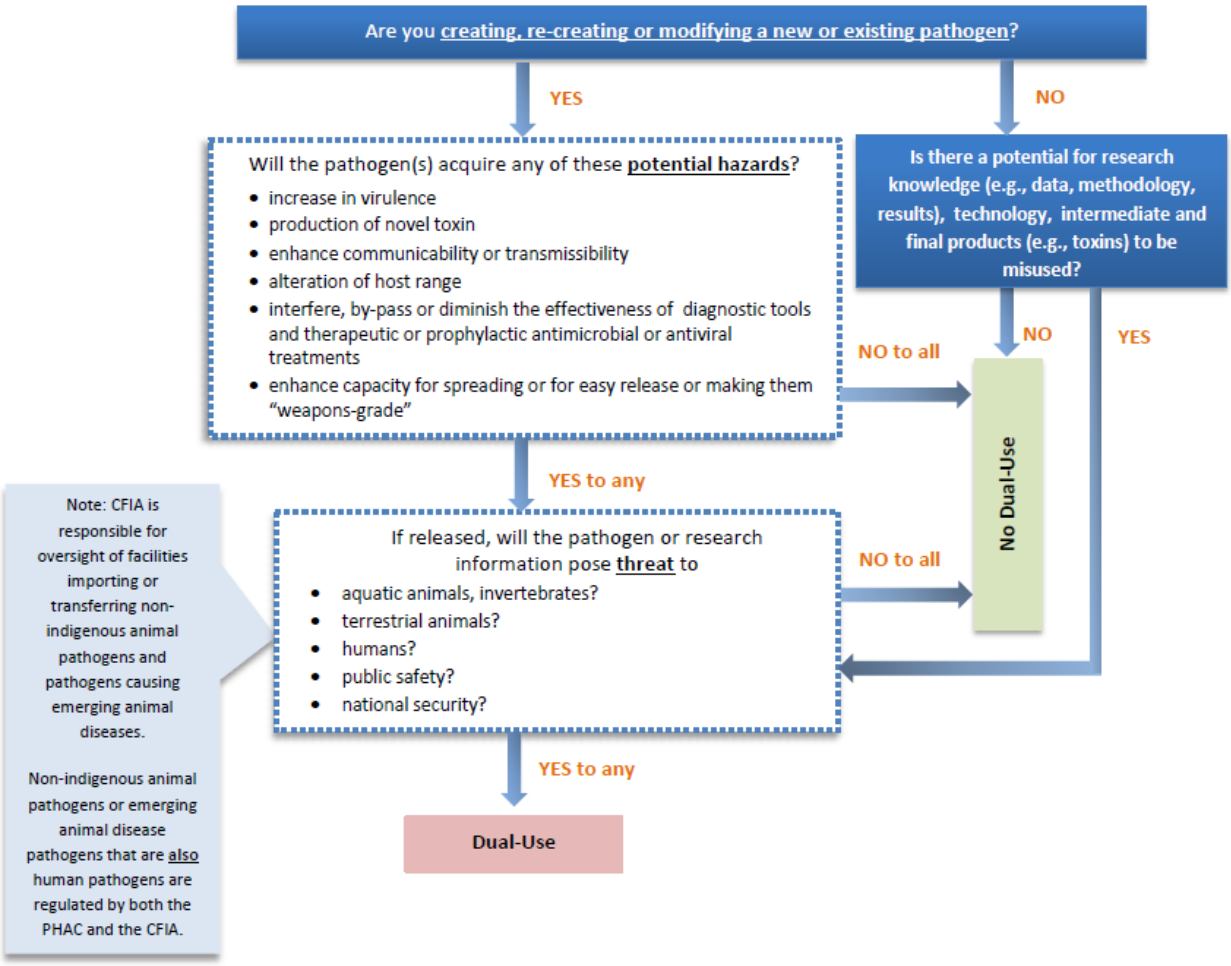


Figure 3. PHAC Decision Tree: Identification of Dual-Use Potential in Life Sciences Research

