Lawson Health Research Institute Biosafety Manual

For Containment Level 1 and 2 Laboratories

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, learners, and volunteers to work in. Lawson's biosafety manual and associated policies and Standard Operating Procedures (SOPs) 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 possible exposure to biological agents or toxins.

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 this legislation in order to communicate any changes to the appropriate 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, learners, 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 (Lawson Visitor guideline).

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

Canada
Canadian Biosafety Standards, Third Edition (CBS)
Canadian Biosafety Handbook, Second Edition (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
Health of Animals Act (HAA)
Health of Animals Regulation (HAR)
Ontario Occupational Health and Safety Act and Regulations (OHSA)

Some of the content of this manual is taken directly from the Canadian Biosafety Standard 3rd Edition (CBS) and is inserted throughout this manual for your convenience. It is still recommended that the CBS be reviewed annually by all faculty, staff, learners and volunteers 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 (SJHC) 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:

Dayna Collins, extension 61456
Email: Dayna.Collins@lhsc.on.ca

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.

Biosecurity refers to 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).

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 a natural product of the metabolic activities of certain microorganism, plants, and animal species and can lead to adverse health effects in humans or animals. In the context of the CBS, the word “toxin” refers only to microbial toxins regulated by the PHAC and the CFIA under the HPTA and are listed in Schedules 1 and 5 of the HPTA.

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 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	**SJHC**	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 Oversight Committee. 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 University to ensure that all affiliated staff and learners are working safely with biohazardous materials in the hospital research laboratories. 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 within the Health and Safety section on the Lawson Health Research Institute Intranet site.

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.

Biological Safety Officer (BSO)

Must have knowledge of microbiology appropriate to the risks associated with the controlled activities attained through a combination of education, training, and experience. They must also have knowledge of the HPTA, the HPTR, and any applicable federal or provincial legislation and applicable biosafety and biosecurity policies, standards and practices appropriate to the risks associated with the controlled activities being conducted.

The functions of the BSO include:

- Complete and submit all required licence applications, verifying accuracy along with all required sections of the Plan for Administrative Oversight (PAO) 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, through annual review;
- 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;
- Communicate 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;
- Conduct 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 the 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).

Principal Investigator is required to:

- 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 exposure to, the loss of or change in risk group to a controlled biohazardous material;
- Ensure that all persons working under their supervision have had appropriate training for working safely with potential biohazardous materials;
- Provide appropriate personal protective equipment (PPE), standard operating procedures (SOPs) and safe work practises (SWP) to laboratory staff/learners/volunteers;
- 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 Containment laboratories;
- Ensure that Local 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;
- Ensure that all biological materials listed on the LBAPP(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 all applicable legislation.

Laboratory Personnel are required to:

- 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 and Lawson Health and Safety;
- 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 Occupational Health and Safety Services (OHSS) and/or Workplace Health (Western staff/learners);
- 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 inadvertently been released or produced;
- Whenever any person who is conducting controlled activities authorized under a licence intends to increase the virulence, pathogenicity, communicability of a human pathogen;
- Intends to increase the resistance of a human pathogen to preventative or therapeutic treatments;
- Intends to increase the toxicity of a toxin;
- An unauthorized person comes into possession of the pathogen or toxin;
- An incident or potential exposure to a pathogen has occurred;
- Possession of a pathogen that is higher than risk group 2;
- A pathogen or toxin has been stolen;
- SSBA has not been received with 24 hrs 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;
- Loss of access card or ID cards
- Cyber security incident has occurred

1.5 Criminal Code of Canada

In 2004, workplace safety legislation (Westray Bill C-45) 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.

217.1 Every one who undertakes, or has the authority, to direct how another person does work or performs a task is under a legal duty to take reasonable steps to prevent bodily harm to that person, or any other person, arising from that work or task."

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.

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

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 Containment Level 2 (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 (RG1) pathogens or those pathogens that require CL 1 facilities are not regulated by PHAC (or the HPTR), and therefore a licence is not required for their importation.

Researchers wishing to import pathogens that require CL 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 CL 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). 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 CFIA 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: Importing Plant Pests.

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 Controls (EC). 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: ECL Export form.

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, compliance with the Transportation of Dangerous Goods Regulation (TDG) administered by Transport Canada is required. 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 (ERAP) 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 diseases are prepared by the CFIA and can be found here:

Animal Disease Fact Sheets

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 pathogens 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 Western University; 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 the Safety Data Sheet (SDS) and chemical, drug or biological questionnaire sheets for the BSO's reference, if applicable.

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.
Note that many links can only be accessed through LHSC or SJHC intranet

SJHC
Waste Management Manual:
https://intra.sjhc.london.on.ca/sites/default/files/pdfs/ohs_waste_management_manual.pdf

Safe Handling of Biomedical Waste:
https://intra.sjhc.london.on.ca/sites/default/files/pdfs/ohs_waste_management_manual.pdf

Chemical Waste Disposal Procedures:
https://intra.sjhc.london.on.ca/sites/default/files/pdfs/ohs_waste_management_manual.pdf

Cytotoxic and Hazardous Drugs
Hazardous Drugs – Safe Management Policy

LHSC
Waste Management:
https://intra.lhsc.on.ca/facilities-management/environmental-stewardship/waste-management

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

Chemical Waste Control Procedure:
https://intra.lhsc.on.ca/facilities-management/environmental-stewardship/waste-management/chemical-waste/chemical-waste-control-procedures

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

2.5 Autoclaves

An autoclave is a piece of decontamination 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 (*Note: Lawson sends all waste offsite to a third party for treatment and disposal).

The Lawson Intranet houses a list indicating the locations of autoclaves throughout Lawson facilities. Annual preventative maintenance is 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 Specialist. As per the CBS section 5.1.4, autoclaves must be validated annually with a representative load and must have the monitoring parameters of the autoclave cycles reviewed and recorded (CBS 3.6.7).

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 ANSI standards 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.

Table 1 - Biological Safety Cabinet Selection

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

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, in fact the inward flow of air can contribute to contamination of samples.

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 type A, B or C 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 AI

Cabinet air may be recirculated via HEPA filtration system back into the laboratory or ducted out of the building. It is able to maintain a minimum average face velocity of 0.38m/s. The air flow splits 6 – 18cm above the work area inside the cabinet where 50% passes through the front grille and the other 50% through the rear grille, then drawn through ductwork, up the back of the cabinet where it is then blown into a positive pressured, contaminated plenum. 70% of the HEPA filtered air is recirculated within the cabinet and 30% of the HEPA filtered air is exhausted out. 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

Class II type A2 is almost identical to Class II A1cabinet with a few additions. The 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 greater inflow velocity and has a minimum average face velocity of 0.51m/s and has a plenum that is under negative pressure. Class II A2 cabinets away have negatively pressured contaminated plenums or positively pressured contaminated ducts/plenums surrounded by negatively pressured ducts/plenums. This cabinet is not suitable for work with low levels of volatile chemicals or radioisotopes unless it is exhausted through the thimble connection (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, > 50% of the air is exhausted out and < 50% of the air is recirculated within the cabinet. It can maintain a minimum average face velocity of 0.51m/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 (Total Exhaust BSC). This cabinet contains

negative pressure air flow and maintains a minimum average face velocity of 0.51m/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.

Class II, Type C1

Moves air by mixing inflow air with the air in the columns of downflow air marked for recirculation. It can use single pass airflow, and when installed in a ducted operating mode, can protect from hazardous chemistry. It developed from the necessity to control infectious material and chemical hazards.

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 -200 Pa or lower, and airflow is maintained by an exterior exhaust system. Operation within the work surface is accessed through rubber glove ports or sealed air locks and maintain an inward directional airflow (IDA) of 0.7m/s when one glove is removed. 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 with risk group 4 pathogens. 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, 3rd 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, learners, volunteers, and the public lies with the principal investigator responsible for conducting controlled biohazardous research.

