University of Toronto

BIOSAFETY POLICIES AND PROCEDURES MANUAL

2007

Emergency Contact Information:

For medical emergencies:	**911**
Weekdays (9am-5pm):	
University Biosafety Office	(416) 978-3981
Office of Environmental Health & Safety	(416) 978-4467
Other times:	
University of Toronto Police:	
Mississauga	(905) 569-4333
Scarborough	(416) 287-7333
St. George	(416) 978-2222

Emergency medical procedure:

1. The exposed site must be washed immediately.

   (i) If needlestick, cut, puncture wound, animal bite or scratch, wash with soap and water after allowing the wound to bleed freely.

   (ii) If mucous (eyes, nose, mouth) membrane or non-intact (cuts, rash, acne or dermatitis) skin contact, flush with water at the nearest faucet or eye wash station.

2. The worker must immediately inform the supervisor / principal investigator of the exposure incident.

3. The worker must seek prompt medical attention at the nearest hospital emergency department or emergency clinic or a medical practitioner of their choosing.

Biohazardous Spills:

1. Evacuate the laboratory for a time sufficient for most aerosols to settle or be dispersed or removed by the ventilation system, (~ 20- 30 min) (respiratory protection should be considered for re-entry*).

2. Pour a strong disinfectant solution (sodium hypochlorite or Wescodyne) around, but not on the spill, and mix the disinfectant with the spilled material cautiously.

3. Remain outside the laboratory for a time expected to be sufficient for decontamination of the mixed material (~20 - 30 min).

4. Carefully absorb the liquid with absorbent paper and place into an autoclavable bag or other container suitable for autoclaving.

5. Decontaminate all surfaces exposed to the spill with a suitable disinfectant.

**Appropriate respiratory protection should be considered depending on the biological agents in use. The selection and use of appropriate respiratory protection can be determined in consultation with the Office of Environmental Health and Safety, and must be used in compliance with the U of T Respirator Standard (http://www.ehs.utoronto.ca/Training/training.htm#Respiratory).*

This page is intended as a quick reference in case of emergency. Please refer to Chapter 5 for additional information on Emergency Procedures and Accident Reporting.

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PREFACE

The Medical Research Council of Canada (MRC) published its Guidelines for the Handling of Recombinant DNA Molecules and Animal Viruses and Cells in 1977. Revised editions of those guidelines were published in 1979 and 1980. The University of Toronto Biosafety Committee (UTBSC), formed in 1977, adopted the MRC Guidelines for local use, but reserved the right to adjust practices and standards published by MRC when this seemed warranted. These guidelines applied to research projects conducted within the University or its affiliated institutions, irrespective of the source of the funds used.

In 1979, UTBSC established standards, similar to those used in research, for teaching laboratories in which animal viruses or cells are used. Two years later, UTBSC approved addition of bacteria, fungi and parasites to the list of agents to which biosafety standards would apply in University research and teaching laboratories and published its Guidelines for the Handling of Recombinant DNA Molecules, Animal Viruses and Cells, Microorganisms and Parasites.

In 1986, MRC and Health and Welfare Canada undertook an extensive revision of the 1980 MRC Guidelines and early in 1990 published Laboratory Biosafety Guidelines. The new document recognized the advantages of reducing the number of physical containment levels and the need to extend containment requirements to agents not included in earlier editions of the MRC Guidelines.

During the fall of 1990, UTBSC began a review of its existing Guidelines and the new Laboratory Biosafety Guidelines. The product of that review was the Biosafety Guidelines at the University of Toronto, 1992.

In 1996, Health Canada published the updated second edition of Laboratory Biosafety Guidelines. The University of Toronto Biosafety Committee subsequently initiated a review of its 1992 Guidelines. In 2004, a third edition of the Laboratory Biosafety Guidelines was released by Health Canada. The University of Toronto Biosafety Policy and Procedures Manual was revised to include these new additions and changes. Although some sections have been added, modified, or expanded with the intent of producing a more informative document for local use, the standards and practices specified herein are identical to, or consistent with, those described in the 2004 Health Canada publication.

Not specifically addressed or included in this document are activities involving:

- Risk Group 4 biological agents requiring Containment Level 4 conditions
- Field trials and deliberate environmental releases of hazardous biological agents

1 INTRODUCTION

This manual is intended to provide direction on the safe handling, transportation and disposal of hazardous biological materials in a laboratory setting. It specifies operational and containment requirements; legislative obligations and emergency protocols for research activities involving the use of biohazardous agents at the University of Toronto.

A biohazardous agent is a biologically derived material that poses a human health risk. The handling of these agents continues to present a risk of infection and mortality, despite increasing awareness of biosafety practices. The application of appropriate safety measurements and containment barriers, as part of a biosafety program, can minimize the risk to laboratory workers and prevent the release of the agents to the surrounding environment. The University of Toronto strives to ensure that all procedures with biohazardous agents are carried out in consideration of the health and safety of employees, students, the public and the environment.

2 BIOSAFETY PROGRAM FRAMEWORK

2.1 University of Toronto Health and Safety Policy

Under the provisions of the Ontario Occupational Health and Safety Act, the University, as an employer, is responsible for ensuring compliance with the Act and regulations and for taking every precaution reasonable in the circumstances for the protection of workers.

The University of Toronto Health and Safety policy has been approved by the Governing Council in compliance with the requirements of the Ontario Occupational Health and Safety Act. Pursuant to this policy, the University has established this formal Biosafety Program to identify, reduce and monitor the risks associated with potentially hazardous biological agents.

This Manual describes the requirements and procedures established by the University for work with potentially hazardous biological agents. It is based on the Health Canada Laboratory Biosafety Guidelines (3rd ed.) and reflects current best practices.

All work conducted by University members with potentially hazardous biological agents on University premises or under the control of the University is to be performed in accordance with the requirements of this manual.

Questions regarding application or interpretation of this manual should be directed to the Biosafety Officer, Office of Environmental Health and Safety, at (416) 978-3981.

2.2 Regulation of Biological Agents at the University

The Governing Council of the University has delegated to the President or his designate (the Vice-President, Human Resources and Equity) responsibility for approval of University regulations and other actions to implement the Occupational Health and Safety Act, the Environmental Protection Act and policies on health and safety approved by the Governing Council.

The Vice-President, Human Resources and Equity, has delegated to the Biosafety Committee, and its Chair, responsibility for the development and promulgation of safety standards for the conduct of research and teaching activities involving potentially hazardous biological agents by members of the University.

The Biosafety Officer in the Office of Environmental Health and Safety is responsible for administering the

Biosafety program on a day-to-day basis, for providing technical advice on safety procedures and equipment, conducting safety inspections, providing biosafety training, and providing guidance and information related to compliance with pertinent regulations.

The Biosafety Officer may also liaise with applicable regulatory bodies, such as the Public Health Agency of Canada (PHAC, formerly Health Canada), Canadian Food Inspection Agency (CFIA), Canadian Nuclear Safety Commission (CNSC) and Transport Canada, in matters involving biological agents and biosafety.

For additional information, please visit the Biosafety website at:

http://www.ehs.utoronto.ca/services/biosafety.htm or telephone the Biosafety Office at (416) 978-3981.

Health and Safety Policy

The University of Toronto is committed to the promotion of the health, safety and wellbeing of all members of the University community, to the provision of a safe and healthy work and study environment, and to the prevention of occupational injuries and illnesses.

The Governing Council, the President and all levels of management will work in consultation and cooperation with University employees, joint health and safety committees, students, contractors and visitors to ensure that the requirements of the Occupational Health and Safety Act and its regulations, other applicable legislation, and the University's Occupational Health and Safety Management System are fully implemented and integrated into all University work and study activities.

Where reasonable, the University will strive to exceed the legislated requirements by adopting the best practices available to protect the University community and to promote a positive health and safety culture. The University will work towards continuous improvement in its health and safety program.

Managers and supervisors, whether academic or administrative, will take responsibility and accountability for the health and safety of those individuals under their direction and those workplaces under their charge. They will advise their employees of the existence of potential or actual workplace hazards, and will ensure that they work safely and in accordance with the Occupational Health and Safety Act and its regulations, and all applicable University policies and procedures. They will take every precaution reasonable in the circumstances for the protection of their employees.

All University employees, including faculty, librarians, and non-unionized and unionized employees, have some responsibility for ensuring health and safety in the workplace. Employees will work safely and in compliance with the Occupational Health and Safety Act and its regulations, and University policies and procedures. Employees will report all unsafe and unhealthy conditions and practices in the workplace to their immediate supervisors so that they may be promptly remedied.

Contractors, tenants and visitors at the University will comply with all relevant legislation, as well as University of Toronto policies and procedures.

While students are not covered by the Occupational Health and Safety Act, the University is also committed to ensuring that health and safety is considered in all aspects of student life. Students are responsible for conducting themselves in a safe manner, and are required to comply with all relevant legislation, University policies and procedures.

The University's Policy for Safety in Field Research addresses health and safety responsibilities for faculty, staff and students engaged in field research beyond the geographical boundaries of the University.

Individuals who fail to meet their obligations concerning health and safety may, depending on the circumstances, face appropriate disciplinary action, up to and including discharge.

All members of the University community are expected to demonstrate their commitment towards a safe and healthy work and study environment by acting in compliance with this Policy.

Approved: March 2004

2.3 The Biosafety Committee

The Biosafety Committee is responsible for setting and enforcing appropriate safety standards for work with potentially hazardous biological agents within University workplaces. The Committee has formally approved the contents of this manual and enforces the standards through the issuance of Biosafety Certificates for all work with potentially hazardous biological agents.

The Biosafety Committee membership includes the Local Biosafety Co-ordinators representing various applicable university departments or offices, the ex officio members and the medical advisor. A current membership list is available in Appendix A.1.

2.3.1 Local Biosafety Co-ordinators

Please refer to Appendix A.2 for a list of Local Biosafety Co-ordinators.

2.4 Office of Environmental Health & Safety

The Office of Environmental Health & Safety (EH&S) provides a broad range of health and safety services to the University community including policy and program development, consulting on the assessment and control of safety hazards, safety training, hazardous waste management, and program audits.

Specific programs include:

- Biosafety
- Laser Safety
- Environmental Protection: Hazardous Waste Management
- Occupational Hygiene & Safety
- Ergonomics
- Radiation Safety

Office of Environmental Health & Safety website: http://www.ehs.utoronto.ca/Home.htm

The EH&S website provides a wide variety of health and safety information to the University community, including communication on University policies, programs, guidelines and procedures; services provided to staff and students; health and safety training courses for staff; access to Material Safety Data Sheets; and links to other health and safety servers on the Internet.

2.5 Health & Well-being Programs and Services

Health & Well-being Programs & Services functions as a centralized resource for all employees at the University of Toronto interested in or needing information on workplace injury, long term disability, workplace accommodation and related issues.

2.5.1 Preventative Programs

Health & Well-being promotes health and safety within the workplace, offering preventative programs in:

- Occupational Disease Prevention
- Hearing Tests
- Spirometry
- Immunization/Screening Tests
- Travel and Field Work

As part of occupational disease prevention, health risk assessments and on-going surveillance programs are available for employees at risk for exposure to:

- Hepatitis A,B,C
- Heavy Metals
- H.I.V.
- Q-fever
- Rabies
- Sensitivities/Allergies
- Tuberculosis
- Vaccinia
- blood and body fluids
- specific viruses/bacteria
- infectious diseases
- animals/non-human primates

Please contact the Health & Well-being office if planning to work with any of these agents or others that may require immunization or an on-going surveillance program (416-978-4476).

2.5.2 Accident Reporting

All work-related accidents, injuries or occupational diseases must be reported to the Office of Health & Well-being Programs and Services within 24 hours (fax: 416-971-3052). Reporting procedures and copies of the University accident/incident form are available for download at:

http://www.ehs.utoronto.ca/resources/wcbproc.htm#Employees

If you require more detailed information, or have any questions, please contact the WSIB administrator at 416-978-8804.

Additional information on Accident Reporting and Accidents involving students or other unpaid work placements is available on the Office of Environmental Health & Safety website:

http://www.ehs.utoronto.ca/resources/wcbproc.htm

University of Toronto Biosafety Committee
Terms of Reference

The University of Toronto Biosafety Committee is charged with ensuring that all activities within the University of Toronto involving infectious biological agents are conducted in a safe manner and in conformity with generally accepted standards. The term "infectious biological agents" excludes biological agents of a strictly chemical nature, but includes viruses, bacteria, fungi, parasites, prions and other micro-organisms / genetic systems that, by virtue of their replicative properties, are potentially harmful to humans and / or other living systems.

The University affirms that the primary responsibility for the safety of all persons in the university community, which includes staff, students and the public, lies with the Principal Investigator using or authorizing the use of such agents. In addition, the University (through the members of the Governing Council and senior administrators) acknowledges a responsibility to provide a policy and procedural framework designed to ensure that work is being conducted safely and in conformity with the relevant acts and regulations.

The Governing Council has delegated to the President and through him or her to the Vice-President, Human Resources and Equity, the responsibility of the approval of regulations and actions with respect to the Occupational Health and Safety Act and the Environmental Protection Act. The Vice-President, Human Resources and Equity, has delegated to the University Biosafety Committee and its Chair, the following functions, powers and duties:

The Committee

1. To develop and promulgate safety standards for the conduct of research and teaching involving infectious biological agents;

2. To specify training requirements and to promote the training of all persons in the university community working with infectious biological agents;

3. To take all reasonable steps to ensure that research and teaching activities of members of the University involving infectious biological agents are performed in compliance with the requirements of the University and any relevant guidelines or legislation;

4. To advise the Vice-President, Human Resources and Equity, on the needs of the University community for biosafety facilities, policies and programs;

5. To decide upon appeals resulting from the refusal to grant a licence or from the withdrawal of a licence by the Chair or his/her designate.

The Chair

2. To approve and licence, at the appropriate containment level(s), all uses of infectious biological agents within the University;

3. To appoint local biosafety co-ordinators to assist in carrying out the mandate of the Chair and the Committee;

University Of Toronto Biosafety Committee
Terms Of Reference

4. To investigate compliance with any applicable regulations, guidelines or safety standards whenever it is believed or complained on reasonable grounds that any breach thereof or other safety hazard may have occurred or be occurring, and for that purpose, to enter any laboratory or other premises on the grounds or under the jurisdiction of the University at any time, and to examine the equipment, operations, materials and systems therein;

5. To withdraw the licence related to any work that is considered to pose an undue biosafety risk and to require the immediate cessation of that work;

6. To delegate the approval and withdrawal of licences and the investigation of compliance to the Vice-Chair or another member of the Committee when necessary due to absence or illness;

7. To report promptly to the Vice-President, Human Resources and Equity, for transmittal to the Business Board, any instances under the jurisdiction of the Committee where:

   (i) an order is issued by the relevant Ministry or any other regulatory authority and there is not full compliance within the specified time;
   (ii) employees refuse to work for reason of danger to their health or safety; or
   (iii) any other significant event occurs where the Business Board should be informed to enable it to perform, on behalf of the Governing Council, its responsibilities pursuant to the Occupational Health and Safety Act, the Environmental Protection Act or any other applicable legislation;

8. To represent the University externally on matters relating to Biosafety;

9. To submit, on behalf of the Committee, by March 31 of each year, an annual report to the Vice-President, Human Resources and Equity, on the activities of the Chair and the Committee, for transmittal to the Business Board.

2.6 Responsibilities

2.6.1 Principal Investigators

The primary responsibility for the safety of staff, students and the public lies with the Principal Investigator in charge of the research. Principal Investigators must be familiar with, follow, and ensure that all individuals working within their laboratories follow the procedures outlined in this manual. In particular, Principal Investigators are responsible for:

- Applying for and renewing biosafety certificates where required.
- Ensuring that all conditions of the certificate are followed.
- Ensuring that the appropriate containment cabinets are functioning properly by having them tested at the stipulated intervals.
- Ensuring that all persons working under their supervision have had appropriate training in working safely with potentially hazardous biological materials.
- Consulting with Occupational Health & Well-being if working with agents that may require immunizations or a surveillance program.
- Providing appropriate personal protective equipment and standard operating procedures.
- Co-ordinating and monitoring decontamination, disinfection and disposal procedures for infectious materials in the facility or laboratory.
- Co-ordinating the movement of infectious material within the facility according to the *Workplace Hazardous Materials Information System* (WHMIS) and *Transportation of Dangerous Goods* (TDG) regulations.
- Ensuring that a record keeping and storage system are in place for all material entering the facility.
- Co-ordinating emergency response activities.
- Liaising with support staff, housekeeping staff and contractors on matters related laboratory biosafety.
- Participating in accident investigations and promoting the reporting of incidents within the laboratory.
- Distributing new and relevant biosafety information to laboratory staff.

2.6.2 Biosafety Officer

The Biosafety Officer is a member of Biosafety committee working with the Committee in the development of internal policies and procedures, for communicating these to the research community and for monitoring compliance with them. The Biosafety Officer is responsible for the administration of the Biosafety Program on a day-to-day basis:

- Representing the University externally on matters related to biosafety.
- Interpreting Biosafety Guidelines and requirements and recommending containment equipment and procedures required to achieve compliance.
- Providing technical advice on safety procedures and equipment.
- Conducting safety and laboratory compliance inspections.
- Providing biosafety training.
- Guidance and information related to compliance with applicable regulations.
- Has the authority to require corrective action, or in the case of immediate danger to employees or students, to close the area.
- Monitoring the operation of all Level 3 Biocontainment facilities at the University on a regular basis to ensure that the operating guidelines for the facility are adhered to and the integrity of the facility is maintained.