Appendix F: Biohazardous Risk Assessment for Lawson Research Areas

Background

Under the requirements of Human Pathogen and Toxin Act and its regulations, all laboratories working with biohazardous agents must have a written biosafety and biosecurity Risk Assessment (RA). The scope of the RA must include a brief over-view of the research, the types of agents used, mitigation strategies and security around the storage of the agents. Table 1 and Table 2 below are used to assist in identifying the risk associated with both cell culture (in-vitro) and animal use (in-vivo) involving biohazardous agents with Risk Group 1 through Risk Group 3 agents. Populate all fillable columns in both table 1 and 2. The Lawson Safety Analyst and Biosafety Officer can assist in helping identify the risk levels based on Risk Matrix used in the RA.

Table 3 is used to outline the standards around the security and storage of the agents used in the research laboratories. Fill in as much detail as possible in this section so the risk and residual risk.

Risk Group vs. Containment Level

Risk Groups (RG)

Classification of organisms according to risk group has traditionally been used to categorize the relative hazards of infective organisms. The factors used to determine which risk group an organism falls into is based upon the particular characteristics of the organism, such as:

- Pathogenicity;
- Infectious dose;
- Mode of transmission;
- Host range;
- Availability of effective preventive measures; and
- Availability of effective treatment.

These classifications presume ordinary circumstances in the research laboratory or growth in small volumes for diagnostic and experimental purposes. Four levels of risk have been defined as follows:

Risk Group 1 (low individual and community risk);
Risk Group 2 (moderate individual risk, low community risk);
Risk Group 3 (high individual risk, low community risk); and
Risk Group 4 (high individual risk, high community risk).

Date of Risk Assessment: _____ Area of Assessment: _____ Auditors: _____

(Department, Campus, Zone, Floor, Room)

_____

Containment Levels (CL)

The classification of organisms according to Risk Groups (RG) is not meant to establish the actual handling of biological hazards in the laboratory setting. For example, the RG system does not take into account the procedures that are to be employed during the manipulation of a particular organism. Containment Levels are selected to provide the end-user with a description of the minimum containment required for handling the organism safely in a laboratory setting. The inherent characteristics of an organism is described as the Risk Group (RG), but the Containment Level (CL) describes the engineering controls, operational controls, technical and physical requirements for manipulating a particular pathogen. These containment levels are applicable to facilities such as diagnostic, research, clinical, teaching and production facilities that are working at a laboratory scale. There are four prescribed Containment Levels in Canada; CL1 to CL4.

Area Information and Biohazards Present

Containment level 1 and 2 (CL1 / CL2) laboratories are located in both controlled-access and uncontrolled -access areas. There are offices, student areas, washrooms, walk-in freezer rooms, storage rooms and autoclave areas in both CL1 and CL2 areas. Often areas do have public access.

The biological hazards present may include:

- Cell lines (including mammalian and human origin, RG1 and 2 used),
- DNA manipulations (plasmids, transfections),
- RNA work (siRNA transfections),
- Use or presence of Security Sensitive Biological Agents (see Annex A);
- Reconstitution and aliquoting of cholera toxin, which is then used as an additive in cell culture growth medium.
- Preparation of cytotoxic agents (Tamoxifen and STZ) for injection into animals (injections are performed on another floor, but risk of needle-stick is still present)
- Human anatomical sample storage and experimentation (including patient biopsies, blood, urine)

Scope of Research and Agents Used

Please write a brief (lay) outline of the scope of research for this area and the type of agents used.