Mandate

- To oversee the Biosafety Program, and provide direction and recommend changes.
- To promote and monitor compliance with all applicable policies, procedures, and legislation as outlined by PHAC and Lawson.
- 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.
- 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.
- 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.
- 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.
- To establish sub-committees as necessary to carry out specific tasks as mandated by the LBOC.
- To collaborate with Western University's Biosafety Committee as needed to maintain consistency between the two organizations.
- 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 disposal of the named agents are followed.

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

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 Specialist or their delegate, and distributed to the committee members.

Records

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

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

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 biohazardous material 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, learners, volunteers, and the public lies with the principal investigator that is conducting controlled biohazardous research.

Mandate

- 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).
- 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.
- 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.
- To generate an Annual Report to be forwarded to the LBOC by May 1st of each year.

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

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 sits 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 Specialist 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.

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

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, monoclonal antibodies, oligonucleotides, 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 Biological Agents Permit Application (BAPA) must be filled out for research being conducted within the facilities at Western University http://www.uwo.ca/hr/safety/topics/biosafety/index.html.

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 Biosafety Officer for review, and then is submitted to the appropriate committee for final approval.

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 Manual and the Canadian Biosafety Standards.

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 submitted by the Principal Investigator.

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 need to be altered or modified (for example request to use a new risk group 2, pathogen or toxin) during the approved period of the current permit, an LBAPP modification form must be completed. This form can be obtained from Lawson's Biosafety Officer, completed by the Principal Investigator and then reviewed by the Lawson Biohazards Sub-Committee.

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; in Canada from 2016 to 2019, 247 exposure incidents were reported. In 2020, 42 exposure incidents involving 57 individuals were reported to Canada's Laboratory Incident Notification Canada surveillance system with none of them resulting in a confirmed or suspected LAI. In 2022 there were 145 laboratory incidents reported to PHAC where 40 of those incidents where confirmed exposure cases involving 93 affected individuals and of those 2 resulted in a laboratory acquired infection

There are a number of ways in which infectious substances can enter the body and cause infection. These include ingestion, inhalation, absorption, 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 from a biohazardous agent may be the greatest exposure risk 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, and that they document this training.

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 one of either a) Lawson's Biosafety Training course or b) Western University's online Biosafety training and provide a certificate after completion. Biosafety training must be refreshed every 3 years. 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
- X-Ray Safety
- Radiation Safety
- Laser Safety
- Supervisor Training
- Health and Safety Awareness
- Laboratory Safety
- Transportation of Dangerous Goods
- Safety Data Sheets (SDS)

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.

WHMIS regulations require that workers have access to information on all hazards in the workplace, including biohazards. SDS for infectious microorganisms (biological agents) have been prepared by the Office of Laboratory Security, PHAC. SDS contain health hazard information, recommended precautions, safe handling methods, decontamination methods and other information that is relevant 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 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 appropriate to the hazards encountered including eye glasses/goggles and respirators (as necessary by risk assessment)
- 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.

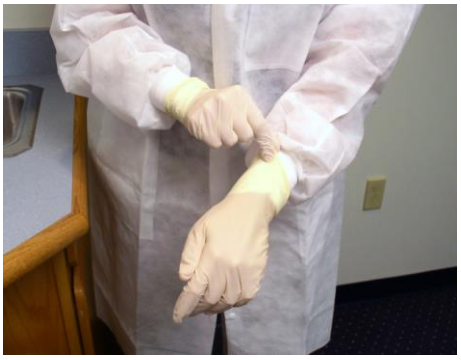

Figure 1 - Glove over cuff technique

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. A Biosafety Risk Assessment must be conducted at the time of LBAPP renewal and will be reviewed annually by the laboratory supervisor (Appendix F). Any changes made must be discussed with the personnel affected. (Chapter 4 of the CBS details the use of PPE in Containment Level 2 laboratories). Proper donning and doffing procedures can be found in the Lawson SOP for PPE use.

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, outlined below, 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.

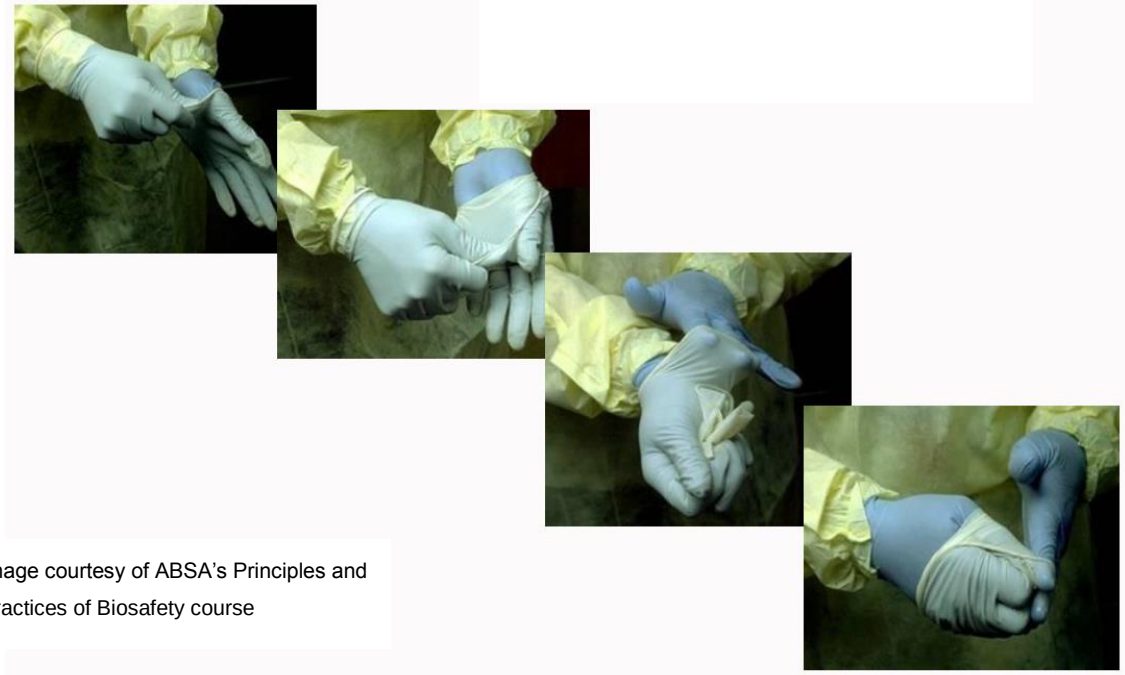

Image courtesy of ABSA's Principles and Practices of Biosafety course

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. Contact of the face or mucous membranes with items contaminated or potentially contaminated with regulated materials must be prevented. Avoid touching nose, face and eyes in the laboratory.

4. Eating, drinking, smoking, vaping, storing of food, personal belongings including cell phones and ear buds/phones, 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.12 & 32 of O.Reg. 67/93).

5. Oral pipetting of any substance is prohibited in any laboratory.

6. Long hair is to be tied back or restrained so that it cannot come into contact with hands, specimens, containers or equipment.

7. Access to laboratory and support areas is limited to authorized personnel.

8. Doors to laboratories must not be left open (this does not apply to an open area within a laboratory).

9. Open wounds, cuts, scratches and grazes should be covered with waterproof dressings.

10. 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.

11. 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.

12. 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.

13. 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.

14. 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.

15. Protective laboratory clothing must not be worn in non-laboratory areas; laboratory clothing must not be stored in contact with street clothing.

16. Rolling up of lab coat sleeves is prohibited. There must be no exposed skin from shoulders down to feet.

17. 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).

18. 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.

19. 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.

20. Contaminated materials and equipment leaving the laboratory for servicing or disposal must be appropriately decontaminated and labelled or tagged out as such.

21. Disinfectants effective against the agents in use must be available at all times within the areas where the biohazardous material is handled or stored.

22. Leak-proof containers are to be used for the transport of infectious materials within facilities (e.g., between laboratories in the same facility).

23. 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.

24. 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.

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.