2.6.3 Persons Working With Potentially Hazardous Biological Materials

- Follow all safety procedures.
- Wear protective equipment.
- Participate in medical surveillance programs when appropriate.

2.7 Licensing and Biosafety Certificates

2.7.1 Requirement for University of Toronto Biosafety Certificate

A University of Toronto Biosafety Certificate is required for all (research and teaching) laboratory activities which involve the use or manipulation of potentially hazardous biological agents, and materials containing such agents (including viruses, bacteria, fungi, parasites, recombinant DNA, prions and other micro-organisms / genetic systems, and human and animal tissues, cells, blood and body fluids), and which are conducted on University premises, or in a building or location administered by or under the control of the University.

All such activities are to be conducted and performed in accordance with the University of Toronto Biosafety Manual and any relevant guidelines or legislation.

All activities meeting any of the above criteria, involving potentially hazardous biological agents, whether directly supported by grants or contracts administered by the University or not, must be identified on the application for a University of Toronto Biosafety Certificate. The release of grants and supporting funds by the University is dependent on a University Biosafety Certificate.

For hospital-based activities, a copy of the approved Hospital Biosafety Certificate must be provided.

The submission of an application for a University of Toronto Biosafety Certificate implies willingness to allow the U of T Biosafety Officer to visit the laboratory sites used by the Biosafety Certificate holder in order to determine compliance with the University of Toronto Biosafety Manual.

2.7.2 Application for a Biosafety Certificate

Application forms for a University Biosafety Certificate with Explanatory Notes to assist the applicant, are available in Appendix B and in electronic format on the EH&S website (Biosafety homepage) at:

www.utoronto.ca/safety/bioshome.html

The completed form may then be signed by the applicant and submitted to the Local Biosafety Co-ordinator for review and approval.

The required information must be typed on the application form. In general, the Principal Investigator may use a single form to identify more than one project if these require similar containment conditions, instead of completing a separate application for a Biosafety Certificate for each project. The project titles must be matched with the corresponding granting agency. The hazardous biological agents to be used (e.g. human whole blood, Hepatitis B virus, chick embryo primary cell culture, CHO cells, E. coli O157) must be identified and specified. Projects and activities requiring Containment Level 3 conditions should be identified on a separate application form to distinguish these from other activities which may require a lower level of containment.

After entering the requested information, Biosafety Certificate application forms should be submitted directly to the Local Biosafety Co-ordinator with jurisdiction over the laboratory location. The Local

Biosafety Co-ordinator will review the application form and forward it for additional review and approval.

Following the review and approval process, a photocopy of the validated Biosafety Certificate will be returned to the applicant. The original will be retained on file in the Office of Environmental Health & Safety. Information will also be entered into RIS (Research Information System) for review by the Office of Research Services. A valid Biosafety Certificate must be on file before the University will release grant funds.

2.7.3 Validation Period

Containment Level 2 and Containment Level 3 (1 year only):

A University Biosafety Certificate for activities requiring Containment Level 2 or Containment Level 3 conditions is valid for one year from the date of approval by the University Biosafety Committee Chair. In the case of multi-year research or recurring teaching programmes, involving potentially hazardous biological agents, the Principal Investigator / Course Instructor must submit a new application form, even if the activities involving biological agents have not been altered or modified since the previous submission.

Containment Level 1 (2 years):

A University Biosafety Certificate for activities requiring only Containment Level 1 is valid for 2 years from the date of approval by the University Biosafety Committee Chair. The Principal Investigator / Course Instructor must submit a new application form even if the activities involving biological agents have not been altered or modified since the previous submission.

2.7.4 Amendments

Only activities and agents identified in the application form can be approved. The use of additional hazardous agents, new personnel and any other significant changes must be reported to the Biosafety Office, to ensure that the amendment is approved and documented. The submission of another application form may not be necessary. Amendments may be submitted as an attachment to the current certificate. Requests for an amendment must include:

- Principle Investigator's name
- Principle Investigator's signature
- current University of Toronto Biosafety Certificate number
- description of requested amendment

Amendment requests must be submitted to the University Biosafety Office.

2.7.5 Information and Enquiries

Explanatory Notes offering more detail are provided with the University of Toronto Biosafety Certificate application form and are available in Appendix B. Should you encounter difficulty or have any questions regarding the completion or submission of your application form, please contact:

Office of Environmental Health & Safety
Biosafety Secretary
TEL: (416) 978-4467

or

Biosafety Officer
TEL: (416) 978-3981

2.8 Material Safety Data Sheets (MSDS)

Material Safety Data Sheets for infectious micro-organisms (biological agents) have been prepared by the Office of Biosafety, Laboratory Centre for Disease Control, Public Health Agency Canada. These are available on the University's Biosafety Program website at:

http://www.ehs.utoronto.ca/services/biosafety.htm

These MSDS contain health hazard information, recommended precautions, and spill clean-up procedures, as well as other information that is relevant specifically to the laboratory setting. They serve as an additional safety resource for laboratory personnel working with biological agents.

3 LEGISLATION, 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.

3.1 Importation, Use and Distribution of Biological Agents

3.1.1 Importation of Biological Agents Affecting Humans

The importation of human pathogens and transfer of specimens within Canada is regulated by the Human Pathogens Importation Regulations (HPIR)(SOR/94-558). Human pathogens requiring containment levels 2, 3 or 4 must have valid Public Health Agency of Canada (PHAC, formerly Health Canada) permits before they can be imported. Containment level 1 pathogens are not regulated by HPIR and consequently do not require importation permits. However, a letter stating that an import permit is not required must accompany the Containment level 1 pathogens being transported.

Permit applications for human pathogens can be obtained through the Office of Laboratory Security at (613) 957-1779 or by downloading the online application form from the Office of Laboratory Security's website at http://www.hc-sc.gc.ca/pphb-dgspsp/ols-bsl/. A copy of the HPIR and frequently asked questions about the importation of human pathogens are also available on the Office of Laboratory Security's website.

Many human pathogens are pathogens of animals as well. Animal pathogens are regulated by the Canadian Food Inspection Agency (CFIA). It is the responsibility of the importer to ensure that all appropriate import permit documentation has been obtained prior to importation of any pathogen into Canada. ***An import permit is required from the CFIA as well as the PHAC for pathogens that are common to both animals and humans.***

Applicants wishing to import and transfer human pathogens must have facilities that comply with the operational practices and physical requirements for a containment laboratory detailed in this manual. For facilities wishing to import pathogens requiring containment level 3, certification by PHAC is required prior to issuance of the permit, to ensure that the laboratory meets the requirements of the Laboratory Biosafety Guidelines (3rd ed.).

Investigators wishing to import pathogens requiring containment level 2 are to perform a self-inspection to ensure that the facility meets the Laboratory Biosafety Guidelines, the applicable CFIA requirements and those of this manual. Prior to signing the permit application, the Biosafety Officer will perform an inspection to ensure that all applicable physical containment and operational requirements are in place. It is important to note that the laboratory is subject to verification by PHAC inspectors at any time, where an importation permit has been requested.

It may also be necessary to obtain permission to transfer listed pathogens within Canada from one scientist or laboratory to another. Requests for single-entry and multiple-entry permits to import infectious substances affecting humans should be directed to:

Office of Biosafety
Laboratory Centre for Disease Control
Public Health Agency of Canada
Ottawa, Ontario
K1A 0L2
(613) 957-1779

A copy of the application for a permit to import biological agents into Canada or to transfer biological agents within Canada must be provided to the University Biosafety Office.

3.1.2 Importation of Biological Agents Affecting Animals

The Health of Animals Act, 1990, and the Health of Animal Regulations give the Canadian Food Inspection Agency (CFIA) the legislative authority to control the use of imported animal pathogens and pathogens associated with reportable animal diseases. These include materials of animal origin that contain potential pathogens.

CFIA approval must be obtained for the importation of every animal pathogen. In the case of pathogens which affect both humans and animals, importation permits are required from both PHAC and the CFIA. If an agent was brought into Canada under an import permit which restricts its distribution, further approval must be obtained before transferring it to another scientist or laboratory. Recombinant organisms and their release into the environment may also be restricted. The CFIA will also establish the conditions under which the animal pathogens will be maintained and the work will be carried out. It is necessary to consider not only the risk to human health, but also the level of containment needed to prevent escape of an animal pathogen into the environment where it may constitute a risk to any indigenous animal species. Please refer to the Health of Animals Act and the Regulations for complete information.

Animal pathogens, including pathogens which affect both humans and animals are under the control of CFIA. The CFIA publication Containment Standards for Veterinary Facilities outlines the minimum design, and physical and operational requirements for Canadian laboratories and animal facilities that import and work with animal or zoonotic pathogens. Laboratories that apply to import animal or zoonotic pathogens must demonstrate that they meet these requirements before the CFIA can issue an import permit. Animal pathogens, including pathogens that affect both humans and animals, under the control of the CFIA are listed in a database maintained by the Biohazard Containment and Safety Division, CFIA. This is a dynamic list that is continuously amended to include emerging pathogens that may require restriction. Animal pathogens considered non-indigenous to Canada form a portion of this database and are severely restricted. Appendix C contains a partial list of these organisms. For each animal pathogen, the CFIA must be consulted for its importation, use and distribution. Information on the status of animal pathogens may be obtained from Biohazard Containment and Safety Division Canadian Food Inspection Agency:

159 Cleopatra Drive
Ottawa, Ontario
K1A 0Y9
Tel.: (613) 221-7068
Fax: (613) 228-6129
http://www.inspection.gc.ca/english/sci/lab/bioe.shtml

Information on the status of plant pathogens under the Plant Protection Act and Regulations can be obtained by contacting:

Plant Health and Production Division
Permit Office
59 Camelot Drive
Ottawa, Ontario
K1A 0Y9
Tel.: (613) 228-2342 (ext. 4334 or 4333)
Fax: (613) 228-6605

A copy of the application for a permit to import biological agents into Canada or to transfer biological agents within Canada must be provided to the University Biosafety Office.

3.2 Export Requirements for Biological Agents

Permits are required for the export of certain micro-organisms and associated equipment from Canada. As a signatory to the 1972 Biological and Toxin Weapons Convention, Canada presently imposes controls on certain toxicological and biological agents, as well as their related equipment, components, materials and technology under item 2007 of the Export Control List. For assistance or advice, contact the Department of Foreign Affairs and International Trade Canada, Export Control Division, tel. (613) 996-2387 or contact their Website at http://www.dfait-maeci.gc.ca/eicb/

A copy of the application for a permit to export biological agents from Canada must be provided to the University Biosafety Office.

3.3 Transportation of Biological Agents

The transportation of infectious substances within Canada is regulated by the Transportation of Dangerous Goods Regulations (TDG) (SOR/85-77), administered by Transport Canada. Transport Canada defines the labeling, 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).

Careful handling, transport and shipment of diagnostic specimens and infectious agents are absolutely essential if Canada is to maintain an effective health care system. Transportation methods must minimize risks to employees of the carrier, the public and the staff of the receiving laboratory. Hazards are compounded by improper packaging; a broken specimen container may lead to contamination of both laboratory and non-laboratory personnel, and an improperly labelled package may be opened inadvertently by secretarial, clerical or other untrained staff.

The efficient and safe transfer of infectious substances requires good co-ordination between the sender, carrier, and receiver to ensure safe and prompt transport and arrival in proper condition. It is important that the sender make advance arrangements with the carrier and the receiver to ensure that specimens will be accepted and promptly processed. In addition, the sender must prepare the appropriate dispatch documents according to the Transportation of Dangerous Goods Act and Regulations. The sender should also forward all transportation data to the receiver. No infectious substances shall be dispatched before advance arrangements have been made between the sender, the carrier and the receiver, or before the receiver has confirmed with national authorities that the substance can be imported legally and that no delay will be incurred in the delivery of the consignment to its destination.

Information may be routinely obtained during working hours from:

- Canadian Transport Emergency Centre (CANUTEC) (613) 992-4624,
  (613) 996-6666 (24 hours per day) for emergencies

Under the Transportation of Dangerous Goods Act and Regulations, biological agents and micro-organisms belonging to Risk Groups 2 and 3 are as identified as “infectious substances” in Class 6.2, with some exceptions. TDG Regulations do not apply to agents that are exempt under Sections 1.30 to 1.42, if they are packaged according to specifications.

More information regarding the transportation of infectious substances within Canada can be obtained by calling Transport Canada, Dangerous Goods Standards, at (613) 990-1059, at the Transport Canada Dangerous Goods Website at http://www.tc.gc.ca/civilaviation/commerce/dangerousgoods/ or
by writing to them at:
Place de Ville, Tower C
330 Sparks St., 4th Floor
Ottawa ON K1A 0N8

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 labeling, 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. For further information regarding international shipping requirements, please contact the ICAO Canadian representative directly:

Chief of Dangerous Goods Standards
Commercial and Business Aviation
Transport Canada
(613) 990-1060

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. A copy of the DGR may be obtained from IATA by calling 1-800-716-6326 or through their Website at http://www.iata.org/ .

Contact the University Biosafety Office if biological agents in Risk Groups 2 or 3 are to be shipped from the University of Toronto.

3.4 Laboratory Animals

All aspects of the proposed use of animals in research and the operational procedures for the care and maintenance of animals must satisfy the Guide to the Care and Use of Experimental Animals of the Canadian Council on Animal Care (CCAC), Containment Standards for Veterinary Facilities, published by the Canadian Food Inspection Agency, the local animal care authority, as well as this manual, if the animals are exposed to or infected with biological agents. This should be done to ensure 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. The University of Toronto holds a CCAC Certificate of Good Animal Practice® as is recommended for Institutions using animals for research, teaching and testing.

Under the Ontario Animals for Research Act and its Regulations, all Principal Investigators who intend to conduct research, testing or teaching projects at the University of Toronto involving the use of animals, must obtain the approval of the University Animal Care Committee before commencing the project. To obtain such approval, the Principal Investigator must submit the University of Toronto Animal Use Protocol Form which is available electronically at:

http://www.research.utoronto.ca/ethics/index.html

The completed protocol form must be signed by the Principal Investigator and must then be submitted to the Chairperson of the appropriate Local Animal Care Committee at the University for review, approval and signature.

Please refer to the practices and procedures in Section 4.2 Working with Laboratory Animals.

3.5 Waste Management

The handling, packaging, transport and disposal of waste in Ontario is governed by municipal, provincial and federal government legislation. To enable compliance with these regulations, the University has

developed programs, procedures and internal services focused on specific waste categories.

The Office of Environmental Health & Safety has prepared a Laboratory Hazardous Waste Management Manual which consolidates existing information and identifies procedures for the packaging, labelling and disposal of biological, chemical, radioactive, sharp, and other hazardous waste at the University of Toronto.

A copy of this document may be obtained from the Office of Environmental Health & Safety at (416) 978-4467 or on the EH&S website at:

www.utoronto.ca/safety/Waste/wmindex.htm

Laboratory waste contaminated with or containing biological agents should be autoclaved or disinfected to inactivate the biological agents prior to disposal. Where on-site functioning autoclaves (steam sterilizers) are not available and the conventional use of chemical disinfectants for the inactivation of hazardous biological agents in laboratory waste is not practicable or not efficacious, other waste handling and disposal methods must be considered.

To provide another alternative, the University of Toronto has negotiated a contract with a commercial firm which is licensed to remove and transport biologically contaminated ("pathological") laboratory waste to a designated disposal site. Specific packaging of waste and special documentation are required and the cost of this service is passed on to the principal investigator of the laboratory generating this waste. To arrange a special pick up, contact the Campus Services at 416-978-0955 or 416-978-3000. For more information, contact the Biosafety Office at (416) 978-3981.

The following chart contains a generalized summary of information excerpted from the Laboratory Hazardous Waste Management Manual and is presented as a convenience.