Date of Risk Assessment: _____ Area of Assessment: _____ Auditors: _____

(Department, Campus, Zone, Floor, Room)

Table 1 - Biosafety Risk Assessment; General

Activity	Performed in Area?	Risk of exposure	Risk Group	Pathogenicity	Route of Exposure	Engineering Controls	Minimum PPE	Residual Risk	Security Sensitive Biological Agents*
	Yes, No, Possible, N/A	See Annex C	1, 2, 2+	See Annex C				See Annex C	See Annex A
Handling of potentially Leaking Samples	**Yes** ☐ **No** ☐ **Possible** ☐ **NA** ☐				Ingestion, contact (open wounds, mucus membranes), injection	BSC (Class II Type A2)	• Lab coat (no cuff) • Nitrile gloves		**Yes** ☐ **No** ☐
Centrifugation	**Yes** ☐ **No** ☐ **Possible** ☐ **NA** ☐				Ingestion, contact (open wounds, mucus membranes), injection	Sealed rotor cups available in some areas; BSCs (Class II Type A2)	• Lab coat (no cuff) • Nitrile gloves		**Yes** ☐ **No** ☐
Decapping tubes	**Yes** ☐ **No** ☐ **Possible** ☐ **NA** ☐				Ingestion, contact (open wounds, mucus membranes), injection	BSC (Class II Type A2)	• Lab coat (no cuff) • Nitrile gloves • If under pressure, safety glasses		**Yes** ☐ **No** ☐
Separating/Aliquoting	**Yes** ☐ **No** ☐ **Possible** ☐ **NA** ☐				Ingestion, contact (open wounds, mucus membranes), injection	BSC (Class II Type A2)	• Lab coat (no cuff) • Nitrile gloves		**Yes** ☐ **No** ☐
Cutting	**Yes** ☐ **No** ☐ **Possible** ☐ **NA** ☐				Ingestion, contact (open wounds, mucus membranes), injection	BSC (Class II Type A2)	• Lab coat (no cuff) • Nitrile gloves		**Yes** ☐ **No** ☐
Grinding	**Yes** ☐ **No** ☐ **Possible** ☐ **NA** ☐				Ingestion, contact (open wounds, mucus membranes), injection	BSC (Class II Type A2)	• Lab coat (no cuff) • Nitrile gloves		**Yes** ☐ **No** ☐

Date of Risk Assessment: _____ Area of Assessment: _____ Auditors: _____

(Department, Campus, Zone, Floor, Room)

Activity	Performed in Area?	Risk of exposure	Risk Group	Pathogenicity	Route of Exposure	Engineering Controls	Minimum PPE	Residual Risk	Security Sensitive Biological Agents*
	Yes, No, Possible, N/A	See Annex C	1, 2, 2+	See Annex C				See Annex C	See Annex A
Internal Transport Systems	Manual only				Contact (minimal)	Sealed, leak-proof containers	• Lab coat (no cuff) • Nitrile gloves		**Yes** ☐ **No** ☐
Shaking/Mixing/ Sonifying/ Homogenizing/ Vortexing	**Yes** ☐ **No** ☐ **Possible** ☐ **NA** ☐				Ingestion, contact (open wounds, mucus membranes), injection	BSC (Class II Type A2)	• Lab coat (no cuff) • Nitrile gloves		**Yes** ☐ **No** ☐
Using Sharps	**Yes** ☐ **No** ☐ **Possible** ☐ **NA** ☐				Injections (needle sticks) and lacerations	BSC (Class II Type A2); safety needles where possible (SEMD)	• Lab coat (no cuff) • Nitrile gloves		**Yes** ☐ **No** ☐
Manipulation of Live Bacterial Cultures (including pure cultures)	**Yes** ☐ **No** ☐ **Possible** ☐ **NA** ☐				Ingestion, contact (open wounds, mucus membranes), injection	RG 1 – Not Required RG 2 - BSC (Class II Type A2)	• Lab coat (no cuff) • Nitrile gloves		**Yes** ☐ **No** ☐
Manipulation of Live Virus Cultures (including pure cultures)	**Yes** ☐ **No** ☐ **Possible** ☐ **NA** ☐				Ingestion, contact (open wounds, mucus membranes), injection	RG 1 – Not Required RG 2 - BSC (Class II Type A2)	• Lab coat (no cuff) • Nitrile gloves		**Yes** ☐ **No** ☐
Manipulation of Cell Lines (including pure cultures)	**Yes** ☐ **No** ☐ **Possible** ☐ **NA** ☐				Ingestion, contact (open wounds, mucus membranes), injection	RG 1 – Not Required RG 2 - BSC (Class II Type A2)	• Lab coat (no cuff) • Nitrile gloves		**Yes** ☐ **No** ☐