- 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.
- 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.
- Provision must be made to ensure that inoculated animals or insects cannot escape.
- 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.
- 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.
- Gloves and safety glasses should be worn by animal care providers while feeding and watering animals or cleaning cages.
- Gloves, boots, floors, walls and cage racks should be disinfected frequently.
- 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.
- The appropriate species must be selected for the animal experiments.
- 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.
- 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.
- 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.
- 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 Blood-borne 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 “blood-borne 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 blood-borne 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 blood-borne 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 blood-borne 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. Therefore, the same safe standards of practice should be used routinely with all samples. 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.
Researchers must assess the risk and identify the strategies that will decrease exposure risk and prevent transmission of microorganisms.
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 of Western University at risk, free of charge. LHSC and SJHC require that Hepatitis B vaccinations be completed prior to employment being offered to hospital employees.

Personal Protective Equipment

PPE is used alone or in combination to prevent exposure, by placing a barrier between the infectious source and one’s own mucous membranes, airways, skin and clothing. The selection of PPE is based on the risk assessment. PPE must be donned prior to entering the containment zone. 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

- 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.
- Eating, drinking, smoking, applying cosmetics or lip balm, and handling contact lenses are prohibited (S.32 of O.Reg. 67/93).
- Jewellery that may become contaminated or compromise PPE must be removed or covered appropriately prior to entering the containment zone (CBS section 4.4.12)
- Clean hands before putting on gloves for a clean/aseptic procedure.
- 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, tissue, secretions, excretions, or equipment and environmental surfaces contaminated with the above. Gloves should also be worn 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.
- Masks and protective eyewear or face shields should be worn to protect mucous membranes of the nose, eyes and mouth during procedures that are likely to generate droplets, sprays, or splashes of blood or other body fluids to prevent exposure of mucous membranes of the mouth, nose, and eyes.
- Gowns or aprons should be worn during procedures that are likely to generate splashes or sprays of blood, body fluids, secretions, or excretions. Protective clothing should be removed before leaving the area.
- 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 and when exiting the containment zone or barrier as per CBS section 4.4.19.
- 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 needle-stick 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.
- Mouthpieces, resuscitation bags, or other ventilation devices should be available for use in areas in which the need for resuscitation is predictable.
- 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 be covered with leak proof bandaging utilizing protective barriers to reduce the risk of exposure.

- Pregnant workers should be especially familiar with and strictly adhere to precautions to minimize the risk of prenatal transmission of blood borne pathogens.

4.6 Medical Surveillance and Immuno-prophylaxis

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. LHSC and SJHC require proof of vaccination before employment begins.
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 -Bloodborne Pathogen Exposure

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 needle-stick, cut, animal bite or scratch, via mucous membrane contact, or via non-intact skin contact.

Worker:

1. The exposed site must be washed immediately.
   1.1. In case of a needle-stick, 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.

   1.2. If mucous (eyes, nose, mouth) membrane or non-intact (cuts, rash, eczema or dermatitis) skin contact, flush with water at the nearest eye wash station or sink for a minimum of ten minutes. In the event of contact with eyes, diphoterine should be used if available.

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 WORS (SJHC) or an online safety 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 learners 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.

4. Inform the BSO at Lawson of the exposure.

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 learners as long as confidential information is omitted.

Workplace Health (Western staff and learners):

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, immuno-prophylaxis 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

St. Joseph's Health Care:

OHSS Department: ext.64332
Urgent Care Centre: ext.67021
Security (for after-hours, non-emergencies): ext.44555
Emergencies: 55555

London Health Sciences Centre:

University Hospital OHSS Department: ext.33201
Victoria Hospital OHSS Department: ext.52286; after hours: 75066
Critical Injury Reporting After Hours Pager: 19915
Security: ext.44555

Emergencies: 55555

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

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, Oxivir/Accel 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, Oxivir/Accel) 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 towel 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 weight/volume) or Oxivir/Accel 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 at minimum:

- The international biohazard warning symbol
- Containment level
- Required PPE
- Entry requirements
- Emergency contact information

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 3).

Laboratory doors must be separated from the public and administrative areas by a lockable door, and only authorized personnel are permitted to enter laboratory work areas via a restricted controlled access system. Any area outside of the containment zone where biohazardous materials are stored must have a biohazard warning sign posted on the equipment containing all the same information that is posted on the containment zone door and be kept locked at all times (e.g., a freezer in a common equipment room that contains risk group 2 pathogens).

Figure 3 - Representative biohazard warning signage

4.10 Containment Level 2 Requirements

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.

- Biohazard signage (see Figure 3) 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.
- Non-laboratory visitors to the CL2 laboratory area must be accompanied at all times and provided with guest lab coats that are to be worn at all times when in the containment zone.

- **Centrifugation of regulated materials that are primarily infectious or transmitted by inhalation to be carried out in sealed safety cups or rotors that are unloaded and opened in a BSC to prevent aerosol and infectious agent release**
- 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 for animals treated with a biological agent must be performed in a BSC.
- All surfaces are to be constructed from non-absorbent materials and able to be cleaned.
- Any potential Dual-Use Agent (SSBAs) must be stored in a secured and locked area when an authorized worker is not present in the immediate area of the agent.
- Lawson provides lab coats for all laboratory workers. Lab coats must be worn at all times while work is being conducted in the laboratory.

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 Standard Operating Procedure for working with lentivirus and lentivirus-based vectors, also refer to site-specific CL2+ procedures Appendix D).

- Access in and out of the virus room should be minimized to prevent inadvertent contamination of personnel or cross-contamination of cell lines. Containment laboratory must be kept locked at all times.
- All surfaces must be impervious to water.
- Must have special ‘sharps precautions’ in place.
- Must have dedicated CL2+ PPE that remains in the containment zone. This includes: safety glasses, 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.
- Infectious materials must be stored in a leak-proof container in a restricted area.
- Where possible, all activities should be conducted in a BSC, any activity that may produce an aerosol must be carried out in a BSC
- If using a centrifuge, closed containers with sealed safety cups must be used, and the rotor must be unloaded in the BSC. Must wait at least 5 minutes after centrifugation stops before opening the lid of the centrifuge.
- Mark the floor (with tape) to indicate dirty and clean sides
- Housekeeping and facilities maintenance workers must not enter the room unless accompanied by a qualified laboratory worker.
- Only lab personnel trained in CL2+ requirements may enter the CL2+ area.
- 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 *more important* 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 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:

Bacteria
Viruses
Fungi
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. ***Note: work with prions is not approved in Lawson laboratories.**

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 RG2 with specific additional physical and operational requirements (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:

- 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.
- 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.
- 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.
- 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.
- 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 and SOPs 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 to be manipulated, 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 and therefore it is important to always follow Universal Precautions.

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 from animal tissues

Primary cells cultured from animal tissue must be handled using Universal Precautions and proper lab techniques. 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

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 Human Cells, 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 components 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, but this is not regulated under the Human Pathogens and Toxins Act.

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;

- Mode of transmission and host range;
- Routes of infection (inhalation, injection, ingestion, contact with skin or mucous membranes);
- 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 PHAC document Canadian Biosafety Guideline - Lentiviral Vectors.
General Laboratory Practices for Working with Viral Vectors:

- Laboratory doors must remain closed.
- All handling of viral vectors must be performed in a certified BSC.
- When centrifuging liquids containing viral vectors/virus, safety-sealed rotor cups should be used, or the entire rotor must have a sealed lid.
- For aspiration of virus-containing liquid, use a serological pipette to manually remove the media and place into a liquid waste container within the BSC. The pipettes used to remove the media should also be disinfected in the BSC before they are removed to the biological waste stream. Lawson's Biological Safety Cabinet Safe Work Practice must be followed (Appendix A).

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. Well known pathogens have had pathogen risk assessments completed by the PHAC and the CFIA and have been assigned an appropriate risk group and containment level. These risk assessments have been developed into technical documents by the PHAC known as 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 and 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.

Included
For the purposes of the Human Pathogens and Toxins Act, a human pathogen or toxin includes:

- A substance that contains a human pathogen or toxin;
- Any synthetic form of the human pathogen or toxin.