LABORATORY HAZARDOUS WASTE MANAGEMENT AND DISPOSAL GUIDE

	HAZARD	COLLECTION	TREATMENT IF CONTAMINATED	PACKAGING & LABELLING	DISPOSAL	
LIQUID WASTE	BIOLOGICAL	FLASK OR BOTTLE	AUTOCLAVING OR DISINFECTION		INTO DRAINAGE SYSTEM	
	CHEMICAL	BOTTLE, JUG, OR CONTAINER	DISINFECTION, INACTIVATION IF BIOLOGICAL HAZARD	CHEMICAL WASTE LABEL APPLIED TO WASTE CONTAINER	TAKE TO WASTE HOLDING FACILITY OR STORE IN LAB	PICKED UP BY EHS CHEM. WASTE TECH.
	RADIOACTIVE	SEGREGATE BY HALF-LIFE	DISINFECTION, INACTIVATION IF BIOLOGICAL HAZARD	INTO PLASTIC JUG OF ABSORBENT MATERIAL PRINT INFO. ON TAG	STORAGE IN LABORATORY	PICKED UP BY EHS RAD. WASTE TECH.
SOLID WASTE NON-SHARP	BIOLOGICAL	AUTOCLAVABLE BAG OR CONTAINER	AUTOCLAVING OR DISINFECTION	INTO BLACK PLASTIC BAG	ON FLOOR BESIDE OTHER GARBAGE	REMOVED BY BLDG. SERVICE WORKERS
	CHEMICAL		DISINFECTION, INACTIVATION IF BIOLOGICAL HAZARD	CHEMICAL WASTE LABEL APPLIED TO WASTE CONTAINER	TAKE TO WASTE HOLDING FACILITY OR STORE IN LAB	PICKED UP BY EHS CHEM. WASTE TECH.
	RADIOACTIVE		DISINFECTION, INACTIVATION IF BIOLOGICAL HAZARD	INTO YELLOW BAG IN WASTE CONTAINER; PRINT INFO. ON TAG	STORAGE IN LABORATORY	PICKED UP BY EHS RAD. WASTE TECH.
SOLID WASTE NEEDLES & BLADES	BIOLOGICAL	YELLOW NEEDLE & BLADE CONTAINER	AUTOCLAVING	INTO WHITE 20 LITRE PAIL	ON FLOOR BESIDE OTHER GARBAGE	REMOVED BY BLDG. SERVICE WORKERS
	CHEMICAL (TRACES)	YELLOW NEEDLE & BLADE CONTAINER	AUTOCLAVING IF BIOLOGICAL HAZARD	INTO WHITE 20 LITRE PAIL	ON FLOOR BESIDE OTHER GARBAGE	REMOVED BY BLDG. SERVICE WORKERS
	RADIOACTIVE (TRACES)	YELLOW NEEDLE & BLADE CONTAINER	AUTOCLAVING IF BIOLOGICAL HAZARD	INTO WHITE 20 LITRE PAIL	ON FLOOR BESIDE OTHER GARBAGE	REMOVED BY BLDG. SERVICE WORKERS
	RADIOACTIVE (SIGNIFICANT)			INTO PLASTIC JUG MARKED “RADIOACTIVE SHARP”	STORAGE IN LABORATORY	PICKED UP BY EHS RAD. WASTE TECH.

LABORATORY HAZARDOUS WASTE MANAGEMENT AND DISPOSAL GUIDE

	HAZARD	COLLECTION	TREATMENT IF CONTAMINATED	PACKAGING & LABELLING	DISPOSAL	
SOLID WASTE GLASSWARE (SMALL)	BIOLOGICAL	CONTAINER OR WHITE 20 LITRE PAIL	AUTOCLAVING OR DISINFECTION	INTO WHITE 20 LITRE PAIL	ON FLOOR BESIDE OTHER GARBAGE	REMOVED BY BLDG. SERVICE WORKERS
	CHEMICAL		DISINFECTION IF BIOLOGICAL HAZARD; WASH & RINSE	INTO WHITE 20 LITRE PAIL	ON FLOOR BESIDE OTHER GARBAGE	REMOVED BY BLDG. SERVICE WORKERS
	RADIOACTIVE		DISINFECTION IF BIOLOGICAL HAZARD; WASH & RINSE	INTO WHITE 20 LITRE PAIL	ON FLOOR BESIDE OTHER GARBAGE	REMOVED BY BLDG. SERVICE WORKERS
SOLID WASTE GLASSWARE (VERY LARGE, INTACT)	BIOLOGICAL		AUTOCLAVING OR DISINFECTION	CARDBOARD BOX CLOSED, TAPED, LABELLED	ON FLOOR BESIDE OTHER GARBAGE	REMOVED BY BLDG. SERVICE WORKERS
	CHEMICAL		DISINFECTION IF BIOLOGICAL HAZARD; WASH & RINSE	CARDBOARD BOX CLOSED, TAPED, LABELLED	ON FLOOR BESIDE OTHER GARBAGE	REMOVED BY BLDG. SERVICE WORKERS
	RADIOACTIVE		DISINFECTION IF BIOLOGICAL HAZARD; WASH & RINSE	CARDBOARD BOX CLOSED, TAPED, LABELLED	ON FLOOR BESIDE OTHER GARBAGE	REMOVED BY BLDG. SERVICE WORKERS

This chart presents a general summary of information pertaining to the handling of hazardous waste. Please contact the EH&S Office or consult the University of Toronto Laboratory Hazardous Waste Management Manual for more information about specific wastes. This document is available on the EH&S website at: http://www.ehs.utoronto.ca/services/biosafety.htm

Biosafety Officer (416) 978-3981
Co-ordinator, Environmental Protection Services (416) 978-7000

3.6 Autoclaves / Steam Sterilizers

Autoclaves can be used for both the disinfection of contaminated materials or for the sterilization of reagents used in laboratory procedures. The use of autoclaves/steam sterilizers for decontamination poses additional hazards from those encountered in sterilization of reagents. Efficacy monitoring of autoclaves used for decontamination of laboratory waste with biological indicators must be done regularly (i.e., consider weekly, depending on the frequency of use of the autoclave), and the records of these results and cycle logs (i.e., time, temperature and pressure) must also be kept on file.

In Ontario, autoclaves / steam sterilizers (15 psi) are regulated through the Technical Standards and Safety Act, Boilers and Pressure Vessels Regulation. Autoclaves / steam sterilizers must have a current certificate of operation issued by a Technical Standards and Safety Authority (TSSA) certified 'competent' Inspector. At the University of Toronto, a Boiler and Machinery Insurance broker provides this service. Design registration, and inspection at the installation location are required before operation, in accordance with The Technical Standards and Safety Act, Boilers and Pressure Vessels Regulation and CSA-B51, The Boiler, Pressure Vessel and Pressure Piping Code.

A certificate of inspection is required to operate any boiler or pressure vessel for a specified period. A TSSA inspection certificate is issued following inspection and approval of:

- The vessel, which must bear a valid Canadian Registration Number (CRN)
- The steam supply piping to the vessel

Detailed procedures for registration and inspection can be found on the TSSA website at: http://www.tssa.org/regulated/boilers/default.asp

Upon completion of certification by the TSSA, this equipment is to be inspected annually by an Inspector representing the Insurer. The scope of inspection will include:

- a visual inspection
- a review of the conditions of operation
- the protective devices such as the pressure relief valves, temperature controls (if any), steam quality control
- the measures being taken by the user for safe and efficient operation

Certificates of inspection authorize equipment usage for 12 months. The certificate of inspection should be posted near the vessel or available for review. The user should not operate any autoclave / steam sterilizer which has steam heating coils with a pressure of 15 psi or higher, without a valid certificate of operation. Persons responsible for the operation should be fully familiar with:

- Ontario Regulation, 220/01, Boilers and Pressure Vessels
- Boilers and Pressure Vessels Code Adoption Document

The University's insurer maintains a list indicating the locations of autoclaves / steam sterilizers. Annual inspections are performed automatically, according to this list. If you have received a new autoclave / steam sterilizer or are using one that has not been inspected during the previous 12 month period, please notify the U of T Risk Management & Insurance Department and provide the information necessary to have this equipment added to the equipment list.

For autoclave / steam sterilizer inspection services or information contact:

University of Toronto
Risk Management & Insurance Dept.
(416) 978-7465
or
Biosafety Office
(416) 978-3981

Regulations, Guidelines & Other Documents

Technical Standards and Safety Act, 2000, Ontario Regulation 220/01, Boilers and Pressure Vessels
http://www.e-laws.gov.on.ca/DBLaws/Regs/English/010220_e.htm

Environmental Protection Act, R.R.O. 1990, Regulation 347, General – Waste Management
http://www.e-laws.gov.on.ca/DBLaws/Regs/English/900347_e.htm

Ontario Regulation 558/00, Environmental Protection Act, Amending Reg. 347, General – Waste Management
http://www.e-laws.gov.on.ca/DBLaws/Source/Regs/English/2000/R00558_e.htm

Guideline C-4, The Management of Biomedical Waste in Ontario
http://www.ene.gov.on.ca/envision/gp/425e.pdf

Guideline C-17
Non-Incineration Technologies for the Treatment of Biomedical Waste (Procedures for Microbiological Testing)
http://www.ene.gov.on.ca/envision/gp/4321e.pdf

3.7 Fume Hoods

Fume hoods should be tested by qualified personnel in accordance with CSA Standard Z316.5-94, or equivalent.

The University of Toronto Fume Hood Standard is available from the Office of Environmental Health & Safety website at:

http://www.ehs.utoronto.ca/resources/manindex/fumehood.htm

The Office of Environmental Health and Safety audits fume hood performance and recalibrates the airflow alarm monitors on an annual basis.

To report fume hood malfunctions or if you require more information, contact:

Facilities & Services Department Building Engineer in / for your building	or	Office of Environmental Health & Safety (416) 978-4467

3.8 Biological Safety Cabinets (BSCs)

Biological safety cabinets (BSCs), when properly used in research and teaching activities involving the manipulation of hazardous biological agents, are effective in containing and controlling particulates and aerosols and complement good laboratory practices and procedures.

Biological safety cabinets used in laboratory activities requiring Containment Level 2 or 3 conditions at the University of Toronto must be inspected, tested and approved for use annually, unless otherwise noted, by trained service personnel to ensure that the cabinet is functioning as intended by the manufacturer.

3.8.1 Installation and Certification

The air curtain at the front of the cabinet is fragile and can easily be disrupted by people walking parallel to it, by open windows, air supply registers or laboratory equipment that creates air movement (e.g., vacuum pumps, centrifuges). BSCs should be installed in accordance with the requirements outlined in the Canadian Standards Association (CSA) Biological Containment Cabinets (Class I and II): Installation and Field Testing. They should be located away from high traffic areas, doors and air supply/exhaust grilles that may interrupt airflow patterns. A minimum unobstructed distance of 40 cm should be provided between the exhaust outlet on top of the cabinet and any overhead obstructions. Whenever possible, a 30 cm clearance should be provided on each side of the cabinet to allow for maintenance access. For ducted cabinets, blowers on the exhaust system should be located at the terminal end of the ductwork; failure of exhaust flow should signal an alarm to the user. To prevent pressurization of the cabinet, an interlock system should be installed to prevent the cabinet blower from operating whenever the exhaust flow is insufficient; an anti-backflow device to prevent reverse airflow through the HEPA filter may be required.

Continuous operation of BSCs helps to control dust levels and other airborne particulates in the laboratory. If BSCs are operated only when needed in order to conserve energy, the balancing of laboratory room air must be considered. In some cases, room exhaust is balanced to include the air exhausted through ducted BSCs, and these cabinets must not be turned off. Biological safety cabinets should not be installed as an integral part of a room air supply and exhaust system in such manner that fluctuations of the room supply and exhaust air cause the biological safety cabinets to operate outside of their design parameters for containment. It is recommended that biological safety cabinets or fume hoods not be used as the sole source of room air exhaust. Room air supply system equipped with dampers to prevent backflow if biological safety cabinets are connected to exhaust ductwork.

If biological safety cabinets are connected to exhaust ductwork, connections are by thimble units where appropriate, and room exhaust ducts are equipped with manual dampers to permit sealing for decontamination. Manufacturers' recommendations for installation should be carefully followed.

The provision of natural gas to BSCs is not recommended. Open flames in the BSC create turbulence, disrupt airflow patterns and can damage the HEPA filter. When suitable alternatives (e.g., disposable sterile loops, micro-incinerators) are not possible, touch-plate microburners that have a pilot light to provide a flame on demand may be used.

The correct operation of BSCs must be verified before they are used and then annually, and after any repairs or relocation, in accordance with the field tests outlined in CSA Z316.3-95, annex F of NSF 49 or other applicable standards. Moving a cabinet can cause damage to the HEPA filter and its seals. These tests include the downward velocity profile, the work access face velocity, the HEPA filter leak test and the airflow smoke patterns. Measuring and testing equipment must be calibrated and maintained in accordance with the CSA standard. A copy of the certification report must be provided to the user and kept on file. A label indicating the date of certification, the date of the next certification, to what standard the tests were performed and the name of the certifier should be affixed to the exterior of the cabinet.

3.8.2 Required Procedures and Tests

Inspection and retesting is mandatory if a biological safety cabinet is relocated. Moves of a minor nature (i.e. within the same room) may be exempt from this requirement if the move is observed by the testing technologist and the cabinet has not been subjected to excessive stress or rough handling which could result in damage. Tests include:

- downward velocity profile
- work access face velocity
- HEPA filter leak test
- airflow smoke patterns

Measuring and testing equipment must be calibrated and maintained in accordance with CSA standard Z316.3-95. ***A copy of the certification report must be provided to the user and kept on file. A label indicating the date of certification, the date of the next certification, to what standard the tests were performed and the name of the certifier should be affixed to the exterior of the cabinet.***

The routine decontamination and testing of used Class II biological safety cabinets shall include the following required procedures and tests which shall be conducted in accordance with, and in the manner described below.

3.8.2.1 Decontamination

Cabinet decontamination with paraformaldehyde vapour shall be conducted prior to the testing of biological safety cabinets which have been used for activities involving biological agents. The biological safety cabinet shall be sealed and decontaminated using the paraformaldehyde vapour technique which is described in NSF Standard 49, and cited in CSA Z316.3-95, or an equivalent procedure acceptable to the University of Toronto. The paraformaldehyde holding / contact time shall be a minimum of 2 hours, after which the paraformaldehyde vapour shall be neutralized or vented to the exterior of the building.

3.8.2.2 Containment System Integrity

Containment system integrity (pressure) testing shall be performed on all biological safety cabinets having air plenums which convey potentially contaminated air at positive pressure and where any portion of these plenums also forms part of the containment shell of the cabinet. The cabinet interior shall be pressurized with air to a differential pressure of 2"w.g. A liquid leak detector shall be applied along all welds, gaskets, penetrations, and seals on the exterior surfaces of the cabinet air plenums. Leakage will be indicated by the presence of bubbles or by the feel or sound of escaping air. Detected leakage shall be corrected using acceptable methods and materials and the repaired area shall be retested to confirm the success of the corrective action.

Note: ***The performance of this test is required at the time of initial cabinet installation, following cabinet relocation, and at least once in every three year period.***

3.8.2.3 Air Velocities and Volumes

Air velocities shall be measured at multiple points on a grid, across the face of the HEPA filters. The location and spacing of the co-ordinates shall be according to the manufacturer's recommendations and / or applicable standards. Additional air velocity measurements may be required by the manufacturer of the cabinet. The blower speed and air dampers shall be adjusted as required so that the final measured and calculated values are within the acceptable ranges indicated by the manufacturer of the biological safety cabinet.

3.8.2.4 HEPA Filter Integrity

All HEPA filters shall have minimum particulate removal efficiency of 99.97% for particles of 0.3 micrometre and shall be installed as close as possible to the source of the hazard to minimize length of contaminated ductwork. They shall be installed in housings with leakproof junctions between filter frame and ducting. They shall aslo be installed in housings which allow *in situ* decontamination and Dioctylphthalate (DOP) leak testing by the scanning method, with access to both the upstream and downstream faces of the HEPA filter. Dampers should be provided at or near the filter plenum to contain the decontaminating vapour and to isolate the plenum during filter replacement. HEPA filters shall be monitored by magnehelic gauges or other appropriate devices.

HEPA filter leak testing shall be performed using sufficient dioctylphthalate (DOP) aerosol (or equivalent) to challenge the air filtration system. The aerosol concentration upstream of the HEPA filters shall be sampled and used as the 100% reference for photometer adjustment prior to testing. All air diffusers and protective grilles downstream of HEPA filters shall be removed to allow direct access to the entire filter surface and perimeter (bond area, gasket, filter frame, and mounting frame) which shall be scanned in overlapping strokes at a traverse rate of not more than 2" per second. Aerosol penetration exceeding 0.01% of the upstream concentration shall be sealed or corrected using generally accepted methods and the repaired area shall be retested to confirm the success of the corrective action.

3.8.2.5 Airflow Smoke Patterns

These tests shall be performed using a source of visible smoke to demonstrate the acceptability of airflows associated with the biological safety cabinet:

Down-flow Supply Air Distribution

The source of visible smoke shall be passed along the ('smoke split') centreline of the work surface, from one side of the workspace to the other. The smoke shall show smooth flow with no dead spots or upward flow. No smoke shall escape from the cabinet.

Supply Air Entrainment / View Screen Retention

The source of visible smoke shall be passed from one side of the cabinet to the other, behind the view screen, and 6" above the top of the front access opening. The smoke shall show smooth downward flow with no dead spots or upward flow. No smoke shall escape from the cabinet.

Intake Air Entrainment / Work Access Opening Retention

The source of visible smoke shall be passed along the entire work access perimeter, about 1.5" outside of the workspace of the cabinet. No smoke shall escape from the cabinet once it is drawn in. For Class II cabinets, no smoke shall pass over the work surface or penetrate the work zone.

Window Seal

The source of visible smoke shall be passed along the perimeter of the view screen, inside the workspace of the cabinet. No smoke shall escape from the cabinet.

3.8.2.6 Other Procedures and Tests

Other procedures and tests (electrical safety, fluorescent and UV lighting intensity, vibration, noise level, etc.) may be recommended or performed, depending on the cabinet design and the circumstances of its installation and usage, but their performance is not required on a routine basis.

3.8.3 Testing Services

Correct operation of BSCs must be verified before they are used and then annually, and after any repairs or relocation, in accordance with the field tests outlined in CSA Z316.3-95, annex F of NSF 49 or other recognized standards.