Date of Risk Assessment: _____ Area of Assessment: _____ Auditors: _____

(Department, Campus, Zone, Floor, Room)

Activity	Performed in Area?	Risk of exposure	Risk Group	Pathogenicity	Route of Exposure	Engineering Controls	Minimum PPE	Residual Risk	Security Sensitive Biological Agents*
	Yes, No, Possible, N/A	See Annex C	1, 2, 2^+	See Annex C				See Annex C	See Annex A
Risk of Aerosolization of biological agents	**Yes** ☐ **No** ☐ **Possible** ☐ **NA** ☐				Inhalation, Ingestion, contact (mucus membranes)	RG 1 / RG 2 - BSC (Class II Type A2)	• Lab coat (no cuff) • Nitrile gloves		**Yes** ☐ **No** ☐
Movement between laboratories	**Yes** ☐ **No** ☐ **Possible** ☐ **NA** ☐				Ingestion, contact (open wounds, mucus membranes), injection	Sealed leak proof containers Sealed cryogenic transport carboys	• Lab coat (no cuff) • Nitrile gloves • Cryogenic gloves and goggles as required		**Yes** ☐ **No** ☐
Waste Management Strategies	**Yes** ☐ **No** ☐ **Possible** ☐ **NA** ☐				Ingestion, contact (open wounds, mucus membranes), injection, lacerations		• Lab coat (no cuff) • Nitrile gloves		**Yes** ☐ **No** ☐
Disinfectant Strategies	**Yes** ☐ **No** ☐ **Possible** ☐ **NA** ☐				Ingestion, contact (open wounds, mucus membranes), injection		• Lab coat (no cuff) • Nitrile gloves		**Yes** ☐ **No** ☐
Other	* If SSBA are involved, or expected to be present in a research sample (in any quantity), indicate which SSBA is present and how employees will be protected against it and any extra security features that are required for the samples.								

Date of Risk Assessment: _____ Area of Assessment: _____ Auditors: _____

(Department, Campus, Zone, Floor, Room)

Table 2 - Biosafety Risk Assessment; Animal Work

Activity	Performed in Area?	Risk of exposure	Risk Group	Pathogenicity (of highest risk present)	Route of Exposure	Engineering Controls	Minimum PPE	Residual Risk	Security Sensitive Biological Agents*
	Yes, No, Possible, N/A	See Annex C	1, 2, 2^+	See Annex C				See Annex C	See Annex A
Handling of small animals (small animal containment)	**Yes** ☐ **No** ☐ **Possible** ☐ **NA** ☐				Bite, Scratch, dermal, mucous membrane, inhalation, ingestion	BSC (Class II Type A2), Fume hood	• Lab coat (no cuff) • Nitrile gloves		**Yes** ☐ **No** ☐
Handling of medium animals (large animal containment	**Yes** ☐ **No** ☐ **Possible** ☐ **NA** ☐				Bite, Scratch, dermal, mucous membrane, inhalation, ingestion		• Lab coat (no cuff) • Nitrile gloves		**Yes** ☐ **No** ☐
Injections (subq, IP, IM, IV)	**Yes** ☐ **No** ☐ **Possible** ☐ **NA** ☐				Bite, Scratch, dermal, mucous membrane, inhalation, ingestion	BSC (Class II Type A2), Fume hood	• Lab coat (no cuff) • Nitrile gloves		**Yes** ☐ **No** ☐
Surgical Sharps	**Yes** ☐ **No** ☐ **Possible** ☐ **NA** ☐				Bite, Scratch, dermal, mucous membrane, inhalation, ingestion	BSC (Class II Type A2), Fume hood	• Lab coat (no cuff) • Nitrile gloves		**Yes** ☐ **No** ☐
Cytotoxic agents	**Yes** ☐ **No** ☐ **Possible** ☐ **NA** ☐				Bite, Scratch, dermal, mucous membrane, inhalation, ingestion	BSC (Class II Type A2), Fume hood	• Lab coat (no cuff) • Nitrile gloves • N95 respirator if BSC not available		**Yes** ☐ **No** ☐
Gavage / Lavage Methods	**Yes** ☐ **No** ☐ **Possible** ☐ **NA** ☐				Bite, Scratch, dermal, mucous membrane, inhalation, ingestion	BSC (Class II Type A2), Fume hood	• Lab coat (no cuff) • Nitrile gloves		**Yes** ☐ **No** ☐