Excluded
The Human Pathogens and Toxins Act does not apply to:

- A human pathogen or toxin that is in an environment in which it naturally occurs if it has not been cultivated or intentionally collected or extracted, including a human pathogen or toxin that
- Is in or on a human suffering from a disease caused by that human pathogen or toxin, or
- Has been expelled by a human suffering from a disease caused by that human pathogen or toxin, or
- Is in or on a cadaver, a body part or other human remains; or
- A drug in dosage form whose sale is permitted or otherwise authorized under the Food and Drugs Act or a human pathogen or toxin contained in such a drug.

6.2.1 Risk Group 1 (low individual and public health 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 immune-compromised individuals. If a worker is identified as being immune-compromised an individual risk assessment will be performed, and ability to complete this level of work will be determined dependent on the assessment and ability to devise an individualized plan to protect the health of the worker. Many biohazardous materials used at Lawson fall into this category, such as *Escherichia coli* DH5 competent bacteria which are widely used in molecular biology experiments.

6.2.2 Risk Group 2 (moderate individual risk, low public health 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 public health 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 public health 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.

As per schedule 5 Variola virus is a prohibited human pathogen.

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. Cultured material is higher risk than primary sample due to increased pathogen load. 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.

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, the disinfectant must be validated and be tested against the biological agent to determine the concentration and contact time required to achieve the objective under the conditions employed. This process must be documented and submitted to the BSO to keep on file.

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 and treated offsite by a third party, Daniels.

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.

6.3.4 Risk Assessments

At the time of LBAPP renewal or modification Principal Investigators will assess the risk of the biological agents and toxins that they are using or proposing to use in their research program using the Lawson Local Biosafety Risk Assessment form (Appendix F). This form identifies the risk group, activities performed, engineering controls, necessary PPE, disposal and decontamination procedures necessary for the safe use of the biological material. Local Risk Assessments should be reviewed annually.

6.4 Biosafety Containment Levels

Containment level refers to the minimum physical containment and operational practices required for a containment zone where infectious material or toxins can be safely handled. 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. The Occupational Health & Safety Act and applicable regulations still outlines safe work requirement. Universal precautions and good microbiological laboratory practices should be listed under section 4.3 of this document must still be followed since RG1 biological material does pose a low risk to the health of individuals or animals.

Physical Containment Recommendations

- Separated from public and administrative areas by door.
- Dedicated computer/paper work stations are segregated from work stations where RG1, animals or biological material is handled
- Size of door openings to allow passage of all anticipated equipment.
- Surfaces to be resistant to scratches, stain, moisture, chemical and heat in accordance with laboratory function (recommended).
- Surfaces to provide impact resistance in accordance with laboratory function (recommended).
- Interior coatings (floors and walls) to be resistant to gas and chemicals in accordance with laboratory function (e.g., will withstand chemical disinfection, fumigation) (recommended).
- Bench tops to have no open seams (recommended).
- Bench tops to contain spills of materials (e.g., with marine edges and drip stops) (recommended).
- Benches, doors, drawers, door handles, etc. to have rounded rims and corners (recommended).
- Backsplashes, if installed tight to wall, to be sealed at wall-bench junction (recommended).
- Reagent shelving to be equipped with lip edges (recommended).
- Drawers to be equipped with catches, i.e., to prevent the drawer from being pulled out of the cabinet (recommended).
- Cabinet doors not to be self-closing (recommended).
- Autoclave or other acceptable means of waste treatment/disposal to be provided (recommended).
- Windows, if they can be opened, to be protected by fly screens.
- Hooks to be provided for laboratory coats at laboratory exit; street and laboratory clothing areas to be separated.
- Hand-washing sinks to be located near the point of exit from the laboratory or in anteroom.
- Lab work areas must be located outside of animal housing rooms
- Animal cages and housing rooms must prevent animal escape

Operational Recommendations

Good microbiological laboratory 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 techniques. The legislation administered by the PHAC and the CFIA does apply to CL2 facilities as outlined below.

Physical Containment Requirements

- Controlled access limited to authorized personnel.
- Laboratory room doors to have appropriate signage (e.g., biohazard sign, containment level, contact information, entry requirements, PPE).
- Where unique hazards exist, project-specific signage to be posted at animal room, animal cubicle, and post mortem room points of entry.
- Doors to the containment laboratory are lockable (this does not apply to areas within the containment laboratory).
- 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.
- Surfaces that come into contact with regulated or infectious material must be continuous with adjacent and overlapping materials
- Surfaces and interior coatings (including walls, case work, 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.
- Floors must be slip-resistant in accordance with function.
- Autoclave or other acceptable means of waste treatment/disposal is to be provided.
- Windows, if they can be opened, are to be protected by fly screens.
- Hooks are to be provided for laboratory coats at laboratory exit; street and laboratory clothing areas are to be separated.
- Hand-washing sinks are to be located near the point of exit from the laboratory or in anteroom.
- Hand-washing sinks are to be provided with "hands-free" capability (recommended).
- 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.
- Decontamination technologies to be provided with monitoring and recording devices that capture operational parameters.
- An autoclave must be capable of operating at the appropriate temperature for decontamination, as determined by validation, if being used as a method of decontamination and calibrated annually. Autoclaves must be validated annual with a representative load.
- Emergency eyewash and shower equipment to be provided in accordance with activities.

- Biological Safety Cabinets (BSCs) and other primary containment devices are to be provided and certified annually. Examples for use include procedures with the potential for producing aerosols and those involving high concentrations, large volumes or particular types of agents.
- 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.
- BSCs must be located as far as possible from high traffic areas, doors, openable windows, and air supply/exhaust diffusers.
- Process equipment, closed systems, and other primary containment devices must be designed to prevent the release of infectious materials/toxins.
- Vacuum systems must be equipped with a mechanism to prevent internal contamination or release of regulated/infectious materials (inline filters).
- 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.

- A biosafety program must be in place for the oversight of safety and containment practices.
- A biosafety representative (i.e., designated BSO) must be designated for the oversight of biosafety and biosecurity practices.
- Contact information provided to the PHAC and the CFIA, as applicable, to be kept up-to-date.
- Program intent must be documented and kept up-to-date.
- An overarching risk assessment must be conducted and documented to identify the hazards present and appropriate mitigation strategies for the proposed activities involving biohazards.
- A biosecurity risk assessment must be conducted and documented.
- A biosecurity plan must be developed, implemented, evaluated and improved as necessary, and kept up-to-date.
- 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.
- A training needs assessment must be conducted.
- 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
- A medical surveillance program must be developed, implemented, and kept up-to-date,
- Emergency medical contact cards to be issued to personnel handling non-human primates or a biohazard identified in an LRA.
- A respiratory protection program (i.e. fit-testing) to be in place where respirators are used.
- A training program to be implemented, evaluated, and improved as necessary, and kept up-to-date.
- 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).
- An Emergency response plan (ERP), to be developed, implemented, and kept up-to-date.
- Containment zone personnel must immediately notify appropriate personnel if an incident occurs that could result in an exposure to a biohazard.

- Good microbiological laboratory practices intended to avoid the release of infectious agents are to be employed.
- 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.
- 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.
- Entry must be restricted to laboratory staff, animal handlers, maintenance staff and others on official business.
- 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.
- 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 following measures, in addition to CL2 requirements, are to be followed.

- There must be a program in place (with appropriate authority to oversee safety and containment practices) for the management of biological safety issues.
- General operational protocols must be supplemented with protocols similar to each project in progress.
- Personnel must have demonstrated proficiency in microbiological practices and techniques.
- 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.
- Personnel entering the containment laboratory must use full coverage protective clothing (i.e., completely covering all street clothing). When a known or suspected exposure may have occurred, all clothing, including street clothing, requires appropriate decontamination.
- 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).
- Centrifugation of infectious materials must be carried out in closed containers placed in sealed safety cups or rotors that are unloaded in a BSC.
- 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.

- 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.