The testing of biological safety cabinets at the University is conducted by an external contractor. Fees for this service are charged to the Principal Investigator / researcher or, in the case of teaching, to the instructor's department. Please contact and make arrangements for testing, servicing or repair of a

biological safety cabinet directly with the service provider.

Contracted Supplier
HEPA Filter Services Inc.
#4-470 North Rivermede Rd.
Concord, Ontario
L4K 3R8
TEL: (905) 669-1991
FAX: (905) 669-4871

Alternate Supplier
Con-Test
P.O. Box 337
Pickering, Ontario
L1V 2R6
TEL: (905) 428-6671
FAX: (905) 428-7703

3.9 Needles and Syringes

Needle and blade waste includes hypodermic, surgical, suture or IV needles, syringes with needles, lancets, scalpels, blades and similar metallic sharp or pointed items for disposal that are capable of causing punctures, cuts, or tears in skin or membranes.

According to the principles of universal blood and body fluid precautions, all needles and blades used in medical care, diagnosis and research, including the manipulation and care of laboratory animals, should be considered potentially infectious. Needles and blades pose a risk to those who use them and needle and blade waste pose a health risk to those involved in its handling, transportation and disposal.

Needle and blade waste contaminated with or containing viable biological agents and trace amounts of hazardous chemical or radioactive material, singly or in any combination, can be collected together in the same yellow container for needle and blade waste. In most cases, the quantity of potentially hazardous material adhering to used needles and blades will be minimal and present in trace amounts only. All liquids containing hazardous chemical or radioactive materials must be drained from disposable syringes and collected for appropriate disposal.

All needle and blade waste for disposal must be carefully collected in an approved needle and blade waste container. Three autoclavable, yellow, plastic containers (B-D Guardian 300439, 300460 and 300466), all complying with CSA Standard Z316.6-95, have been selected and approved for the collection and disposal of needle and blade waste generated at the University of Toronto. Their capacities range from 1.4 to 7.6 litres. These containers are available at University of Toronto Medstore: http://www.uoftmedstore.com/ .

If needle and blade waste is contaminated with or contains viable biological agents, it must be treated to inactivate the biological agents. The designated yellow containers for needle and blade waste are autoclavable. The filled container may be steam sterilized along with other laboratory waste. Steam sterilization is however generally not recommended for laboratory waste contaminated with or containing a combination or mixture of viable biological agents and significant amounts of hazardous chemical or radioactive materials. These situations will be handled on a case-by-case basis.

For more information regarding sharp waste management, please refer to the Laboratory Hazardous Waste Management Manual.

4 SAFETY PRACTICES AND PROCEDURES

4.1 General Laboratory Safety Practices

The following requirements are basic for any laboratory using hazardous biological agents.

1. ***Training:*** All laboratory personnel and others whose work requires them to enter the laboratory must understand the chemical and biological hazards with which they will come in contact during their normal work in the laboratory and be trained in appropriate safety precautions and procedures. Personnel must be required to know, understand, and follow standard practices and procedures. Training in laboratory safety shall be provided by the laboratory director / principal investigator and competence in safe technique must be demonstrated before work is allowed with hazardous agents or toxic material.

2. ***Safety Procedures Manual:*** A laboratory safety manual must be prepared or adopted. It is the responsibility of the laboratory director / principal investigator to ensure that it identifies known and potential biological hazards and specifies the practices and procedures to eliminate or minimize such risks. The manual must contain an emergency response plan.

3. The laboratory must be kept neat, orderly and clean, and storage of materials not pertinent to the work must be minimized.

4. ***Laboratory clothing:*** Protective laboratory clothing (uniforms, coats, gowns) must be available; worn by all personnel including visitors, trainees, and others entering or working in the laboratory; and be properly fastened. Protective laboratory clothing must not be worn in non-laboratory areas. The U of T Protective Clothing Standard is available at:
   http://www.ehs.utoronto.ca/Assets/ehs3/documents/ClothingStd2004.pdf.pdf

5. ***Footwear:*** Suitable footwear with closed toes and heels and preferably with non-slip soles must be worn in all laboratory areas. The U of T Protective Footwear Standard is available at:
   http://www.ehs.utoronto.ca/Assets/ehs3/documents/FootwearStd2004.pdf.pdf

6. ***Gloves:*** must be worn for all procedures that might involve direct skin contact with chemicals, toxins, blood, infectious materials or infected animals. Rings or hand jewellery interfering with glove use must be removed before gloving. The wearing of jewellery in the laboratory should be discouraged. Gloves must be removed carefully and decontaminated with other laboratory wastes before disposal. Reusable gloves (e.g. insulated, chemical resistant, etc.) may be used where necessary and must be appropriately decontaminated after use. Metal mesh gloves can be worn underneath the glove. The U of T Protective Glove Standard is available at:
   http://www.ehs.utoronto.ca/Assets/ehs3/documents/FootwearStd2004.pdf.pdf

7. ***Face and eye protection:*** (e.g., glasses, goggles, face shields, or other protective devices) Must be worn when necessary to protect the face and eyes from splashes, impacting objects, harmful substances, UV light, or other rays. The U of T Protective Eye and Face wear Standard is available at: http://www.ehs.utoronto.ca/resources/manindex.htm

8. Eating, drinking, smoking, storing food or utensils, applying cosmetics, and inserting or removing contact lenses are not permitted in any laboratory work area. Contact lenses are not protective devices, and must be used only in conjunction with appropriate protective eyewear in eye hazard areas. Wearing jewelry is not recommended in the laboratory.

9. Oral pipetting is prohibited in any laboratory.

10. Long hair must be tied back or restrained.

11. ***Hand-washing:*** Hands must be washed before leaving the laboratory, and after handling materials known or suspected to be contaminated, even when gloves have been worn.

12. ***Decontamination:*** Work surfaces must be cleaned and decontaminated with the appropriate disinfectant at the end of the day and after any spill of potentially hazardous material. Loose or cracked work surfaces must be repaired or replaced.

13. All technical procedures must be performed in a manner that minimizes the creation of aerosols.

14. ***Waste disposal:*** All contaminated or infectious liquid or solid materials must be decontaminated before disposal or reuse. Contaminated materials that are to be autoclaved or incinerated at a site away from the laboratory must be double-bagged or placed into containers, the outsides of which are disinfected.

15. ***Laboratory Access:*** Access to Containment Level 3 laboratories must be strictly limited. Decisions regarding entry into Level 1 and 2 laboratories must be at the discretion of the laboratory director / principal investigator (e.g. only persons who have been advised of the potential hazards and meet any specific entry requirements such as immunization should be allowed to enter the laboratory area). Persons under the age of 16 years should not be permitted in the laboratory or support areas. Pregnant women and immunocompromised people who work in or enter the laboratory must be advised of the associated risks.

16. ***Signage:*** Hazard warning signs, indicating the containment level or the risk group of the agent used, must be posted outside each laboratory operating at Containment Level 2 or 3. Where the infectious agent used in the laboratory requires special provisions for entry, the relevant information must be included in the door sign. The agent(s) must be identified in the information provided for signing along with the names of the laboratory supervisor and other responsible person(s), and any special conditions for staff entry.

17. ***Needles and Syringes:*** The use of needles and syringes and other sharp objects should be strictly limited. Hypodermic needles and syringes must be used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles. Extreme caution must be used when handling needles and syringes to avoid autoinoculation and the generation of aerosols during use and disposal. Needles must not be bent or sheared. Disposable needles and syringes must not be replaced in their sheath or guard. They must be placed into a puncture-resistant yellow container and autoclaved, in accordance with CSA standard Z316.6-95(R2000).

18. ***Incident Reporting:*** All spills, accidents (needlesticks, punctures, cuts, etc.) and overt or potential exposures must be reported in writing to the laboratory supervisor or acting alternate as soon as circumstances permit. This person must file this report with the University of Toronto, Health & Well-Being Programs & Services. Appropriate medical evaluation, surveillance, and treatment must be sought and provided as required. Actions taken to prevent future occurrences should be documented.

19. ***Medical surveillance:*** Baseline sera or other specimens shall be collected from laboratory and other at-risk personnel and stored when deemed necessary. Additional serum specimens may be collected periodically, depending on the agent handled or the function of the facility. Baseline and periodic serum or other specimens shall be collected and maintained by the University's Health & Well-Being Programs & Services or an equivalent health service. Confidentiality will be maintained according to the legal obligations of the Regulated Health Disciplines Act, or its subsequent revision. Tests will not be performed without the informed consent of the donor.

    Laboratory workers should be protected by appropriate immunization where possible. Levels of antibody considered to be effective should be documented. Appropriate immunization or evidence of exposure should be maintained in a confidential manner. Particular attention must be given to

individuals who are or may become immunocompromised, as vaccine administration may be different than for immunologically competent adults. Principal Investigators should contact the University of Toronto Health & Well-Being Programs & Services for information and advice on appropriate immunization and medical surveillance.

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

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

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

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

24. An effective pest control program (e.g., rodents and arthropods) must be maintained.

4.2 Working with Laboratory Animals

4.2.1 General Requirements

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) suspected of containing these agents. Under these circumstances, the animals should be kept at the containment level appropriate to the risk of the agent, recognizing that 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 an aerosol. 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. There is also a need to address, in the equipment and practices of animal facilities, the issues of cross-contamination between animals and of keeping adventitious agents from inadvertently infecting experimental animals (also referred to as "barrier" facilities).

In all situations, it is the responsibility of the laboratory director / principal investigator and the Biosafety Committee, in consultation with Government agencies and the animal care authorities, to determine the risk levels inherent in the proposed activity.

Animal facilities for work with small and large animals should be designed and operated in accordance with the *Containment Standards for Veterinary Facilities*, published by the Canadian Food Inspection Agency (CFIA), the *Guide to the Care and Use of Experimental Animals*, published by the Canadian Council on Animal Care and other CCAC guidelines and policies. Institutions using animals for research, teaching and testing should consider obtaining a CCAC Certificate of GAP (Good Animal Practice®). There are other international recommendations which can provide further assistance with the assessment of hazards associated with the care and use of research animals.

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 4.1 and should include the following precautions.

1. Infected animals and insects should be segregated from uninfected animals wherever possible, and it is preferable to separate any handling area from the holding area.

   Ideally, animal facilities should be a physically separated unit, but if they adjoin the laboratory the animal rooms should be separated from other activities in the laboratory to allow for isolation and decontamination as required.

   Animal rooms for small animals should be designed for ease of cleaning and disinfection, and have a minimum of built-in equipment.

   A small preparation area, storage area and hand washing sink are usually all that are required.

   As well, the design should facilitate the use of containment caging systems and support facilities for animal procedures, cage washing, waste disposal and food/bedding storage.

2. Animals or insects in use in an experiment must be maintained at a level of containment which is at least equivalent to the containment level for the biological agent with which it has been infected or treated.

   As general protocols cannot anticipate the specific requirements of each experiment, specific entry and exit protocols for scientific staff animal handlers, animals, biological samples, equipment, feed and wastes should be developed for each project.

3. Provision must be made to ensure that inoculated animals or insects cannot escape.
   Physical barriers, restraints and gating systems should be designed and used to prevent such injuries. The handler must have knowledge of the animal's general characteristics, such as mentality, instincts and physical attributes.

4. Dead animals or insects and the refuse from the animal room and cages (e.g. bedding, faeces and food) must be placed in a leak-proof container and autoclaved or incinerated.

5. All cages must be properly labelled, and procedures in the holding area must minimize the dispersal of dander and dust from the animals and cage refuse.

6. At least one-fifth of people who work with laboratory rodents, guinea pigs and rabbits develop allergies. Allergic conditions may result from contact with animal fur or hair, bedding and animal wastes. The allergy may manifest itself immediately or may be acquired over a succession of exposures to the allergen. Symptoms range from mild rashes to severe asthma. Unnecessary exposure to these allergens can be minimized through engineering controls, ventilation, use of isolators and containment caging systems, and appropriate use of respiratory and other personal protection.

7. Personal protective equipment, appropriate chemical restraint or other appropriate restraint devices (e.g. pole and collar) are required for the handling of non-human primates.

   Particular attention must be given to the use of protective clothing and equipment by staff entering an animal cubicle contaminated with large volumes of infected animal waste. Floor drains connected to an effluent sterilization system are employed at containment levels 3 and 4 to effectively remove and treat infected animal wastes. Special care must also be taken to avoid serious injuries (e.g., crushing) that could occur when handling large animals.

8. Gloves should be worn by animal care providers while feeding and watering animals or cleaning cages.

9. Gloves, boots, floors, walls and cage racks should be disinfected frequently.

In addition to the preceding, the following must also be satisfied:

1. 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, and local animal care authorities.

2. The appropriate species must be selected for the animal experiments.

3. 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.

4. All incidents, including animal bites and scratches or cuts from cages or other equipment must be documented and the employee should report to the Health & Well-Being Programs and Services for medical assessment and follow-up.

5. Small laboratory rodents or other small animals that escape from their cages should be killed when captured, their carcasses incinerated, and the area should be thoroughly decontaminated. In the event that animals escape through the containment perimeter, the relevant authorities must be notified promptly and appropriate action initiated.

6. Unexpected illness or deaths among animals must be reported to the principal investigator and the veterinarian, who will be responsible for final disposition. Animals should not be touched until instructions are given by the person in charge.

   Recent technological improvements have been incorporated into a wide variety of housing systems to provide control of microenvironmental factors such as temperature, air exchange and humidity.

   Containment facilities for large animals are unique, in part because of the large quantity of infectious microorganisms that may be present in the animal cubicle. Unlike a laboratory room, where the BSC provides primary containment, the large animal cubicle serves as both the primary and secondary barrier.

4.2.2 Non-human primates

Working with non-human primates presents unique hazards related to their physical strength and naturally occurring pathogenic zoonotic organisms they may harbour. Certain species possess long canine teeth and powerful jaws that can inflict serious and painful wounds. Many species also have sharp fingernails that can scratch and abrade the skin of handlers. They are generally very messy, noisy and destructive animals, characteristics that must be considered when designing animal rooms used to house them. Infectious hazards to people handling non-human primates include bacterial diseases (*Salmonella*, *Shigella*, *Campylobacter,* tuberculosis), viral diseases (hepatitis A virus, simian immunodeficiency virus and especially *Cercopithecine herpesvirus 1* (CHV-1), also known as herpes B virus), protozoan and metazoan parasites (*Entamoeba*, *Blastocystis*, *Trichomonas*, *Balantidium*) and other agents.

CHV-1 is an enzootic virus present in up to 70% of captive macaques, including rhesus and cynomolgus

non-human primates. Although the virus causes oral lesions in its natural simian host, asymptomatic shedding from the buccal mucosa and urogenital tract (though rare) and the presence of the virus in conjunctival fluid can occur without such clinical signs. Guidelines are available for working safely with macaques, for the prevention of CHV-1 infection and for the treatment of such infections in exposed people, and these should be consulted.

Risk of exposure to pathogenic agents can be reduced through appropriate use of personal protective equipment and through an adequate animal health surveillance program, with emphasis on identification and treatment, isolation or euthanasia of diseased animals. Animal handlers should be enrolled in a health and medical surveillance program.

Protection against aerosol exposure, scratches, abrasions or splashes of mucous membranes is provided by appropriate personal protective equipment which must be used at all times by handlers or anyone entering animal rooms where non-human primates are housed. Reusable, protective clothing that has been in contact with non-human primates should be decontaminated before being sent to the laundry. Animal handlers must be instructed to cleanse immediately and thoroughly any bites, scratches, splashes or abraded skin and to report these exposures at once. Post-exposure procedures must also be instituted. All reports should be made to facility management and to Health & Well Being Programs and Services for medical assessment and follow-up.

Anyone who handles non-human primates must be adequately trained. Training is designed and implemented to foster the safe handling of non-human primates and to prevent injury or exposure to zoonotic disease. Training includes basic biology and behaviour, physical hazards associated with working with non-human primates, zoonotic disease, the use of personal protective equipment, appropriate methods of restraint in conjunction with introduction to caging features which aid in safe non-human primate handling and post-exposure prophylaxis. Additional training is required if non-human primates are to be handled without chemical restraint. New investigative staff should work in tandem with more experienced staff until competency has been demonstrated. Investigative staff should work in teams of at least two at all times especially when working with larger species.

When feasible, chemical restraint should be used before removing non-human primates from cages, especially in the case of macaques and other larger species. Caging features such as squeeze mechanisms facilitates the safe handling of non-human primates. Where chemical restraint is not feasible, knowledge of normal non-human primate behaviour is necessary for safe handling. Behaviour conditioning can be effectively used in combination with restraint devices such as pole and collar to achieve safe handling of conscious animals. Caging and other equipment should be free of sharp edges and corners that may cause scratches or wounds.

Facilities for housing non-human primates should conform to the recommendations for small animal containment facilities in the Containment Standards for Veterinary Facilities. Unless experimentally infected with or known to have an infectious organism requiring a higher containment level, non-human primates can be handled in containment level 2 animal facilities with the additional practices and personnel precautions described above for working safely with these animals. It is recommended that all macaque colonies be treated as naturally infected with CHV-1, even those that have been shown to be free of CHV-1 antibody. The Guide to the Care and Use of Experimental Animals also provides information on housing and handling requirements specific to non-human primates.

Generally, housing for non-human primates requires the following:

1. Consideration to be given to the behavioural, emotional and social needs of laboratory primates when planning their housing.