Activity	Performed in Area?	Risk of exposure	Risk Group	Pathogenicity (of highest risk present)	Route of Exposure	Engineering Controls	Minimum PPE	Residual Risk	Security Sensitive Biological Agents*
	Yes, No, Possible, N/A	See Annex C	1, 2, 2^+	See Annex C				See Annex C	See Annex A
Intubation Methods	**Yes** ☐ **No** ☐ **Possible** ☐ **NA** ☐				Bite, Scratch, dermal, mucous membrane, inhalation, ingestion	BSC (Class II Type A2), Fume hood	• Lab coat (no cuff) • Nitrile gloves		**Yes** ☐ **No** ☐
Catheterization	**Yes** ☐ **No** ☐ **Possible** ☐ **NA** ☐				Bite, Scratch, dermal, mucous membrane, inhalation, ingestion	BSC (Class II Type A2), Fume hood	• Lab coat (no cuff) • Nitrile gloves		**Yes** ☐ **No** ☐
Carcass Handing / Disposal	**Yes** ☐ **No** ☐ **Possible** ☐ **NA** ☐				Dermal, mucous membrane, inhalation, ingestion	BSC (Class II Type A2), Fume hood	• Lab coat (no cuff) • Nitrile gloves		**Yes** ☐ **No** ☐
Contaminated bedding	**Yes** ☐ **No** ☐ **Possible** ☐ **NA** ☐				Dermal, mucous membrane, inhalation, ingestion	BSC (Class II Type A2), Fume hood	• Lab coat (no cuff) • Nitrile gloves • N95 respirator if BSC not available		**Yes** ☐ **No** ☐
Waste Management Strategies	**Yes** ☐ **No** ☐ **Possible** ☐ **NA** ☐				Dermal, mucous membrane, inhalation, ingestion, parenteral	BSC (Class II Type A2), Fume hood	• Lab coat (no cuff) • Nitrile gloves		**Yes** ☐ **No** ☐
Other	* If SSBA are involved, or expected to be present in a research sample (in any quantity), indicate which SSBA is present and how employees will be protected against it and any extra security features that are required for the samples.								

Table 3 - Biosecurity Risk Assessment

Condition	Condition Description	Risk of Access	Mitigation Strategies	Residual Risk	Security Sensitive Agents in Storage
		See Annex C		See Annex C	See Annex A
Access Control / Physical Barriers	Example: Swipe Card access room		Example: keep doors locked at all times		**Yes** ☐ **No** ☐
Secondary Containment	Example: Locked -80C freezer		Example: issue freezer keys only to authorized users		**Yes** ☐ **No** ☐
Effective Signage					**Yes** ☐ **No** ☐
Inventory Management System					**Yes** ☐ **No** ☐
Loss Control Reporting and Documentation					**Yes** ☐ **No** ☐
Spill Strategies					**Yes** ☐ **No** ☐
Exposure Controls and Process					**Yes** ☐ **No** ☐
Public Access to area					**Yes** ☐ **No** ☐