6.5.2 Animal Work Considerations

- Viewing of animals through containment barrier windows by unauthorized individuals is to be prevented
- Procedures used must minimize injury to personnel (includes scratches, bites, kicks, crushing injures, inadvertent self-inoculation)
- Handle animals in the assigned regulated containment level
- Animals must be contained in a primary containment device to prevent accidental release.
- Animals moved within the containment zone must be moved safety and securely to prevent escape and contamination
- Inoculation, surgical or necropsy procedures with animals in small animal zones to be carried out in a BSC
- Primary containment caging or animal cubicles housing regulated animals must be labelled identifying regulated or infectious material used
- Procedures involving regulated or infectious materials must be carried out in manner that prevents aerosols and dissemination of dust or other particulate matter from cages
- Animal carcasses must be moved in a manner that prevents spread of contamination such as a labelled, leak proof, impact resistant container that has been surface decontaminated

REFERENCES

Canadian Biosafety Standards,3rd Edition, 2023
Canadian Biosafety Handbook, 1st Edition, 2015
University of Western Ontario Biosafety Manual, 2015
American Biological Safety Association: Principles and Practices of Biosafety, 2015
Public Health Agency of Canada website, 2022

Appendix A: Use of the Biological Safety Cabinet

BACKGROUND

This Standard Operating Procedure (SOP) will provide details and instruction on the safe operation of biological safety cabinets (BSC) in Lawson research laboratories. 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) cell lines must be performed in a certified BSC, in a containment level 2 (CL2) laboratory.

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.

PROCEDURE

This SOP applies to all staff and affiliates working within Lawson Health Research Institute (Lawson) who use a BSC for 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. This training can be provided by Lawson Health and Safety or the supervisor and must be documented. Refresher training should be provided annually by the supervisor. Personnel should also have a basic understanding of the different classes/types of BSCs and how they operate.

1. **Pre-Start-Up Activities**
   - 1.1. Don the appropriate personal protective equipment (PPE) as determined by the nature of the work and the local risk assessment.
   - 1.2. Check that the sash is at the appropriate height. Adjust seat height so that the user's underarms are level with the bottom of the sash.
   - 1.3. 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).
   - 1.4. Check the pressure gauges to verify that readings are within the acceptable range and indicated in the manual.
   - 1.5. If present, test the air flow alarm and ensure it is switched to the "on" position.
   - 1.6. Confirm inward flow by holding a tissue at the middle of the edge of the sash to establish that it is drawn in.
   - 1.7. Disinfect the interior surfaces with a disinfectant effective against the infectious material and toxins used in the laboratory, allowing for the appropriate contact time.
   - 1.8. Assemble all materials required for the experiment and load into the BSC. Care should be taken not to overcrowd or block the front or rear grilles.

1.9. Place aerosol-generating equipment (e.g. vortex mixer, sonicator) towards the back of the BSC, without blocking the rear grille.

1.10. After loading material in the BSC, allow sufficient time for the air to purge and the airflow to stabilize before initiating work (at least 10 minutes).

2. **Working in the BSC**

2.1. Work in a BSC should only be conducted by one (1) person at a time.

2.2. Perform all operations as far to the rear of the work area as possible. Ensure that elbows and arms do not rest on the grille or work surface.

2.3. Avoid excessive movement of hands and arms through the front opening. These movements disrupt the air curtain at the front of the BSC, which can allow contaminants to enter or escape the BSC.

2.3.1. Arms should enter and exit the BSC slowly and perpendicular to the front opening.

2.4. Keep a bottle of an appropriate disinfectant in the BSC while work is performed to avoid having to move hands outside of the BSC.

2.5. Segregate non-contaminated (“clean”) items from contaminated (“dirty”) items. Work should always flow from clean to dirty areas.

2.6. Material should be discarded in a waste container located towards the rear of the cabinet. Do not discard materials in containers outside of the cabinet.

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

2.8. If a spill occurs, decontaminate the surfaces of all objects in the cabinet with an appropriate disinfectant while the cabinet is still in operation.

3. **Completion of Work in the BSC**

3.1. Upon completion of work, allow sufficient time for the air in the BSC to purge before disrupting the air curtain by removing hands or unloading material.

3.2. Close and cover all containers.

3.3. Surface decontaminate items before removing them from the BSC, including the exterior of the waste containers.

3.4. Remove contaminated gloves, and dispose of as contaminated waste.

3.4.1. Wash and dry hands thoroughly.

3.5. Don clean gloves and using a suitable disinfectant, clean all interior surfaces of the BSC, including the sides, back, lights, and interior of the glass, allowing for the appropriate contact time.

3.6. The BSC should undergo a periodic thorough cleaning. See the “Cleaning the BSC” SOP for instructions.

NOTE: The use of Ultra Violet (UV) lights in the BSC must be carefully considered, and is in fact strongly discouraged as the sole means of decontamination. UV germicidal lamps have a very limited effectiveness as a surface disinfectant. 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.

DEFINITIONS

Affiliates – Individuals who are not employed by the organization but perform specific tasks at or for the organization, including:

- Credentialed Professional Staff with a hospital appointment (e.g. physicians, midwives, dentists),
- Learners,
- Volunteers*,
- Contractors or contracted workers who may be members of a third-party contract or under direct contract with the organization, and
- Individuals working at the organization but funded through an external source.

REFERENCES

Canadian Biosafety Standard, Third Edition - Canada.ca
Canadian Biosafety Handbook, Second Edition, 2015

Corporate
Waste Management Manual
Hand Hygiene

Departmental
Donning and Doffing Personal Protective Equipment
Cleaning the Biological Safety Cabinet

Appendix B: Lawson Autoclave Safe Work Practice

PURPOSE

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. All autoclave users should be trained by a competent trainer and all training should be documented.

DEFINITIONS

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

SCOPE

This Safe Work Practice (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.

RESPONSIBILITIES

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

PROCEDURE

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

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).

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!!! 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 and bottles should not be overfilled. 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

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**

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, 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:

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 polypropylene (PP) and **do not** have good steam permeability; adding some water to the bag before autoclaving will facilitate steam production inside of 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.

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

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.

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.

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

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 the 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., steri-pouches 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.

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

BACKGROUND - Best Microbiological Practices

The Public Health Agency of Canada categorizes various diseases in levels of biohazard: Level 1 being minimal risk and Level 4 being extreme risk. Containment zones are designated to handle different levels of biohazardous agents. Containment level 1 zones are use to perform research mostly on non-infectious microbes using standard equipment and routine lab safety procedures. Containment Level 2 zones are used to handle bacteria and viruses that cause only mild disease to humans, or are difficult to contract via aerosol in a lab setting.

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 Biological Safety Cabinets (BSC) and Personal Protective Equipment (PPE). A risk assessment should be conducted to identify any potential deficiencies in the practices of the laboratory workers, and where extra protection measures need to be implemented.

Careless procedures and practices are a serious concern in the laboratory, because it can compromise any safeguards in place and increase the risk of pathogen exposure to everyone. In order to reduce the inherent risks while working with biohazardous agents, staff and learners require:

- Laboratory and biosafety training;
- Knowledge of the agent and the hazards associated with it;
- Experience in proper handling of the agent;
- Knowledge of the hazards associated with the procedures being used (aliquoting, centrifuging, etc.);
- Knowledge of safe laboratory practices; and
- Concern for their health, as well as all the other laboratory staff.

New laboratory staff and learners will require supervision, training and mentoring to acquire these important skills. Laboratory directors or principal investigators must train and retrain new research team members until they are confident in the workers' abilities to complete the techniques safely, efficiently and correctly on their own.

There are 3 main elements of safe containment of microorganisms.
1. Good lab practices and technique
2. Safety equipment
3. Facility design

Best microbial safe practices include:

- Legs must be completely covered when human or zoonotic pathogens are handled and lab coats must be worn
- Shoes must completely cover the feet
- Jewelry that compromises PPE or elevates the danger of exposure should be removed
- Long hair must be pulled back to prevent entry into the working area
- Gloves must be worn at all times while working in the BSC when handling Risk Group 2 and higher pathogens

- Personal items and electronics are not be used or taken into CL2 laboratories

1. Treat all microorganisms as potential pathogens. Workers who have compromised immune systems or recent extended illnesses should talk with their supervisor before working in a microbiology lab.