2. Contact information of experienced primate handlers and the person responsible for the facility, to be provided throughout the facility.

3. Animal rooms to be provided with a vestibule or other arrangement to ensure that there are

always two doors between the non-human primate cage and the building corridor; provision should be made to observe all cages before entering the room to ensure that animals are not loose.

4. All lighting, electrical fixtures and exposed plumbing in non-human primate rooms to be protected against tampering by the animals.

5. Because of the requirement for daily sanitation of animal rooms, floors to be constructed of slip-proof materials and workers to wear footgear that provides traction on wet, slippery floors. As well, walls and ceilings to be designed with a finish to withstand wash-down cleaning and disinfection procedures.

6. Shower and changing facilities to be provided for workers having substantial animal contact to shower at the end of the work day.

7. Animal rooms and cages to be kept locked at all times and to be accessible only to authorized people. Security locks and closing devices must take into consideration the persistent, creative, destructive and intellectual capacities of most non-human primates.

8. The movement of equipment items (e.g., carts, scales, feed containers and scoops, gloves) between animal rooms to include proper disinfection practices on exit from each room as appropriate to the containment level of the work.

9. Cages to have sufficient strength that they cannot be damaged by the non-human primates and to be maintained in proper working condition.

10. Cages to be equipped with a squeeze mechanism to facilitate examination and immobilization. Transfer boxes and other special restraint apparatus can be used to hold primates safely while primary cages are being cleaned or to move primates from one room to another.

11. For group caging, such factors as compatibility between animals and the population dynamics of the species to be considered in order to minimize fighting.

4.3 Working with Human Pathogens

Some micro-organisms (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 micro-organism due to differences in physiology, metabolism, biochemistry, etc. 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 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
- auto-inoculation via a cut, laceration or puncture with a contaminated instrument.

4.3.1 Human Bloodborne Pathogens

Human blood, a potential source of pathogenic micro-organisms, presents a risk to workers. Although the hepatitis B virus (HBV) and the human immunodeficiency virus (HIV) are often cited as examples, a “bloodborne pathogen” is any pathogenic micro-organism that is present in human blood or other potentially infectious materials that can infect and cause disease in persons who are exposed to blood containing the pathogen.

“Other potentially infectious materials” means material which has the potential to transmit bloodborne pathogens. This includes infected human tissues and the following body fluids: semen, vaginal secretions, cerebrospinal fluid, synovial fluid, pleural fluid, peritoneal fluid, pericardial fluid, amniotic fluid, saliva in dental procedures, and any other body fluid that is visibly contaminated with blood.

The biosafety requirements identified for research laboratories may not always be applicable to all workplace settings where workers handle or are exposed to human blood, body fluids or other materials potentially containing biological agents.

4.3.2 Universal Blood and Body Fluid Precautions

Between 1982 and 1988, the Centers for Disease Control (Atlanta, Georgia) published a series of recommendations and precautions for the protection of healthcare workers (physicians, nurses, phlebotomists, dentists, laboratory workers, etc.) who have, or are likely to have, contact with human blood and certain body fluids and may be at risk of exposure to bloodborne pathogens such as hepatitis B virus (HBV) and human immunodeficiency virus (HIV). These recommendations became known as “Universal Blood and Body Fluid Precautions” or simply, “Universal Precautions”.

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 certain other body fluids from all humans be considered potentially infectious and that precautions be taken to minimize the risk of exposure. This approach, called "Universal Precautions", is a method of infection control, intended to prevent parenteral, mucous membrane, and non-intact skin exposure of workers to bloodborne pathogens. All human blood, certain human body fluids, and other materials are considered potentially infectious for hepatitis B virus (HBV), human immunodeficiency virus (HIV), and other bloodborne pathogens. Precautions must be consistently used.

Universal blood and body fluid precautions apply to:

- blood
- body fluids containing visible blood
- semen
- vaginal secretions
- cerebrospinal fluid
- synovial fluid
- pleural fluid
- peritoneal fluid
- pericardial fluid
- amniotic fluid.

Universal precautions generally do not apply to faeces, breast milk, nasal secretions, sputum and saliva, sweat, tears, urine, and vomitus unless they contain visible blood. Although these materials are not implicated in the transmission of bloodborne pathogens, it is prudent to minimize non-intact skin and mucous membrane contact with these materials.

Hepatitis B immunization is recommended as an adjunct to universal precautions for workers who have occupational exposure to human blood or other potentially infectious materials. This immunization is provided to employees at risk, free of charge, by the University of Toronto Health & Well-Being Programs and Services.

In the hospital setting, HBV immunization is recommended for personnel in these occupational groups:

medical technologists, operating room staff, phlebotomists and intravenous therapy nurses, surgeons and pathologists, dialysis unit staff, emergency room staff, nursing personnel, physicians, and students in schools of medicine, dentistry, nursing, laboratory technology and other allied health professions.

Outside the hospital setting, HBV immunization is recommended for healthcare workers who may have exposure to human blood and other potentially infectious materials, such as dental professionals, laboratory and blood bank technicians, dialysis centre staff, emergency medical technicians, morticians, workers in clinical / diagnostic laboratories, and workers in research facilities that study, produce or manipulate human blood which may contain HBV and HIV.

General Precautions

1) All workers should routinely use appropriate barrier precautions to prevent skin and mucous membrane exposure when contact with human blood or other body fluids is anticipated.

2) Eating, drinking, smoking, applying cosmetics or lip balm, and handling contact lenses are prohibited.

3) Gloves should be worn when touching blood and body fluids, mucous membranes, or non-intact skin, for handling items or surfaces soiled with blood or body fluids, and for performing venipuncture and other vascular access procedures. If a glove is torn or damaged during use, it should be removed and a new glove used as promptly as safety permits. Disposable gloves should not be washed or disinfected for reuse. Washing with surfactants may enhance penetration of liquids through undetected holes in the glove. Disinfecting agents may cause deterioration of the glove material.

4) Protective eyewear, appropriate respiratory protection or face shields should be worn during procedures that are likely to generate droplets of blood or other body fluids to prevent exposure of mucous membranes of the mouth, nose, and eyes.

5) Gowns or aprons should be worn during procedures that are likely to generate splashes of blood or other body fluids. Protective clothing should be removed before leaving the area.

6) Hands and other skin surfaces should be washed immediately and thoroughly if contaminated with blood or other body fluids. Hands should be washed immediately after gloves are removed since no barrier is 100% effective.

7) Workers should take precautions to prevent injuries caused by needles, scalpels, and other sharp instruments or devices during procedures, when cleaning used instruments, during disposal of used needles, and when handling sharp instruments after procedures. Needles and syringes should be used only in those situations when there is no alternative. To prevent needlestick injuries, needles should not be recapped, purposely bent or broken by hand, removed from disposable syringes, or otherwise manipulated by hand. After they are used, disposable syringes and needles, scalpel blades, and other sharp items should be placed in puncture-resistant containers for disposal. The puncture-resistant container should be located as close to the use area as practical. Contaminated reusable pointed and sharp objects such as large bore needles and scalpels should be placed in a puncture resistant container for transport to the reprocessing area.

8) Mouthpieces, resuscitation bags, or other ventilation devices should be available for use in areas in which the need for resuscitation is predictable.

9) Workers who have exudative lesions, weeping dermatitis, cuts, open wounds or other breaks in the skin should either refrain from all direct contact with blood and other body fluids until the condition resolves, or utilise protective barriers to reduce the risk of exposure.

10) Pregnant workers should be especially familiar with and strictly adhere to precautions to minimize the risk of perinatal transmission of bloodborne pathogens.

Additional Precautions for Clinical Laboratories

1) All blood and body fluid specimens should be in a well constructed container with a secure lid to prevent leaking during transport.

2) Gloves should be worn by all persons processing blood and body fluid specimens. Gloves should be removed and replaced and hands should be washed upon completion of specimen processing since no barrier is 100% effective.

3) Masks and protective eyewear or a face shield should be worn if mucous membrane contact with blood or body fluids is anticipated.

4) A biological safety cabinet is not necessary for routine procedures such as histologic and pathologic studies or microbiological culturing. However, biological safety cabinets should be used whenever procedures involve activities that have a high potential for generating aerosol droplets (blending, sonicating, vigorous mixing, etc.)

5) Mechanical pipetting devices should be used for manipulating all liquids in the laboratory.

6) Laboratory work surfaces should be decontaminated with an appropriate chemical germicide after a spill of blood or other body fluids and when work activities are completed.

7) Hands should be washed after completing laboratory activities and protective clothing should be removed before leaving the laboratory area.

8) Equipment and instruments should be decontaminated and cleaned before being repaired in the laboratory or transported to the manufacturer or repair shop.

9) Contaminated materials should be decontaminated before processing for reuse. Disposable contaminated wastes must be collected in the appropriate containers.

Additional Precautions for Autopsies or Morticians' Services

1) All persons performing or assisting in postmortem procedures should wear gloves, masks, protective eyewear, gowns, and waterproof aprons.

2) Instruments and surfaces contaminated during postmortem procedures should be decontaminated with an appropriate chemical germicide. Gloves should be worn during the cleaning and decontaminating procedure.

Additional Precautions for Dentistry

1) All dental workers should wear surgical masks and protective eyewear or plastic face shields during dental procedures in which splashing, or spattering of blood, saliva or gingival fluids is likely.

2) Handpieces, ultrasonic scalers, air / water syringes, etc., should be sterilized or disinfected after use with each patient and according to the manufacturer's recommendations.

3) Contaminated materials, impressions, and intra-oral devices should be disinfected before they are handled in the dental laboratory and before they are placed in the patient's mouth.

4) The surfaces of dental equipment that may become contaminated but are difficult to disinfect, should be wrapped or covered with impervious paper, aluminum foil or clear plastic wrap which is replaced after use with each patient.

These recommended "universal blood and body fluid precautions" are excerpted from:

Morbidity and Mortality Weekly Report	volume 37, number 24
(Centers for Disease Control)	volume 37, number 15
	volume 36, number 2S
	volume 34, number 45
	volume 32, number 34
	volume 31, number 43

4.4 Decontamination

It is a basic biosafety principle that all contaminated materials be decontaminated prior to disposal. Decontamination includes both sterilization (the complete destruction of all microorganisms, including bacterial spores) and disinfection (the destruction and removal of specific types of microorganisms). It is the responsibility of all laboratory workers to ensure the effective use of products for decontamination of materials, equipment, and samples from containment zones; of surfaces and rooms; and of spills of infectious materials. These procedures represent a critical containment barrier whereby failure in the decontamination procedure can result in occupational exposure to infectious agents and/or the unintentional release of agents from a containment facility.

All contaminated materials must be decontaminated before disposal or cleaning for reuse, as mentioned in Section 4.1 of this manual. The choice of method is determined by the nature of the material to be treated. This may include, but is not limited to, laboratory cultures, stocks and clinical specimens; laboratory equipment, sharps and protective clothing; and other items that have come into contact with infectious materials. Laboratory bench tops and surfaces are to be decontaminated after any spill of potentially infectious materials and at the end of the working day. Laboratory rooms and large pieces of equipment may also require decontamination (i.e., prior to servicing, maintenance, transfer to other settings or reassignment). Specific written protocols are available in the University's Laboratory Hazardous Waste Management Manual. Employees must be trained in all decontamination procedures specific to their activities and should know the factors influencing the effectiveness of the treatment procedure, as discussed briefly below.

4.4.1 Disinfection Agents

Chemical Disinfectants

Chemical disinfectants are used for the decontamination of surfaces and equipment that cannot be autoclaved, such as specimen containers and other items removed from containment, and for clean up of spills of infectious materials, rooms and animal cubicles, and a variety of other items for which heat treatment is not feasible.

The initial choice of a chemical disinfectant depends upon the resistance of the microorganisms of concern. The most susceptible are vegetative bacteria, fungi and enveloped viruses. Mycobacteria and

non-enveloped viruses are less susceptible; bacterial spores and protozoan cysts are generally the most resistant. Consideration should also be given to practicability, stability, compatibility with materials and health hazards.

The effectiveness of the disinfectants can be influenced by a number of factors: presence of organic material (e.g., blood, serum, sputum) that decreases the effect of hypochlorites; temperature; relative humidity; concentration; and contact time. In some cases, it may be beneficial for laboratories to conduct in-use disinfectant efficacy testing to evaluate a product's performance in the field, under conditions of use. A basic method to evaluate surface disinfectants involves the artificial contamination of a surface and immersion in the appropriate dilution of the disinfectant; thereafter the disinfectant is neutralized by dilution and checked to determine whether all microorganisms have been killed. A similar protocol can be used to verify the effectiveness of disinfectants used in discard containers: an inoculum is added to the disinfectant, which after a predetermined contact time is neutralized by dilution, and an aliquot is examined for growth. The active components of disinfectants belong to relatively few classes of chemicals, and understanding the capabilities and limitations of each class of chemicals (e.g., hypochlorites, quaternary ammonium compounds, phenolics, iodines, alcohols) will allow choice of a product based on relative effectiveness.

Gaseous Decontamination of Rooms

Gaseous decontamination of rooms is generally only necessary at containment levels 3 and 4 under particular circumstances (e.g., after a spill or accidental release of infectious materials, for removal of large equipment items from containment, before maintenance work on contaminated systems, before retesting of HVAC control systems).

Because of the potential for exposure to the hazardous chemicals (formaldehyde) used, gaseous decontamination of rooms should be done only by highly trained personnel. The two-person rule should always apply to this operation, and both individuals should be trained and fitted in the use of appropriate respiratory protection.

Irradiation

Gamma irradiation (e.g., 60Co) can be used for the decontamination of heat-sensitive materials and is an effective means of decontaminating chemicals and solvents removed from a containment facility. The efficacy of the treatment technology depends on the penetration of the treated items by gamma irradiation and, therefore, on the density of the treated substance as well as the strength of the irradiation source.

Microwave irradiation is not widely used for decontamination in containment facilities. As in steam autoclaving, heat is the critical factor for eliminating viable microorganisms. The factors that affect microwave treatment include the frequency and wavelength of the irradiation, the duration of exposure and the moisture content of the material to be decontaminated.

Ultraviolet irradiation (UV) should not be relied upon as the sole method of decontamination for materials removed from containment facilities. UV has limited penetrating power and is primarily effective against unprotected microbes on exposed surfaces or in the air. It can be effective in reducing airborne and surface contamination provided that the lamps are properly cleaned, maintained and checked to ensure that the appropriate intensity is being emitted.

4.5 Medical Surveillance and Immunoprophylaxis

The University of Toronto, Health & Well-Being Programs and Services provides several health surveillance, testing and immunoprophylaxis programs for University employees.

Animal Care Workers Surveillance

Communicable Disease Surveillance:	Faculty of Dentistry Faculty of Nursing Student Health Services Research Laboratories HIV Research Laboratory personnel
Immunoprophylaxis Programs:	Hepatitis A Virus Hepatitis B Virus Influenza Virus Measles, Mumps and Rubella Rabies Virus Tetanus, Diphtheria, Polio Virus
Testing Programs:	Anonymous HIV testing Epstein Barr Virus testing Hepatitis C Virus testing Mantoux testing (Tuberculosis screening) Q Fever serological testing

Immunizing agents are available for the protection of laboratory workers against the etiologic agents of the following diseases:

Anthrax	Lyme disease	Rabies
Botulism	Measles	Rubella
Cholera	Meningococcus	Tetanus
Diphtheria	Mumps	Tuberculosis (BCG)
Hemophilus influenzae type b	Pertussis	Typhoid
Hepatitis A	Plague	Vaccinia
Hepatitis B	Pneumococcus	Varicella
Influenza A	Polio	Yellow fever
Japanese encephalitis		

Immunoprophylaxis and information pertaining to the availability and the advisability of immunizing agents are available through Health & Well-Being Programs and Services at 416-978-2149.

Laboratory personnel should be protected against laboratory-acquired infections 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. The University of Toronto offers and provides hepatitis B immunization free of additional cost to its employees through the Health & Well-Being Programs and Services.

Students may obtain immunizations through the University Student Health Service.

5 EMERGENCY PROCEDURES

5.1 Emergency Contact Information

For medical emergencies:	***911***
Weekdays (9am-5pm):	
University Biosafety Office	(416) 978-3981
Office of Environmental Health & Safety	(416) 978-4467
Other times:	
University of Toronto Police:	
Mississauga	(905) 569-4333
Scarborough	(416) 287-7333
St. George	(416) 978-2222

5.2 Spills and Other Uncontrolled Releases

Emergency response plans required at Containment Levels 2 and 3 must include procedures for dealing with spills and other laboratory incidents that can 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. Effective disinfectants must be available in the laboratory at all times and for immediate use.

In the event of a spill or container breakage resulting in the unintentional release of a biological agent:

6. Evacuate the laboratory for a time sufficient for most aerosols to settle or be dispersed or removed by the ventilation system, (~ 20- 30 min) (respiratory protection should be considered for re-entry*).

7. Pour a strong disinfectant solution (sodium hypochlorite or Wescodyne) around, but not on the spill, and mix the disinfectant with the spilled material cautiously.

8. Evacuate the laboratory for a time expected to be sufficient for decontamination of the mixed material (~20 - 30 min).

9. Carefully absorb the liquid with absorbent paper and place into an autoclavable bag or other container suitable for autoclaving.