Annex A - Security Sensitive Biological Agents

Security Sensitive Biological Agents List – Viruses

Andes virus	Chapare virus	Chikungunya virus
Choclo virus	Congo-Crimean haemorrhagic fever virus	Dobrava-Belgrade virus
Eastern equine encephalitis virus	Ebola virus	Guanarito virus
Hantaan virus	Hendra virus (Equine morbillivirus)	Highly pathogenic avian influenza virus
Japanese encephalitis virus	Junin virus	Kyasanur Forest virus
Laguna Negra virus	Lassa fever virus	Louping ill virus
Lujo virus	Machupo virus	Marburg virus
Monkey pox virus	Murray Valley encephalitis virus	Nipah virus
Omsk haemorrhagic fever virus	Oropouche virus	Powassan virus
Rift Valley fever virus	Rocio virus	Sabia virus
Seoul virus	Sin nombre virus	St Louis encephalitis virus
Tick-borne encephalitis virus (Russian Spring-Summer encephalitis virus)	Variola virus	Venezuelan equine encephalitis virus
Western equine encephalitis virus	Yellow fever virus	

Security Sensitive Biological Agents List – Bacteria

Bacillus anthracis	Brucella abortus	Brucella melitensis
Brucella suis	Burkholderia mallei (Pseudomonas mallei)	Burkholderia pseudomallei (Pseudomonas pseudomallei)
Chlamydophila psittaci (formerly known as Chlamydia)	Chlamydophila psittaci (formerly known as Chlamydia psittaci)	Coxiella burnetii
Francisella tularensis	Rickettsia prowazekii	Yersinia pestis

Security Sensitive Biological Agents List – Toxins (trigger quantity)

Alpha toxin (5 mg)	Botulinum neurotoxin (0.5 mg)	Cholera toxin (20 mg)
Clostridium botulinum C2 and C3 toxins (5 mg)	Clostridium perfringens Epsilon toxin (5 mg)	Hemolysin (10 mg)
Shiga-like toxin (verotoxin) (1 mg)	Shigatoxin (1mg)	Staphylococcus enterotoxins, Type B (1 mg)
Staphylococcus enterotoxins, types other than Type B (10 mg)	Staphylococcus aureus Toxic shock syndrome toxin (5 mg)	

Security Sensitive Biological Agents List – Fungi

Coccidioides immitis	Coccidioides posadasii

Laboratories that work with strains of bacteria that produce SSBA toxins are not captured by the SSBA designation as long as the SSBA toxin is not produced to levels above the trigger quantity. If work with strains of bacteria that produce SSBA toxins results in the production of quantities of SSBA toxins that exceed the SSBA toxin trigger quantities, the work would be subject to the SSBA designation.

If you exceed the toxin trigger quantity, you will need a security clearance

Annex B - Area Map

(to be filled in with map of assessed area)

Annex C - Risk Assessment Guidance

Score and Prioritise Matrix					
	2. How likely is it to be that bad? [Probability of event]				3. Prioritize the action needed
1. How severely could it hurt someone or how ill could it make someone? [Severity of event]	***Very likely*** Could happen at any time	***Likely*** Could happen sometime	***Unlikely*** Could happen, but very rarely	***Very unlikely*** Could happen, but probably never will	***Action Priority***
Kill or cause permanent disability or ill health	**1**	**1**	**2**	**3**	***1 = Urgent*** → **Act now; Shutdown area or task. Notify Manager / Coordinator immediately. Area leadership to notify H&S immediately. Action immediately.**
Long term illness or serious injury	**1**	**2**	**3**	**4**	***2 = High Priority*** → **Isolate affected. Notify Manager / Coordinator immediately. Action within the week (7 days)**
Medical attention and several days off work	**2**	**3**	**4**	**5**	***3 = Medium Priority*** → **Action required within 30 days.**
First aid needed	**3**	**4**	**5**	**6**	***4 = Low Priority*** → **Action required within 90 days.**
Insignificant	**4**	**5**	**6**	**7**	***5/6/7 = Monitor Risk*** → **If hazard increases in risk, take action. Action required within 12 months**