2. Familiarize yourself with the location of safety equipment in the lab.

3. Set up the Biological Safety Cabinet (BSC) to allow for movement of items from a clean side to a dirty side (Figure 1). This will assist with keeping the employee and the work area free from contamination.

4. Label everything clearly. If hazardous, label the items with the proper warning and hazardous information.

5. Clean up spills with care, following the spill procedure.

6. Doff all PPE in the appropriate area inside the containment zone.

7. Before leaving the containment zone, the employee must perform effective hand hygiene with soap and water.

8. Hang the lab coat in a designated area away from personal belongings to avoid cross contamination of personal clothes, chairs, paperwork etc.

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

BACKGROUND

This Standard Operating Procedure (SOP) will provide details and instruction on the safe production, use and handling of all human pathogenic lentivirus-based vectors. All lentiviral work will be conducted under containment level 2 conditions with additional operational precautions (CL2+). The viruses have the ability to integrate into host chromosomes, can infect non-dividing and terminally differentiated cells and have high rates of mutation. The major risk from working with human pathogenic lentivirus-based vectors is the generation of replication-competent lentivirus, 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.

This SOP applies to all staff and affiliates that work within Lawson Health Research Institute 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.

PROCEDURE

1. A designated CL 2+ room must be assigned and equipped with the necessary equipment before work with lentivirus may commence. The room must contain:
   1.1. Functional BSC that is inspected annually.

   1.2. Methods for disposal of waste.

   1.3. Door that must be lockable.

   1.4. Sink for hand washing.

   1.5. Dedicated area for personal protective equipment (PPE) storage.

2. Principal Investigators are responsible for reviewing this SOP and the hazards of working with lentivirus with their staff and affiliates prior to work commencing.
   2.1. SOP should be reviewed annually with research staff handling lentivirus.

   2.2. All necessary PPE must be provided, including:
      2.2.1. A dedicated lab coat or solid front disposable gown.
      2.2.2. Nitrile gloves (double gloved).
      2.2.3. Safety glasses.

3. Staff and affiliates handling lentivirus should review and understand this SOP before commencing work with Lentivirus.
   3.1. Staff and affiliates must understand that handling of lentivirus must be done in a Biological Safety Cabinet (BSC), and 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.

   3.2. Vacuum lines are NOT to be used as a method of aspiration of media off of the cells infected with lentivirus. Media must be manually aspirated and disposed of in a waste container

containing 10% bleach for a minimum of 30 minutes before it can be disposed of down the drain.

3.3. Long pants must be worn. NO shorts, skirts or capris. Closed–toe/heel shoes are to be worn in the containment zone; long hair must be tied back; all jewelry must be removed before entering the containment zone.

4. Access in and out of the room should be minimized to prevent inadvertent contamination of personnel or cross-contamination of cell lines.

5. Containment laboratory must be kept locked at all times.

6. Whenever possible, work with lentivirus during normal working hours to enable the best response possible to an adverse event. (If it is not possible, the working alone policy for Lawson must be followed.)

7. Experiments should be carefully reviewed for all reagents and supplies necessary before initiating any work to minimize trips into the room.

8. Only items used in the virus room should be stored in the virus room.

9. No organisms used in the virus room may be used or transferred to any other tissue culture facility within the building until the virus is deemed inactive by passage or decontamination.

10. No food/drink/cosmetics are allowed in the designated room.

11. All personnel using the virus room must receive training from a qualified instructor, with experience using the specific class of viral agent to be used, prior to initiating any experiments.

12. All individuals using the virus room should be trained in sterile technique and standard tissue culture procedures by their supervisor prior to receiving training on lentivirus handling.

13. A list of approved users will be posted on the entry door. (List will be updated as users are added/removed).

14. Prepare decontamination solutions as specified prior to commencing work.

15. BSC should be decontaminated before and after use.
    15.1. When finished work inside the BSC, decontaminate the outer surface of everything that is inside.

    15.2. Remove all items from the cabinet.

    15.3. Wash the BSC thoroughly with 70% alcohol or another approved disinfectant.

    15.4. Note: the use of ultra-violet light to disinfect the hood is not recommended because it is not effective unless the bulb is properly maintained.

16. All materials should be surface decontaminated before removing from the BSC.

17. Do not touch anything outside of the BSC without first doffing the outer pair of gloves.

18. If the BSC is not working properly, work must be put on hold and the Biosafety Officer (BSO) should be notified immediately.

19. Refrigerators are only for reagents, stock solutions, and media used in the virus room.

20. All stock solutions and media must be properly labeled with the chemical name, user name and date of preparation or purchase.

21. All containers used to store samples should be labeled with a biohazard symbol and the name of the organism

22. All reagents, stock solutions and media no longer in use must be properly discarded.

23. The compound microscope is dedicated to work in the virus room only. (If the microscope is not working properly, contact the BSO).

24. All disposable lab ware and PPE generated in the virus room should be disposed of in a biohazard waste bin or autoclaved within the room (Autoclave use requires monitoring and regular verification of representative loads).
    24.1. Anyone that operates the autoclave must be trained by an authorized autoclave use instructor, and the training must be documented on a log sheet.

25. Always transport biohazardous material outside the virus room in an unbreakable, well-sealed primary container placed inside of a sealed, leak-proof, and unbreakable secondary container. The container should be labeled with the contents.

26. If a spill occurs, follow the appropriate spill response (see Biological Spill SOP).

27. If staff and/ or affiliate are exposed:
    27.1. On their skin, then wash the affected area thoroughly using antimicrobial soap and report the incident to the BSO.
    27.2. In their eyes, immediately run water for 15 minutes using an eyewash and hold eye lids open to ensure effective wash behind the eyelids. Report the incident to the BSO.
    27.3. By a needle stick or puncture wound, wash the affected area thoroughly using antimicrobial soap for 5 minutes and report to the BSO.
    27.4. By inhalation, report to the BSO and if feeling unwell seek medical assistance.

DEFINITIONS

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

Affiliates – Individuals who are not employed by the organization but perform specific tasks at or for the organization, including:

- Credentialed Professional Staff with a hospital appointment (e.g. physicians, midwives, dentists),
- Learners,
- Volunteers,
- Contractors or contracted workers who may be members of a third-party contract or under direct contract with the organization, and
- Individuals working at the organization but funded through an external source.

REFERENCES

Canadian Biosafety Standard, Third Edition - Canada.ca
Canadian Biosafety Guideline: Lentiviral Vectors - Canada.ca
http://en.wikipedia.org/wiki/Lentivirus

Departmental

Biological Spill Response Guide
Working Alone or in Isolation

Appendix E: Lawson Biosecurity Plan

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 (University Hospital and Victoria Hospital) has a licence for each hospital for all research using controlled biohazardous materials. Section 4.1.8 of the Canadian Biosafety Standard (CBS) requires that facilities using biological agents have a biosecurity plan in place.

Lawson has two levels of Biosafety oversight, consisting of a Biosafety Oversight Committee and a Biohazards Sub-Committee. Membership is described in Chapter 3 of the Biosafety Manual. 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 SJHC 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.

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:

- 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;

[1] Canadian Biosafety Standard, Third Edition, 2023
[2] NSABB: *Enhancing Responsible Science – Considerations for the Development and Dissemination of Codes of Conduct for Dual Use Research*, 2010

- 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 for bioterrorism. 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 (trigger quantity) in Section 10(2) of the HPTR. A toxin present in a facility in a quantity below the trigger quantity is not an SSBA; however, it remains a regulated toxin and subject to the requirements in the CBS.

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).

1. **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:

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

2. **Designation of a License Holder**

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

The Licence Holder, SJHC/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, exposure, 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.

3. **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.

3.1. **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).

3.2. **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.

3.3. **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.

3.4. **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 SJHC 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 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 SJHC London or LHSC.