10. Decontaminate all surfaces exposed to the spill with a suitable disinfectant.

If a spill kit is available:

1. Absorb the spilled liquid with a spill control pillow or other absorbent material.

2. Place the used pillow or absorbent material into an autoclavable bag or covered container and autoclave it.

3. Decontaminate all surfaces exposed to the spill with a suitable disinfectant.

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*Appropriate respiratory protection should be considered depending on the biological agents in use. The selection and use of appropriate respiratory protection can be determined in consultation with the Office of Environmental Health and Safety, and must be in compliance with the U of T Respirator Standard (http://www.ehs.utoronto.ca/Assets/ehs3/documents/RespiratorStd2004.pdf.pdf).

If the incident involves a large amount of biological material (high concentration or large volume), affects a large area, or results in overt exposure of laboratory personnel, the incident must be reported to the laboratory supervisor or Principal Investigator of that laboratory as soon as possible.

Affected laboratory workers should promptly seek medical attention.

5.3 Emergency Medical Procedures

In life-threatening situations requiring immediate medical attention, telephone the University of Toronto Police Office and they will contact the appropriate authorities and co-ordinate the response.

The following emergency procedures shall be followed by a worker exposed to:

- blood or body fluids (via a needlestick, cut or puncture wound, via mucous membrane contact, or via non-intact skin contact)
- infectious or communicable disease agents (via inhalation, a needlestick, cut or puncture wound, via ingestion or mucous membrane contact, or via non-intact skin contact)
- zoonotic agents (via a needlestick, cut, animal bite or scratch, via mucous membrane contact, or via non intact skin contact)

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1. ***The exposed site must be washed immediately.***

   (i) If needlestick, cut, puncture wound, animal bite or scratch, wash with soap and water after allowing the wound to bleed freely.

   (ii) If mucous (eyes, nose, mouth) membrane or non-intact (cuts, rash, acne or dermatitis) skin contact, flush with water at the nearest faucet or eye wash station.

2. ***The worker must immediately inform the supervisor / principal investigator of the exposure incident.***

3. ***The worker must seek prompt medical attention at the nearest hospital emergency department or emergency clinic or a medical practitioner of their choosing.***

5.4 Incident Reporting

The worker must provide information for a University Accident/Incident/Occupational Disease 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.

5.4.1 Supervisor / Principal Investigator

1. Supervisors / principal investigators must complete and sign the University Accident / Incident / Occupational Disease Report (http://www.ehs.utoronto.ca/resources/wcbproc.htm).

2. The supervisor must ensure that exposure incidents are reported within 24 hours to the Office of Health & Well-Being Programs and Services fax: 416-971-3052.

3. The supervisor must refer the affected worker(s) to the nearest hospital emergency department or emergency clinic, or preferably, to the University.

5.4.2 Health & Well-Being Programs and Services

The Office of Environmental Health & Safety shall be forwarded a copy of the Accident / Incident Occupational Disease Report by Health & Well-Being Programs and Services.

If the worker refuses appropriate post-exposure prophylaxis and / or testing, this shall be documented in the medical record by the Health & Well-Being Programs and Services and countersigned by the employee, or a refusal document should be signed and forwarded to the Health & Well-Being Programs and Services.

5.4.2.1 Animal Bites and Scratches

1. Health & Well-Being Programs & Services shall confer with the affected individual(s) and / or attending physician(s) / caregiver(s).

2. Counselling regarding potential exposure and infection, immunoprophylaxis and follow-up testing shall be offered to any worker if her / his exposure is determined to be of a nature that may transmit zoonotic agents.

5.4.2.2 Human Blood and Body Fluids

1. The Health & Well-Being Programs & Services shall confer with the affected individual(s) and / or attending physician(s) / caregiver(s) to determine whether the exposure is of a nature that may transmit HBV, HIV or other bloodborne pathogens.

2. Counselling regarding potential HBV, HCV, HIV or other bloodborne pathogen exposure and infection, chemo / immunoprophylaxis and follow-up testing shall be offered to any worker if her / his exposure is determined to be of a nature that may transmit HBV, HCV, HIV or other bloodborne pathogens. A hepatitis B vaccine or other appropriate post-exposure prophylaxis shall be offered if the worker has not been immunized previously or does not demonstrate adequate antibodies.

5.4.2.3 Exposure to Infectious and Communicable Disease Agents

1. The Health & Well-Being Programs & Services shall confer with the affected individual(s) and / or attending physician(s) / caregiver(s) to determine whether the exposure is of a nature that may transmit the biological agent and result in infection.

2. Counselling regarding potential exposure and infection, chemo / 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 the biological agent.

6 CLASSIFICATION OF BIOLOGICAL AGENTS

6.1 General

The standards and practices described in this manual apply to all laboratory research and teaching activities conducted within the University and its affiliated institutions where such activities involve the use of known biological agents or cultures, or when an agent has been recently isolated or is suspected to be present in the material handled.

Judgements of the inherent risks of a pathogen are made on the basis of such factors as the severity of the disease it causes, the routes of infection, its virulence and infectivity. This judgement should take into account the existence of effective therapies, immunization, the presence or absence of vectors, quantity of agent and whether the agent is indigenous to Canada, as well as possible effects on other species, including plants and animals. Due to their unknown characteristics, emerging pathogens and novel agents may require more stringent specialized practices and procedures for their safe handling.

Biological agents are classified according to Risk Groups, which are analogous to the Containment Levels described in Section 7.3. These classifications presume ordinary circumstances in the laboratory, or growth in small volumes for experimental, diagnostic or teaching purposes.

The classifications of biological agents reflect the judgements made on their inherent risks. The general criteria are indicated in Section 6.4 and agents categorized according to these criteria are listed in Appendix D. The agents were chosen because they seem to be frequently used in Canada. Agents which are not listed will be classified on the basis of similarity to those listed; Risk Groups for others will be identified on request to the Office of Biosafety, Laboratory Centre for Disease Control.

Large volumes and high concentrations of a biological agent in growth media may pose greater risks than smears of the same agent on a microscope slide. Other unusual manipulations may also increase the hazard. Where deemed appropriate, Appendix D includes annotations indicating changes in classification acceptable to Health Canada when certain agents are used in defined ways.

6.2 Genetically Engineered Organisms and Cell Lines

The biological hazards associated with the use of mammalian or other cells in culture, and an appropriate Risk Group, will be influenced by the following criteria. Micro-organisms that are demonstrated to be non-pathogenic, containing no adventitious agents and having a long history of safe industrial use are not considered here.

1. Primary cultures of mammalian or other cells may harbour infectious agents or integrated DNA originally present in the animal or human from which the cultures were derived. Whenever possible, the donor should be tested for suspect pathogens prior to the preparation of the culture, and the culture should be considered to be contaminated until proven to be free of the suspect agents. Such primary cultures should be handled in a manner appropriate to the Risk Group of the suspected contaminant, and precautions should be taken to protect laboratory personnel.

2. Cell lines known to contain infectious agents or integrated DNA should be handled in a manner appropriate to the Risk Group for the agent.

3. Cell lines that are deemed to be free of infectious agents rarely pose a biological hazard. If there is unintentional parenteral inoculation, normal immune response should provide protection, prevent progressive growth, and cause rejection of accidentally transplanted cells.

For activities involving genetically engineered organisms,

- the host organism should be non-pathogenic, with no adventitious agents, a history of safe use, and limited ability to survive in the environment
- vectors with known inserts should be well characterized and free of sequences that result in adverse effects to humans, animals, plants, or the environment
- the genomic insert should be limited in size to the smallest sequence required and should not increase the stability of the gene product in the environment.

Resistance markers should be transferred with caution, to prevent acquisition of resistance that might compromise the therapeutic use of antimicrobial agents. The resulting recombinant organism should be non-pathogenic or alternatively posses limited survival characteristics and be without adverse environmental consequences.

6.2.1 Recombinant DNA and Genetic Manipulation

Genetic methods such as selection, cross breeding, conjugation and transformation have been used for many years to alter animals, plants and microorganisms. These methods have recently been supplemented with newer and much more efficient ones, of which the best known are the techniques of recombinant DNA. Some newer techniques include:

- the production of transgenic plants and animals
- the cloning of microbial toxin or other virulence genes in an expression vector or in a host background in which it may be expressed
- the production of full-length infectious viral clones, including the reconstruction of infectious virions from recombinant constructs (reverse genetic engineering)

For the purposes of this document, recombinant DNA includes:

- DNA molecules produced outside living cells by joining natural or synthetic DNA segments to DNA molecules capable of replication in living cells,
- DNA molecules produced in living cells by joining enriched or natural segments to intracellular DNA, and,
- DNA molecules resulting from 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 which might be created or used in the laboratory. The vast majority of this research involves only the remotest 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.

Factors to consider when determining the containment level of a recombinant organism should include:

- containment level of the recipient organism
- containment level of the donor organism
- replication competency of the recombinant organism
- property of the donor protein to become incorporated into the recombinant particle
- potential pathogenic factors associated with the donor protein

In general, containment levels for activities involving recombinant DNA will be assigned according to the following criteria and considerations:

1. If none of the components of the genetic manipulation (DNA, vector, host) presents any known hazard and none can be reasonably foreseen in their combination, then no restrictions beyond the requirements of Containment Level 1 are necessary.

2. If one of the components used in the procedure is hazardous, then, in general, determination of the containment level required will begin at the level appropriate to the known hazard. The level of containment may be increased or decreased depending on the particular gene transferred, the expression of the gene in the recombinant organism, the envisaged interactions between the transferred gene and the host-vector system, and other relevant factors.

3. In any activity involving genes coding for hazardous products, host-vector systems with limited ability to survive outside of the laboratory (affording biological containment) should be used. Their use may reduce the level of physical containment required.

4. The containment level may be reduced if it is known that the DNA or vector is mutant and defective in their disease-causing or replication characteristics.

5. In the case of animal virus vectors, including retroviruses, one must consider the nature of the helper cells and the likelihood that replication-competent viruses may be produced.

Each case needs to have a risk assessment, as it is not realistic to try to define in advance all the possible genetically engineered organisms that might be created or used in the laboratory. Assistance with the risk assessment can be provided by the Office of Laboratory Security, telephone (613) 957-1779. The vast majority of recombinant research involves only the remotest possibility of creating a hazard, because the source of the DNA being transferred, the vector and the host are all innocuous. However, some genetic manipulation does raise significant possibility of risk.

6.2.2 Transgenic Plants

There is considerable potential for commercial production of biological products in transgenic plants and animals. The potential release of transgenic organisms into the environment and transmission of novel genes to other plants and animals must be considered when designing both the production system and facilities to contain the transgenic organisms. In each case, the risk level should be determined in consultation with the appropriate Government agency.

Transgenic plants may transmit novel characteristics to other plants, thereby modifying the gene pool of existing species. Since this transmission is mediated by pollen, transgenic plants should be made sterile or contained in a growth chamber or greenhouse designed to prevent pollen release via air or insects. If plants are allowed to mature, care must be taken to contain seeds in the growth chamber or greenhouse.

Transgenic animals should be handled according to the Guidelines of the Canadian Council for Animal Care. An important consideration is the ability of the animal to transmit genes by breeding with another animal of the same or a related species. Transgenic animals must be adequately contained to prevent the unintentional spread of genetic modifications. It is recommended that transgenic animals be produced using methodology which restricts the potential for transmission of genes to another host.

If viable micro-organisms are used as vehicles for transfection, the containment level for the plants or animals inoculated with these viable recombinant micro-organisms must be at least as high as that required for work with that specific micro-organism. Transgenic plants and animals produced by microinjection, by use of replication defective vectors, or other sequences that are not normally horizontally transmitted, generally may be handled at Containment Level 1. The following recommendations should be considered prior to the initiation of transgenic experiments.

- Complete copies of the replication competent genome should not be used.
- The constructs should not contain genes capable of causing neoplastic transformation in animals.
- The probability of recombination with extraneous micro-organisms should be minimal or non-existent.

6.3 Animal Cells, Blood and Body Fluids, and Fixed 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.

The following should be handled at Containment Level 2:

- Primate cell lines derived from lymphoid or tumour tissue
- all cell lines exposed to or transformed by a primate oncogenic virus
- all samples of human tissues and fluids
- all primate tissues
- all cell lines new to the laboratory (until proven to be free of adventitious agents)
- all virus-containing primate cell lines
- and all mycoplasma-containing cell lines

These are factors that influence the containment level required:

- 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
- the use of additional precautions

Non-recombinant cell lines

For every new cell line that is manipulated in a laboratory, a detailed risk assessment must be done in order to determine the appropriate level of precautions to be taken. A detailed risk assessment should include, but is not limited, to the following:

- source of cell line: the closer phylogenetically to humans, the greater the potential risk (highest to lowest risk: human autologous, human heterologous, primate, other mammalian, avian, invertebrate);
- source tissue: provides an indication of possible contaminants and latent (oncogenic) viruses;
- type of cell line highest to lowest risk: primary cell cultures, continuous cell cultures, intensively characterized cell cultures;
- quantity of cells per culture;
- source population of the specimen from which the cell line was derived.

Recombinant cell lines (in addition to the above criteria)

- properties of the host cell line (in the case of hybridomas, the properties of each of the contributing cells must be considered);
- vector used for transformation (may increase containment level requirements);
- transfer of viral sequences (may increase containment level requirements);
- transfer of virulence factors (may increase containment level requirements);
- activation of endogenous viruses (may increase containment level requirements);
- recombinant gene product (may increase containment level requirements);
- helper virus presence (may increase containment level requirements).

Once all the relevant information regarding the cell line has been obtained, including any hazards associated with the media to be used during manipulation of the cell culture, it can be assessed to ascertain the hazards posed by manipulating the particular cell line. The cell line is to be handled at the

containment level appropriate to the level of risk determined by the assessment.

6.3.1 Animal Cells

Primary cell cultures and animal tissues

The following containment requirements apply to primary cell cultures and tissues from human, non-human primate and non-primate animal sources when handled in the laboratory or used for animal passage. Cells and tissues known or suspected to be contaminated or infected with any of the agents included in Appendix D must be handled at the containment level appropriate to those agents.

Human and non-human primate material: Containment Level 2
Non-primate animal material: Containment Level 1

Established cell lines

Human or other animal cell lines known not to be contaminated or infected with any of the agents included in Appendix D may be handled at Containment Level 1. Cultures known or suspected to be contaminated or infected with any of the agents included in Appendix D must be handled at the containment level appropriate to those agents.

6.3.2 Blood and Body Fluids

The need for precautionary measures extends also to situations in which human blood, saliva, urine and other body fluids or faeces 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 Containment Level 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.

Culturing of specimens in research laboratory

Blood or blood fractions and other body fluid specimens of human or animal origin that are known or suspected to contain any of the agents included in Appendix D 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.

Routine clinical diagnostic work in laboratory

For routine clinical diagnostic work with specimens of human blood, serum and other body fluids (urine, cerebrospinal fluid, etc.) from the **general population**, Containment Level 1 may be acceptable if the activity does not involve culturing of the specimen beyond the volumes necessary to allow clinical analysis. However, in such cases the workers must be made fully aware of the potential hazards and should take additional precautions. Pre-exposure immunization against Hepatitis A and B viruses and the use of appropriate face and eye protection and gloves are recommended.

For routine clinical diagnostic work with specimens which are known to be from **infected individuals**, the containment level appropriate to the agent must be maintained.

6.3.3 Fixed Tissues and Tissue Sections

Tissues and tissue sections from human and animal sources are routinely fixed by treatment with

chemical agents to preserve structures for later examination and study. Generally, these chemical treatments inhibit all biological activity. Most, but not all, intracellular and intercellular biological agents are inactivated during this treatment. A notable exception is the group of unconventional agents known as 'prions'.

In general, fixed tissues and tissue specimens should be handled under at least Containment Level 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.

Where a biological agent which usually requires a higher level of containment is present in the tissue, the laboratory director / principal investigator should provide documentation to the University of Toronto Biosafety Committee to support a request for a lower level of containment.

6.3.4 Cell Line Contamination with Infectious Agents

Bacteria and fungi
Cell lines contaminated with bacteria and fungi are readily identified when grown in antibiotic-free media because they quickly overgrow the cells.

Viral contamination
Unlike bacteria and fungi, viruses are not readily identified and so can pose a significant hazard to those manipulating primary cell lines. Because of the varying risks associated with cell line material, the World Health Organization proposed a classification of cell lines based on each line's likelihood of carrying viruses pathogenic to humans.

Low likelihood: cell lines derived from avian and invertebrate tissues.
Medium likelihood**:** mammalian nonhematogenous cells, such as fibroblasts and epithelial cells.
High likelihood**:** blood and bone marrow cells derived from human or non-human primates; human pituitary cells, caprine and ovine cells, especially those of neural origin;and hybridoma cells when at least one fusion partner is of human or non-human primate origin.

Both viral and cellular oncogenes have been recognized, most notably the human T-cell leukemia virus (HTLV-I). HTLV-I is a human oncogenic virus that transforms normal cells into malignant cells. Cell lines with known or potential viral contaminants are to be handled at the containment level appropriate for the contaminating agent of the highest risk. One of the primary hazards of manipulating cell cultures is the expression of latent viruses. Endogenous viral sequences have been found in a variety of cell lines derived from mammalian species, including humans. Cell lines can be grown in an altered manner by applying various treatments (e.g., change in pH, serum level, temperature, medium supplements, cocultivation).

These treatments may cause altered expression of oncogenes, expression of latent viruses, interactions between recombinant genomic segments or altered expression of cell surface proteins. Manipulations that may alter the "normal" behaviour of cell lines to a more hazardous state are to be conducted at a containment level appropriate to the new hazardous state.

The biological hazards associated with primate cell lines must also be taken into consideration when determining the level of containment required. Primary cell lines derived from the genus *Macaca* may harbour *herpesvirus simiae* (Cercopithecine herpes virus, B-virus), and therefore tissues from *Macaca* must be manipulated as follows:

Containment level 2 is to be used when handling tissues or body fluids from macaques. If material is suspected or known to contain herpesvirus simiae, containment level 3 is required. *In vitro* primary diagnostic tests are to be done at containment level 3. All propagation (culturing) of the virus is to be done at containment level 4.

Prions
The protein-only infectious particle, or prion, is accepted as the causative agent of transmissible spongiform encephalopathies, such as bovine spongiform encephalopathy (BSE). Cell cultures derived from bovine sources known or suspected to be BSE positive, and *in vitro* primary diagnostic tests of cell cultures derived from bovine sources known or suspected to be BSE positive are to be handled using TSE specific guidelines. Information and the TSE guidelines can be found by contacting CFIA, Biohazard Containment and Safety Division directly at (613) 221-7074 or accessing their Web site:
http://www.inspection.gc.ca/english/sci/bio/bioe.shtml

Mycoplasmas
Although mycoplasmas have commonly been identified as sources of cell culture contamination, mycoplasma-contaminated cultures have not yet been reported as a source of a laboratory-acquired infection. However, because of the presence of biologically active mycoplasma products and the stability of mycoplasma antigens as well as the fact that a number of mycoplasmas are human pathogens, they are considered hazardous in cell cultures. Cell lines with mycoplasma contaminants are to be handled at the containment level appropriate for the contaminating agent of the highest risk.

Parasites
Freshly prepared primary cell lines may be at risk of parasite contamination if the cell line was obtained from a specimen known or suspected to be infected with a human parasite. Parasites have many life cycle stages, and not all stages are infective. This must be taken into consideration when determining the appropriate level of containment. Cell lines in which the life-cycle stage of the infecting parasite is not known are to be manipulated at the containment level appropriate for the contaminating agent of the highest risk.

6.4 Biological Agent Risk Group Criteria and Categories

A risk group classification 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 the 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 I (low individual and community risk)

A biological agent that is unlikely to cause disease in healthy workers or animals.

Risk Group 2 (moderate individual risk, limited community risk)

A pathogen that can cause human or animal disease, but under normal circumstances is unlikely to be a serious hazard to healthy 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 3 (high individual risk, low community risk)

A pathogen that usually causes serious human or animal disease, or which can result in serious economic consequences but does not ordinarily spread by casual contact, from one individual to another, or that can be treated by antimicrobial or antiparasitic agents.

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

A pathogen that usually produces very serious human or animal disease, 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.

Appendix D contains a listing of Risk Group categories for biohazardous agents. For the current risk group classification of an agent, contact the Office of Laboratory Security directly at (613) 957-1779 or access their Web site at http://www.phac-aspc.gc.ca/ols-bsl/.

It is recommended that the Canadian Food Inspection Agency (CFIA), Biohazard Containment & Safety Division, also be contacted ((613) 221-7068).

NOTE: ***Risk Group 4 agents are not approved for use at the University of Toronto and shipments including such agents should not be accepted.***

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. Agents with similar pathogenic characteristics but which are not included in the following lists, should be considered to belong in the same risk group.

Certain biological agents which are animal pathogens are considered not indigenous to Canada and are subject to control by the CFIA. Appendix C contains a partial listing of agents not indigenous to Canada.

7 CONTAINMENT OF BIOLOGICAL HAZARDS

7.1 Introduction

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 that 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.

7.2 Risk Assessment

Risk assessment is a critical step in the selection of an appropriate containment level for the microbiological work to be carried out. Upon review of the biosafety certificate, the biosafety committee will conduct a detailed local risk assessment to determine whether work requires containment level 1, 2, 3 or 4 facilities and operational practices.

Available information can be used as a starting point to assist in the identification of risk factors, including the recommended Risk Group of the organism. In addition to the Risk Group classifications, which are based on the risk factors inherent to the organism, the following factors associated with the laboratory operation should also be examined:

- potential for aerosol generation
- quantity and concentration of the agent
- agent stability in the environment (inherent biological decay rate)
- type of work proposed (e.g., *in vitro*, *in vivo*, aerosol challenge studies)
- use of recombinant organisms (e.g., gene coding for virulence factors or toxins; host range alteration;
- oncogenicity; replication capacity; capability to revert to wild type).

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.

7.2.1 Aerosol Generation

An aerosol consists of airborne particles and surrounding gases. The particle phase may include solids and liquids. The gas phase normally includes air and water vapour and may include vapours of organic and inorganic compounds as well as contaminant gases.

A laboratory worker may unwittingly be exposed to the aerosolized form of the hazardous biological materials 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, opening a tube containing a lyophilized agent, releasing the vacuum on a freeze dryer, and opening a tube within which the air pressure may differ from that of the room, such as 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 specialized

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 safety cabinet. Also, blenders are available which prevent the escape of aerosols produced during use. 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 high efficiency particulate air (HEPA) filters or removed from the laboratory by local and room ventilation methods. A chemical fume hood or a containment cabinet provides a partial barrier and some operator protection against airborne materials, including aerosols, while a gas-tight Class III biological safety cabinet provides an absolute barrier between the material and the worker. A partial barrier for a centrifuge, fermenter or freeze-drying apparatus may be devised by positioning an exhaust hood or canopy over the apparatus provided that there is sufficient exhaust air movement to sweep away aerosols resulting from an incident.

7.2.2 Large Quantities and Concentrations

The risks to laboratory personnel or the environment may increase as the volume or concentration of the biological agent increases. The procedures described in this manual relate primarily to small scales of operation normally encountered in University laboratories. The large scale production (normally, volumes greater than 10 litres) of micro-organisms is addressed in Appendix G.

7.2.3 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.

7.2.3.1 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 air flow patterns through the room so that all parts of the room are swept by the air flow. The influence of opening and closing doors on these air flow patterns is of particular importance. Decontamination of air is best achieved with a high efficiency particulate air (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 air flow due to loading or when irreparable leaks are detected.

Vacuum lines also serve as a conduit through which air may leave the laboratory. These must be protected in a manner commensurate with the other air exhausts. Reusable filter holders with replaceable elements and sealed disposable filter cartridges are available for this application.

7.2.3.2 Liquids

Some liquid wastes, particularly those in which agents have been cultured, will require sterilization or disinfection to inactivate the agent before disposal to the sewage system.

Hazardous chemical and radioactive liquid wastes may require an additional procedure to inactivate viable biological agents before removal from the laboratory. It is dangerous and illegal to dispose of hazardous chemicals and radioactive materials into drains and the sewage system.

Autoclaving (steam sterilization) is generally the surest method of inactivating biological agents and should be used whenever possible. Liquid waste containers designed to withstand autoclaving

temperatures must be used. Containers of liquid waste must be placed into a tray or pan of sufficient capacity to contain all liquid in the event of vessel failure or breakage inside the autoclave chamber.

Although some chemical disinfectants can be used for the inactivation of many biological agents, others may be less effective against particular micro-organisms, or may be suitable only for some of the types of disinfection required in the laboratory (disinfection of work surfaces or instruments, clean up after spills or accidents, and disinfection of liquid wastes). Before adoption, it is recommended that a disinfectant be tested against the biological agent to determine the concentration and contact time required to achieve the objective under the conditions employed.

7.2.3.3 Solids

Reusable items such as glassware should be sterilized by autoclaving whenever this is 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 incinerated or must be autoclaved or chemically disinfected before disposal.

Disposable sharp waste (sharp or pointed items capable of causing punctures, cuts, or tears in skin or mucous membranes and including hypodermic, surgical, suture, or IV needles, syringes with needles, lancets, scalpels and blades) must be carefully collected in a puncture-resistant waste container.

Needles and blades for disposal must be collected in a designated, yellow, puncture-resistant container. After autoclaving, if required, the filled container of needles and blades must be placed into a white plastic 20 litre pail which is provided for the collection of broken and intact glassware for disposal.

Intact and broken glassware for disposal must be collected in puncture-resistant containers. White plastic 20 litre pails are provided for this purpose.

If the material collected in the waste container is contaminated with viable hazardous biological agents, the waste must be decontaminated, preferably by autoclaving, to inactivate the biological agents. Chemical disinfection of sharp waste is generally not recommended since it requires additional handling.

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 bags. After autoclaving and cooling, these bags of waste must be placed into black plastic garbage bags for disposal.

Hazardous chemical and radioactive solid wastes may require an additional procedure 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.

7.2.4 Pipetting

Pipetting accidents can be reduced by using commercially available pipetting devices. 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.

7.2.5 Storage

Agents stored and maintained for on-going research or as part of a culture collection should have

adequate physical protection. Agents should be stored securely, in consideration of the containment level of the agent itself and should have restricted access. An inventory of stored agents should also be maintained so that pathogen storage locations are tracked, and also so that it is clear who is responsible for the pathogens. Documentation procedures should include proper labelling, tracking of internal possession, inactivation and disposal of cultures after use and transfers within and outside the facility. Other records on who has access to the agents, who has access to where the agents are stored or used and transfer documents, should also be kept.

7.3 Physical Containment Levels

Four levels of containment (1 - 4), appropriate to the four risk groups for potentially hazardous biological agents, are defined. These levels of containment are to be regarded as adequate for most laboratory uses of the listed agents. It remains the responsibility of the principal investigator or laboratory director and the University of Toronto to require a higher level of containment for specific manipulations, if these appreciably increase the possibility of infection.

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 Appendix D, 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. Four containment levels are described as follows:

7.3.1 Containment Level 1

This level applies to the basic laboratory for the handling of Risk Group 1 agents. Containment Level 1 requires no special design features beyond those suitable for a well designed and functional laboratory. Biological safety cabinets 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.

7.3.1.1 Physical Requirements

- A room separated from public areas by a door is required. There are no particular restrictions on locating the facility near public or heavily travelled corridors; however, doors should remain closed.
- Coatings on walls, ceilings, furniture, and floors should be cleanable. Windows that can be opened should not be near working areas or containment equipment and should be equipped with fly screens.
- There are no special air handling requirements beyond those concerned with proper functioning of the biological safety cabinets, if used, and those required by building codes.
- Handwashing facilities must be provided, preferably near the point of exit to public areas.
- Separate locations should be provided for hanging street clothing and laboratory coats at the entrance/exit.
- Eye wash stations should be available.

7.3.1.2 Operational Requirements

- The basic laboratory safety practices described in Section 4.1 must be followed.

- In addition, 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.

7.3.2 Containment Level 2

Agents requiring containment level 2 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 handwashing sinks and decontamination facilities (autoclaves).

Containment Level 2 is suitable for most procedures with agents in Risk Group 2. In addition to the requirements of Containment Level 1, the following are required.

7.3.2.1 Physical Requirements

- A biohazard sign with appropriate information must be posted on the entrance to the laboratory; if biological 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.

- Laboratory furnishings and work surfaces should be impervious and readily cleanable. Laboratory surfaces to be scratch, stain, moisture, chemical and heat resistant in accordance with laboratory function.

- An autoclave must be available in or near the laboratory.

- The laboratory should be located away from public areas, general offices, and patient care areas. Access limited to authorized persons and separated from public areas by door. Entrance doors to containment laboratory should be self-closing and lockable.

- Emergency eyewash facilities to be provided in accordance with applicable regulations (ANSI Z358.1-1998).

- Emergency shower equipment to be provided in accordance with applicable regulations (ANSI Z358.1-1998).

7.3.2.2 Operational Requirements

- Class I or II biological safety cabinets (see Appendix E) are required for all manipulations of agents which may create an aerosol. The biological safety cabinet must have been tested and certified within the previous 12 months according to accepted standards (see Section 3.8).

- Inspection and retesting is mandatory if the cabinet is relocated. Moves of a minor nature may be exempt if the move is supervised by the testing technologist to ensure that the equipment has not

been subjected to undue stress. At the time certification is carried out, the testing technologist should ascertain that the users are familiar with the containment capability of the equipment under various operating conditions and familiarize such individuals with precautions to be taken in its use.

- Air from these cabinets may be recirculated to the room only after passage through a high efficiency particulate air (HEPA) filter.

- Good microbiological laboratory practices intended to avoid the release of biological agents are to be employed. Centrifugation must be conducted with closed containers or aerosol proof safety heads or cups. These should be opened only in the biological safety cabinet.

- Organisms which have been experimentally infected must remain in the laboratory or appropriate animal containment facility.

- An emergency plan for handling spills of infectious materials must be developed and be ready for use whenever needed. Laboratory workers must be educated about the emergency plans. A record must be made of other people entering the facility during an emergency.

- Vacuum lines used for work involving the agent must be protected from contamination by HEPA filters or equivalent equipment.

- Laboratory coats should be worn only in the laboratory area. Either front-button coats or wrap around gowns are acceptable. These coats shall not be worn outside the containment laboratory.

- Special care should be taken to avoid contamination of the skin with infectious materials. Gloves must be worn when handling infected organisms or when hands may be exposed to biological agents.

- Contaminated glassware must not leave the facility. Decontamination must be carried out using procedures demonstrated to be effective. If there is no autoclave or incinerator in the laboratory, contaminated materials must be disinfected chemically or be double bagged and transported to the autoclave or incinerator in durable, leakproof containers which are closed and wiped on the outside with disinfectant before leaving the laboratory.

- All people working in the containment area must be trained in and follow operating protocols for the project in process. Service personnel and cleaning staff entering the facility must be informed of the hazards that might be encountered. Cleaning staff should clean only the floors. The laboratory personnel have the responsibility for rendering the facility safe for routine cleaning. Periodic intensive cleaning must be done at regular intervals. Cleaning and maintenance staff should receive appropriate immunization and medical surveillance.

- Entry must be restricted to laboratory staff, animal handlers, maintenance staff and others on official business.

7.3.3 Containment Level 3

Containment Level 3 is suitable for most procedures with agents in Risk Group 3. The operational requirements for the Level 3 laboratory are substantially greater than those for Levels 1 and 2 and the laboratory staff must receive specific training in the safe handling and manipulation of the agents used in this laboratory. The Containment Level 3 laboratory is designed to minimize environmental release of hazardous materials and provide enhanced worker protection. Annual performance testing and verification is required.

Containment level 3 emphasizes additional primary and secondary barriers to minimize the release of infectious organisms into the immediate laboratory and the environment. Additional features to prevent transmission of containment level 3 organisms include appropriate respiratory protection, HEPA filtration of exhausted laboratory air and strictly controlled laboratory access.

A Containment Level 3 laboratory requires specialized design and construction. Those responsible for biosafety in an institution should maintain close control and seek expert advice in, and remain in close communication throughout, all phases of design, construction, initial and annual performance testing and verification, operation and maintenance.

The following are additional to the requirements of Containment Levels 1 and 2.

7.3.3.1 Physical Requirements

- The laboratory must be located away from general work areas and have a controlled access system (e.g. card key) or equivalent. This is accomplished by entry through a lockable changing room with self-closing doors. A body shower should be provided within the containment perimeter (i.e. between 'dirty' and 'clean' change anterooms)

- Office areas to be located outside of containment laboratory. Paperwork stations for data collection can be within the containment laboratory provided they are located away from laboratory work areas.

- Entry to laboratory to be provided via an anteroom.

- Anteroom door(s) located between the clean and dirty change rooms not to be opened simultaneously with either the containment laboratory door or the clean change entry door. Interlocked doors, if present, to have manual override for emergency exit.

- Entry to laboratory zone to be provided with clothing change areas separating personal and laboratory clothing dedicated to that zone.

- Laboratory furnishings should be kept to a minimum. Work surfaces should be impervious, readily cleanable, and resistant to chemical disinfectants.

- All penetrations for services in the floors, walls, and ceiling of the laboratory must be sealed. Surfaces to be impact resistant in accordance with laboratory function. Surfaces to be continuous and compatible with adjacent and overlapping materials; wall and floor welded seams are acceptable in level 3 laboratories. Interior surfaces to minimize movement of gases and liquid through perimeter membrane. Interior coatings to be cleanable. Bench tops to have to open seams.

- Laboratory windows must be sealed and unbreakable.

- 100% outside air to be supplied.

- The laboratory must be held at a negative pressure relative to the surrounding area at all times such that a directional airflow is created, with air ingressing towards areas of higher containment. A control system must be provided to ensure that the Level 3 laboratory does not become positively pressurized relative to the surrounding area. Visual pressure differential monitoring devices to be provided at entry to containment laboratory.

- Supply air to be HEPA filtered.

- The laboratory should be provided with a dedicated air supply and exhaust system which is

sealed. The air discharged from the laboratory cannot be recirculated back into the air supply system of either the laboratory or the building or adjacent buildings. In most cases, exhaust air from containment level 3 laboratories should be HEPA filtered prior to discharge.

- Supply air system to be interlocked with exhaust air system, to prevent sustained laboratory positive pressurization.