Figure 1: Risk Assessment Matrix[3]

4. Biosecurity Risk Mitigation Strategies

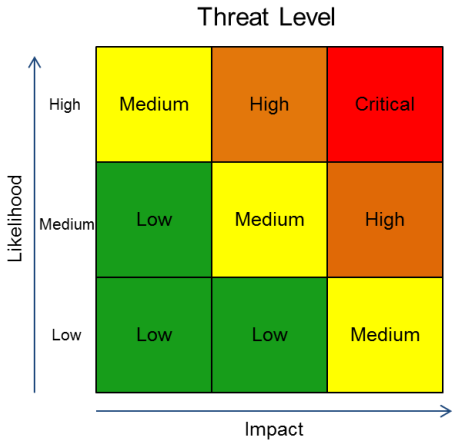
4.1. Physical Protection and Security

Lawson, at SJHC and LHSC, implements graded protection based on the biosecurity risk of materials. Methods include:

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

4.2. 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. 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.

4.3. 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;

[3] ABSA Principles and Practices of Biosafety, 2015

Record of transfers within and outside Lawson labs (at SJHC 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).

4.4. 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 SJHC or LHSC Disaster and Emergency Response Manuals for information on biohazard spill response procedures and other emergency procedures (e.g., fire, evacuation).

The SJHC Workplace Occurrence Reporting 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 online safety system 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.

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;
- 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.

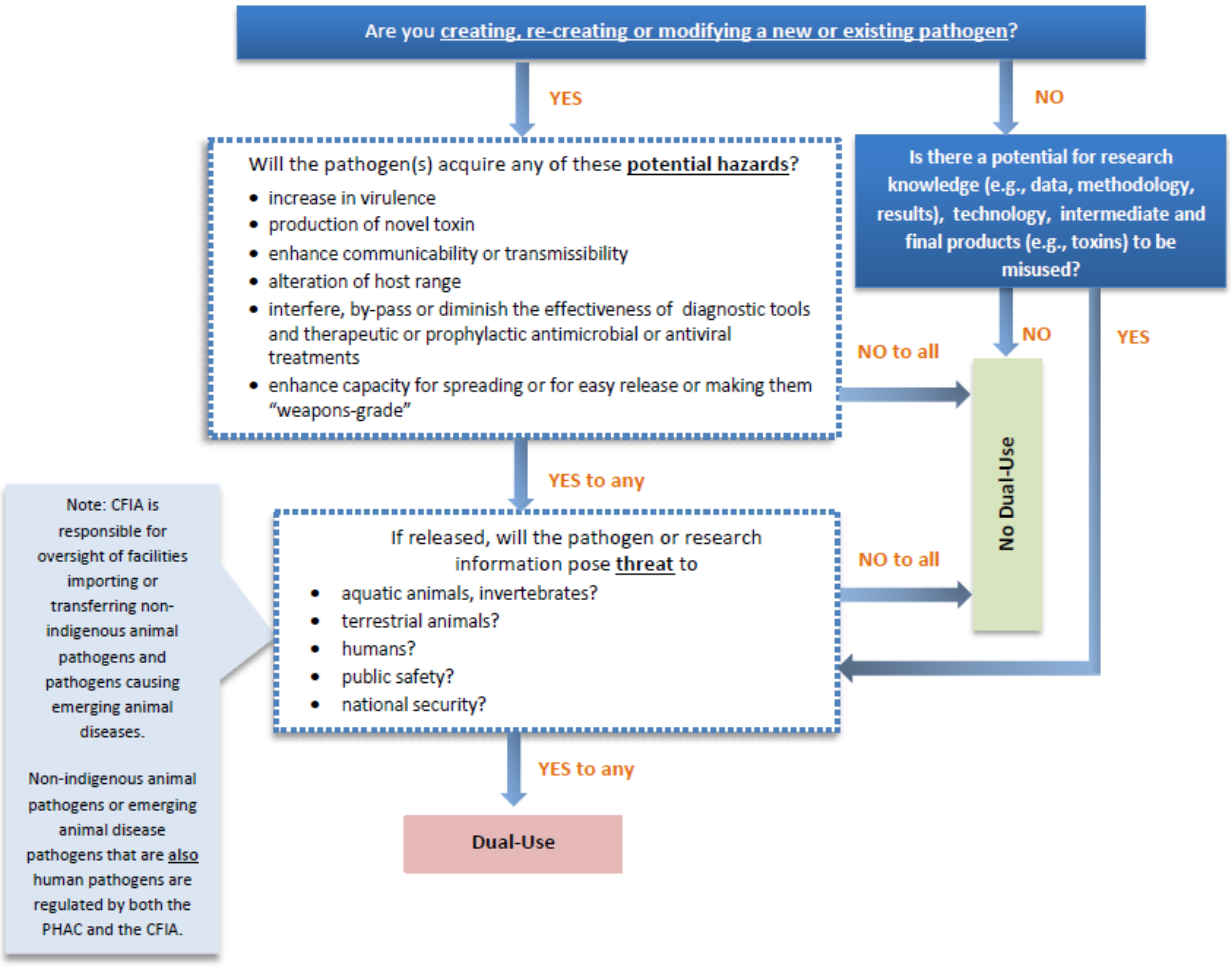


Figure 3. PHAC Decision Tree: Identification of Dual-Use Potential in Life Sciences Research

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.

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).

Appendix G - Local Biosafety Risk Assessment

Type of Agent Used	Risk Group/ Containment Level	Activities (pipetting, vortexing, centrifuging, etc.) and Location	Potential Routes of Exposure / Frequency of contact (Please select all that apply)	Mitigation Strategies and Decontamination Procedures / PPE required(Please select all that apply)
Human tissue/primary cells/body fluid			☐ Inhalation ☐ Ingestion ☐ Skin ☐ Eye contact ☐ Injection/needle stick ☐ Routine/daily ☐ Weekly ☐ Random/ monthly/yearly	☐ Biological Safety Cabinet ☐ Centrifuge sealed rotors ☐ SOP required (please specify) ☐ Lab coat ☐ Disposable gown ☐ Gloves: (single/double) Eye protection: ☐ Safety glasses ☐ Safety goggles ☐ Face shield ☐ N95 Respirator ☐ Other (please specify) ☐ Bleach ☐ Autoclave ☐ 70% Ethanol ☐ Other(please specify)
Primate tissue/primary cells/body fluid			☐ Inhalation ☐ Ingestion ☐ Skin ☐ Eye contact ☐ Injection/needle stick ☐ Routine/daily ☐ Weekly ☐ Random/ monthly/yearly	☐ Biological Safety Cabinet ☐ Centrifuge sealed rotors ☐ SOP required (please specify) ☐ Lab coat ☐ Disposable gown ☐ Gloves: (single/double) Eye protection: ☐ Safety glasses ☐ Safety goggles ☐ Face shield ☐ N95 Respirator ☐ Other (please specify) ☐ Bleach ☐ Autoclave ☐ 70% Ethanol ☐ Other(please specify)

Sheep tissue/primary cells/body fluid			☐ Inhalation ☐ Ingestion ☐ Skin ☐ Eye contact ☐ Injection/needle stick ☐ Routine/daily ☐ Weekly ☐ Random/ monthly/yearly	☐ Biological Safety Cabinet ☐ Centrifuge sealed rotors ☐ SOP required (please specify) ☐ Lab coat ☐ Disposable gown ☐ Gloves: (single/double) Eye protection: ☐ Safety glasses ☐ Safety goggles ☐ Face shield ☐ N95 Respirator ☐ Other (please specify) ☐ Bleach ☐ Autoclave ☐ 70% Ethanol ☐ Other(please specify)
Pig tissue/primary cells/body fluid			☐ Inhalation ☐ Ingestion ☐ Skin ☐ Eye contact ☐ Injection/needle stick ☐ Routine/daily ☐ Weekly ☐ Random/ monthly/yearly	☐ Biological Safety Cabinet ☐ Centrifuge sealed rotors ☐ SOP required (please specify) ☐ Lab coat ☐ Disposable gown ☐ Gloves: (single/double) Eye protection: ☐ Safety glasses ☐ Safety goggles ☐ Face shield ☐ N95 Respirator ☐ Other (please specify) ☐ Bleach ☐ Autoclave ☐ 70% Ethanol ☐ Other(please specify)
Rodent tissue/primary cells/body fluid			☐ Inhalation ☐ Ingestion ☐ Skin ☐ Eye contact ☐ Injection/needle stick ☐ Routine/daily ☐ Weekly ☐ Random/ monthly/yearly	☐ Biological Safety Cabinet ☐ Centrifuge sealed rotors ☐ SOP required (please specify) ☐ Lab coat ☐ Disposable gown ☐ Gloves: (single/double) Eye protection:

				☐ Safety glasses ☐ Safety goggles ☐ Face shield ☐ N95 Respirator ☐ Other (please specify) ☐ Bleach ☐ Autoclave ☐ 70% Ethanol ☐ Other(please specify)
Adenovirus			☐ Inhalation ☐ Ingestion ☐ Skin ☐ Eye contact ☐ Injection/needle stick ☐ Routine/daily ☐ Weekly ☐ Random/ monthly/yearly	☐ Biological Safety Cabinet ☐ Centrifuge sealed rotors ☐ SOP required (please specify) ☐ Lab coat ☐ Disposable gown ☐ Gloves: (single/double) Eye protection: ☐ Safety glasses ☐ Safety goggles ☐ Face shield ☐ N95 Respirator ☐ Other (please specify) ☐ Bleach ☐ Autoclave ☐ 70% Ethanol ☐ Other(please specify)
Lentivirus			☐ Inhalation ☐ Ingestion ☐ Skin ☐ Eye contact ☐ Injection/needle stick ☐ Routine/daily ☐ Weekly ☐ Random/ monthly/yearly	☐ Biological Safety Cabinet ☐ Centrifuge sealed rotors ☐ SOP required (please specify) ☐ Lab coat ☐ Disposable gown ☐ Gloves: (single/double) Eye protection: ☐ Safety glasses ☐ Safety goggles ☐ Face shield ☐ N95 Respirator ☐ Other (please specify) ☐ Bleach ☐ Autoclave ☐ 70% Ethanol ☐ Other(please specify)

Retrovirus			☐ Inhalation ☐ Ingestion ☐ Skin ☐ Eye contact ☐ Injection/needle stick ☐ Routine/daily ☐ Weekly ☐ Random/ monthly/yearly	☐ Biological Safety Cabinet ☐ Centrifuge sealed rotors ☐ SOP required (please specify) ☐ Lab coat ☐ Disposable gown ☐ Gloves: (single/double) Eye protection: ☐ Safety glasses ☐ Safety goggles ☐ Face shield ☐ N95 Respirator ☐ Other (please specify) ☐ Bleach ☐ Autoclave ☐ 70% Ethanol ☐ Other(please specify)
Bacteria Level 1	1		☐ Inhalation ☐ Ingestion ☐ Skin ☐ Eye contact ☐ Injection/needle stick ☐ Routine/daily ☐ Weekly ☐ Random/ monthly/yearly	☐ Biological Safety Cabinet ☐ Centrifuge sealed rotors ☐ SOP required (please specify) ☐ Lab coat ☐ Disposable gown ☐ Gloves: (single/double) Eye protection: ☐ Safety glasses ☐ Safety goggles ☐ Face shield ☐ N95 Respirator ☐ Other (please specify) ☐ Bleach ☐ Autoclave ☐ 70% Ethanol ☐ Other(please specify)
Bacterial Level 2	2		☐ Inhalation ☐ Ingestion ☐ Skin ☐ Eye contact ☐ Injection/needle stick ☐ Routine/daily ☐ Weekly ☐ Random/ monthly/yearly	☐ Biological Safety Cabinet ☐ Centrifuge sealed rotors ☐ SOP required (please specify) ☐ Lab coat ☐ Disposable gown ☐ Gloves: (single/double) Eye protection:

				☐ Safety glasses ☐ Safety goggles ☐ Face shield ☐ N95 Respirator ☐ Other (please specify) ☐ Bleach ☐ Autoclave ☐ 70% Ethanol ☐ Other(please specify)
Plasmids			☐ Inhalation ☐ Ingestion ☐ Skin ☐ Eye contact ☐ Injection/needle stick ☐ Routine/daily ☐ Weekly ☐ Random/ monthly/yearly	☐ Biological Safety Cabinet ☐ Centrifuge sealed rotors ☐ SOP required (please specify) ☐ Lab coat ☐ Disposable gown ☐ Gloves: (single/double) Eye protection: ☐ Safety glasses ☐ Safety goggles ☐ Face shield ☐ N95 Respirator ☐ Other (please specify) ☐ Bleach ☐ Autoclave ☐ 70% Ethanol ☐ Other(please specify)
Primary plant or insect cells			☐ Inhalation ☐ Ingestion ☐ Skin ☐ Eye contact ☐ Injection/needle stick ☐ Routine/daily ☐ Weekly ☐ Random/ monthly/yearly	☐ Biological Safety Cabinet ☐ Centrifuge sealed rotors ☐ SOP required (please specify) ☐ Lab coat ☐ Disposable gown ☐ Gloves: (single/double) Eye protection: ☐ Safety glasses ☐ Safety goggles ☐ Face shield ☐ N95 Respirator ☐ Other (please specify) ☐ Bleach ☐ Autoclave ☐ 70% Ethanol ☐ Other(please specify)

Established cell lines (Level 1)	1		☐ Inhalation ☐ Ingestion ☐ Skin ☐ Eye contact ☐ Injection/needle stick ☐ Routine/daily ☐ Weekly ☐ Random/ monthly/yearly	☐ Biological Safety Cabinet ☐ Centrifuge sealed rotors ☐ SOP required (please specify) ☐ Lab coat ☐ Disposable gown ☐ Gloves: (single/double) Eye protection: ☐ Safety glasses ☐ Safety goggles ☐ Face shield ☐ N95 Respirator ☐ Other (please specify) ☐ Bleach ☐ Autoclave ☐ 70% Ethanol ☐ Other(please specify)
Established cell lines (Level 2)	2		☐ Inhalation ☐ Ingestion ☐ Skin ☐ Eye contact ☐ Injection/needle stick ☐ Routine/daily ☐ Weekly ☐ Random/ monthly/yearly	☐ Biological Safety Cabinet ☐ Centrifuge sealed rotors ☐ SOP required (please specify) ☐ Lab coat ☐ Disposable gown ☐ Gloves: (single/double) Eye protection: ☐ Safety glasses ☐ Safety goggles ☐ Face shield ☐ N95 Respirator ☐ Other (please specify) ☐ Bleach ☐ Autoclave ☐ 70% Ethanol ☐ Other(please specify)
Biological Toxins			☐ Inhalation ☐ Ingestion ☐ Skin ☐ Eye contact ☐ Injection/needle stick	☐ Biological Safety Cabinet ☐ Centrifuge sealed rotors ☐ SOP required (please specify)

			☐ Routine/daily ☐ Weekly ☐ Random/ monthly/yearly	☐ Lab coat ☐ Disposable gown ☐ Gloves: (single/double) Eye protection: ☐ Safety glasses ☐ Safety goggles ☐ Face shield ☐ N95 Respirator ☐ Other (please specify) ☐ Bleach ☐ Autoclave ☐ 70% Ethanol ☐ Other(please specify)
Hormones			☐ Inhalation ☐ Ingestion ☐ Skin ☐ Eye contact ☐ Injection/needle stick ☐ Routine/daily ☐ Weekly ☐ Random/ monthly/yearly	☐ Biological Safety Cabinet ☐ Centrifuge sealed rotors ☐ SOP required (please specify) ☐ Lab coat ☐ Disposable gown ☐ Gloves: (single/double) Eye protection: ☐ Safety glasses ☐ Safety goggles ☐ Face shield ☐ N95 Respirator ☐ Other (please specify) ☐ Bleach ☐ Autoclave ☐ 70% Ethanol ☐ Other(please specify)

Please add rows as required

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)
Coxiella burnetii	Chlamydophila psittaci (formerly known as Chlamydia psittaci)
Rickettsia prowazekii	Francisella tularensis
Yersinia pestis	
Note: these are all RG3 for humans and animals	

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 the toxin trigger quantity is exceeded, an HPTA security clearance is needed.