- Alarm (visible or audible) to be provided in the laboratory and outside laboratory area to signal air handling systems failure.

- The air supply and exhaust system should be equipped with manual dampers at the room perimeter which may be closed as required to permit gas decontamination.

- All vent lines should be provided with HEPA filters or equivalent protection. Where HEPA filters are used for backdraft protection in accordance with local risk assessment, supply HEPA filter housings to be designed to withstand structural change at applied pressure of 2500Pa.

- Exhaust HEPA filter housings to be designed to withstand structural change at applied pressure of 2500 Pa and to be provided with a method of isolation and decontamination.

- Exhaust air system to be independent of other laboratory areas. CL3 exhaust can be combined with areas of lower containment when provided with a HEPA filter upstream from the connection.

- HEPA filters installed into the supply and exhaust system to conform to the requirements of IEST-RP-CC001.3.

- Where backdraft protection is required in accordance with local risk assessment, supply air ductwork that is outside the containment perimeter (e.g. between containment perimeter and HEPA filter or bubble tight backdraft damper) to be sealed airtight in accordance with SMACNA Seal Class A.

- Exhaust air ductwork that is outside the containment perimeter (e.g. between containment perimeter and HEPA filter or bubble tight backdraft damper) to be sealed airtight in accordance with SMACNA Seal Class A.

- Airflow control devices and duct sensors to be located downstream of the exhaust HEPA filter and upstream of the supply bubble tight backdraft damper or HEPA filter, or if located upstream, duct penetrations to be sealed with SMACNA Seal Class A.

- Bubble tight backdraft dampers and HEPA filters to be located in close proximity to the containment perimeter.

- Biological safety cabinets must be installed in a manner which does not interfere with the air balance of the cabinet or the room. Thimble unit connections are recommended.

- The laboratory must have a dedicated handwashing sink with foot, knee or automatic controls located near the exit.

- The laboratory must have a pass-through or stand alone autoclave located in the work zone. Where physical constraints preclude the installation of an autoclave in an existing Level 3 laboratory, alternative technologies may be used for sterilization of contaminated materials. Barrier autoclave to be equipped with interlocking doors, or visual or audible alarms to prevent both doors from opening at the same time.

- Autoclave condensate drains should have closed connections and go directly to the sanitary

sewer. For CL3, open connection is allowable if located within the containment barrier.

- For materials that cannot be autoclaved (e.g., heat sensitive equipment, samples, film) other proven technologies for waste treatment (e.g., incineration, chemical, or gas) to be provided at containment barrier.

- All penetrations to be sealed with non-shrinking sealant at containment barrier.

- All conduit and wiring to be sealed with non-shrinking sealant at the containment barrier

- Windows positions on containment barrier to be sealed in place; window glazing material to provide required level of security.

- Water supplied to the laboratory must be provided with reduced pressure backflow preventers.

- Dunk tanks may be provided at the containment perimeter.

- Sink and floor drains from this suite should be piped separately to the main building drain and be appropriately labelled. Floor drains are not generally recommended. Infectious materials must never be placed in sinks or floor drains.

- Drainage traps to be provided to required deep seal depth in consideration of air pressure differentials.

- Plumbing vent lines to be independent of lower containment plumbing vent lines, or combined with lines from lower containment when provided with a filter of efficiency equivalent to that of HEPA upstream from the connection.

- Handwashing sinks to be provided with “hands-free” capability.

- BSCs and other primary containment devices to be provided.

- When it is not possible to limit the quantities of hazardous chemicals within the laboratory, emergency shower equipment to be provided n accordance with applicable regulations (i.e., ANSI Z358.1-1998).

- Drain lines and associated piping (including autoclave condensate) to be separated from lower containment laboratory areas and to go directly to main building sanitary sewer at point of exit from building (downstream of all other connections).

- In animal care facilities for small animals, the disposal of wastes will not differ from other contaminated laboratory materials. Large animals producing quantities of infectious wastes require special facilities which must be designed accordingly.

- Portable vacuum pumps must be fitted with in-line HEPA filters or equivalent equipment. No vacuum lines may exit through the containment perimeter.

- Emergency lighting to be provided.

- Life safety systems, lighting, HVAC systems, BSCs, security systems and other essential equipment to be supported with emergency back-up power.

- Circuit breakers to be located outside biocontainment area.

- Laboratory to be equipped with a communication system between containment area and outside

support area.

- System (e.g., fax, computer) to be provided for electronic transfer of information and data from laboratory area to outside laboratory perimeter (Note: paperwork from the containment laboratory may be removed after appropriate decontamination, i.e., autoclaving, irradiation, microwaving; such practices are generally not recommended for use on a routine basis).

7.3.3.2 Operational Requirements

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

- A protocol specific to the operation of the laboratory must be developed and read by personnel. This should include entry and exit protocols for people, animals, equipment, samples and waste. General protocols must be supplemented with protocols specific to each project in progress. Signed, written records of lab personnel training must be maintained

- Laboratory staff must be trained in the handling of pathogenic and other hazardous material and in the use of safety equipment, disposal techniques, handling of contaminated waste, and emergency response.

- Personnel working in the containment area must have knowledge of the physical operation and design of the facility (e.g., air pressure gradients between zones, directional airflow patterns, alarm signals for air pressure failure, containment perimeter).

- Written protocols must be provided and posted within the laboratory outlining operational protocols, waste disposal, disinfection procedures, and emergency response. When a known or suspected aerosol exposure may have occurred, protocols based on a local risk assessment must be in place to determine whether showering is required on exit from the laboratory. In the event of life-threatening emergencies, personal health and safety are a priority; 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).

- Staff are required to change into dedicated, solid front laboratory clothing on entry to the facility. This laboratory clothing must be removed on completion of work and autoclaved prior to laundering.

- Personal protective clothing which may include head covers and dedicated shoes or foot covers must be used while in the containment facility and removed on leaving.

- Appropriate respiratory protection should be considered depending on the biological agents in use.

- Body showering may be required depending on biological agents used and manipulations involved.

- Personal effects may not be taken into or stored in the laboratory.

- Gloves must be worn when handling infectious or potentially infectious materials including animals or waste.

- All activities involving infectious materials are conducted in biological safety cabinets or other appropriate combinations of personal protective and physical containment devices.

- Centrifugation must be conducted with closed containers using aerosol proof safety heads or cups which are opened and loaded and unloaded in the biological safety cabinet.

- Effective disinfectants must be available at all times in the laboratory.

- All Risk Group 3 agents must be stored within the Containment Level 3 facility.

- An effective pest control program must be implemented.

- The facility must have a medical surveillance program appropriate to the agents used, which includes serum storage for all personnel working in the containment laboratory.

- The laboratory must have a reporting system for accidents and exposures to biological agents or other incidents or unusual occurrences in the operation of the laboratory.

- Authorized maintenance and service personnel must abide by the same operational protocols as laboratory staff and be accompanied by laboratory personnel when entering and working in the laboratory.

- The Containment Level 3 facility and its systems must be tested for containment capability upon completion of construction and at least annually thereafter.

- Smoke testing (i.e., using a smoke pencil held at the door between the anteroom and the containment facility, and other doors as required) should be done periodically to verify correct airflow; a containment check must be performed before entering the containment laboratory (e.g., verify correct reading on the pressure monitoring device).

- People entering a containment facility must be well prepared and bring all materials they will need with them; if something has been forgotten, established traffic patterns must still be adhered to (i.e., do not go back to get it; either phone for someone to bring it or exit using proper protocols).

- Routine laboratory cleaning must be done by persons using the containment facility or by specific persons dedicated and trained for this task.

- The containment laboratory must be kept locked.

- Drainage traps must be filled with liquid (i.e., through regular sink usage, automatic primers or by filling traps in areas that are not frequently used).

- Laboratory samples and supplies may be carried into the containment laboratory or passed in through a pass-box; if the barrier autoclave is used to pass materials into the laboratory, the autoclave must have been cycled before the outer "clean side" door is opened.

- Organisms that have been experimentally infected must remain in the laboratory or appropriate animal containment facility.

- Infectious agents should be stored inside the containment laboratory; agents stored outside of the zone must be kept locked, in leakproof containers; emergency response procedures are to take into account the existence of such infectious agents outside of the containment level 3 laboratory.

7.3.3.3 Commissioning, Certification and Recertification

Commissioning is a requirement for the certification of containment levels 3 laboratories. Building systems commissioning is a process designed to ensure that the finished facility, equipment and systems will operate in accordance with the design intent and construction documents. It is recommended that commissioning be implemented early in the planning phase through to the construction and certification. To ensure that the physical requirements for the intended containment level and use of the facility have been met, each laboratory must undergo a detailed commissioning regimen. This requires verification and documentation of critical containment components, equipment start-up, control system calibration, balancing and performance testing. A complete set of drawings and specifications, an understanding of the intended use and work to be performed, a list of equipment requirements, all test results, and an understanding of the intent of the systems' operation are all part of the commissioning process.

A matrix of critical containment components to be verified during initial certification is available in Health Canada's Laboratory Biosafety Guidelines (3rd ed.). Operational protocols must also be established before work with pathogens at the specified containment level can be carried out. Training of personnel is a critical aspect of this process and may involve initial work with pathogens normally requiring a lower containment level. Users must understand the containment systems and their operation in addition to scientific procedures. Detailed records of the certification process and test results must be maintained.

Recertification of certain containment components should also be performed, the nature and frequency of which depend on a variety of factors. For example, verification of directional airflow, detection of any visual leaks in the room perimeter, recalibration of sensitive controllers and gauges, and monitoring of the efficacy of sterilization systems such as autoclaves can all be performed on a routine basis without disruption to the operation of the containment facility. Monitoring the resistance across a HEPA filter (e.g.., using pressure monitoring devices) installed into air handling systems will provide information as to the necessity and frequency of replacing HEPA filters. Retesting the integrity of the room perimeter and ductwork is necessary after any structural change. Retesting of the HVAC control systems for fail-safe operation is not necessary unless the system has undergone logic changes or upgrades.

It is the responsibility of the facility director to ensure that the requirements of certification and re-certification are met. Any testing required for certification or maintenance of the Containment level 3, shall be arranged by the facility director, but must be conducted by independent bodies approved by the university.

7.4 Laboratory Decommissioning

At least 30 days prior to the expected date of vacating the laboratory or laboratory space, the Principal Investigator (PI) must notify, in writing, the Biosafety officer and the Environmental Protection Services.

The Principal Investigator must ensure that appropriate decontamination measures have been taken prior to the relocation or disposal of laboratory equipment used for the manipulation of biological agents.

After decontamination, the laboratory equipment may be recycled through the Facilities and Services Department. The University's "Safe-To-Work" tag should be completed and attached to the equipment, prior to it being removed from the laboratory. The tag may be obtained from the Office of Environmental Health and Safety at (416) 978-4467.

Inspection and retesting is mandatory if a biological safety cabinet is relocated. Moves of a minor nature (i.e. within the same room) may be exempt from this requirement if the move is observed by the testing technologist and the cabinet has not been subjected to excessive stress or rough handling which could result in damage.

7.5 Animal Biohazard Containment Facilities

Laboratory facilities must provide containment for laboratory animals exposed to or harbouring biological agents which is appropriate to the risk level of the biological agents involved. In addition to the physical containment requirements identified in Section 7.3, 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 to the Care and Use of Experimental Animals of the Canadian Council on Animal Care and the local animal care authority, as well as this manual in order to ensure 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.

8 SECURITY

8.1 General

Biological agents have a dual-use potential. They can be used in research for the advancement of science and the diagnosis of disease, but can also be misused, stolen or intentionally released. The handling of infectious disease agents requires a security plan to ensure that biological agents are used as intended and stored securely.

8.2 Physical Protection

The physical protection risk assessment should include all levels of a security review: perimeter security, facility security, laboratory security and agent specific security, and outline procedures for securing the area, e.g., card access, key pads, locks etc.

All laboratories should adopt security practices to minimize opportunities for unauthorized entry into laboratories, animal and storage areas, as well as the unauthorized removal of infectious materials from their facility. The aim is to have a dedicated and controlled access into the laboratory limited to authorized personnel, laboratory staff, and maintenance staff. Within the laboratory, access to biological agents should be controlled as well. The containment perimeter (i.e., doors, windows) should provide the required level of security and should be kept closed. Similarly, information security for data and electronic technology needs to be addressed.

8.3 Personnel Reliability/Suitability

Background checks and security clearances may be required before employees are granted access to containment facilities. It may be appropriate to use photo identification badges for employees and temporary badges for escorted visitors to identify individuals with clearance to enter restricted areas. Procedures are needed for approving and granting visitors access to controlled areas. In this capacity the access to agents and storage facilities is limited to legitimate use/individuals only. Biosafety training should include address security issues and must be provided to all personnel who are given access. Personnel must demonstrate that they have understood the biosecurity training provided. For Containment Level 3 laboratories, the training must be documented and signed by both the employee and the supervisor.

8.4 Pathogen Accountability

A system for proper labeling, tracking of internal possession, inactivation and disposal of cultures after use, and transfers within and outside the facility should be developed. These controls also assist in the tracking of pathogen storage locations and in clarifying under whose responsibility the pathogens lie.

Inventories should be updated regularly to include new additions as a result of diagnosis, verification of proficiency testing, or receipt from other locations as well as to remove agents after transfers or appropriate inactivation and disposal mechanisms have been used. Disposal of agents after use should include all sub-cultures of that agent as well. The record keeping should include pathogen inventories, who has access to agents, who has access to areas where agents are stored or used, as well as transfer documents. A record of culture collections and other agents not currently used for research should be included in inventory lists as well. A notification process for identifying, reporting, and remediation of security problems, i.e., inventory discrepancy, equipment failure, breach of security, release of agents, etc., should be in place.

8.5 Storage

Agents stored and maintained for on-going research or as part of a culture collection should have adequate physical protection. Agents should be stored securely, in consideration of the containment level of the agent itself and should have restricted access. An inventory of stored agents should also be maintained so that pathogen storage locations are tracked, and also so that it is clear who is responsible for the pathogens. Documentation procedures should include proper labelling, tracking of internal possession, inactivation and disposal of cultures after use and transfers within and outside the facility. Other records on who has access to the agents, who has access to where the agents are stored or used and transfer documents, should also be kept.

APPENDIX A AGENTS NOT INDIGENOUS TO CANADA

The following partial list is provided as an example of animal pathogens which are not indigenous to Canada and which are subject to control by the Canadian Food Inspection Agency (CFIA). The CFIA will determine the conditions under which these agents are used and maintained. This list of non-indigenous agents is not complete.

BACTERIA

Mycoplasma agalactiae
Mycoplasma mycoides
Rickettsia ruminantium

PARASITES

Besnoitia besnoiti
Theileria annulata
Theileria bovis
Theileria hirci
Theileria lawrencei
Theileria parva
Trypanosoma equiperdum
Trypanosoma evansi
Trypanosoma vivax

VIRUSES

Bornaviridae
- Borna disease virus

Bunyaviridae
- Nairobi sheep disease virus
- Rift Valley fever virus

Caliciviridae
- Swine vesicular disease virus
- Vesicular exanthema virus

Flaviviridae
- Hog cholera virus

Herpesviridae
- Pseudorabies virus

Iridoviridae
- African swine fever virus

Orthomyxoviridae
- Fowl plague virus

Paramyxoviridae
- Rinderpest, Newcastle disease virus: mesogenic, velogenic strains
- Peste des petits ruminants

Picornaviridae
- Genus Aphthovirus
- Foot-and-mouth disease virus
- Genus Enterovirus
- Teschen disease virus

Poxviridae
- Chordopoxvirinae (poxviruses of vertebrates)
- Genus Orthopoxvirus
- Smallpox (Alastrim) virus
- Genus Capripoxvirus
- Sheeppox virus
- Goatpox virus
- Lumpy skin disease virus
- Genus Suipoxvirus
- Swinepox virus
- Camelpox virus

Reoviridae
- Genus Orbivirus
- Bluetongue virus
- African horsesickness virus

Rhabdoviridae
- Genus Vesiculovirus
- Ephemeral fever virus
- Vesicular stomatitis virus (animal inoculation)

Togaviridae
- Louping ill virus (animal inoculation)
- Wesselsbron disease virus
- Venezuelan equine encephalitis (VEE) virus

APPENDIX B RISK GROUP CATEGORIZATION OF AGENTS

RISK GROUP 1

low individual and community risk

This group includes those micro-organisms, bacteria, fungi, viruses and parasites which are unlikely to cause disease in healthy workers or animals.

Many agents are referred to in the literature by a variety of names and, before assuming that an unlisted agent is assigned to Risk Group 1, its characteristics and pathogenicity must be verified in consultation with the University of Toronto Biosafety Committee or the Office of Biosafety, Public Health Agency of Canada.

RISK GROUP 2

moderate individual risk, limited community risk

A pathogen that can cause human or animal disease but, under normal circumstances, is unlikely to be a serious hazard to healthy 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.

BACTERIA, CHLAMYDIA, MYCOPLASMA

Actinobacillus: all species
Actinomyces pyogenes (C. pyogenes)
Bacillus cereus
Bartonella bacilliformis, B. henselae, B. quintana, B. elizabethae
Bordetella pertussis, B. parapertussis and B. bronchiseptica
Borrelia recurrentis, B. burgdorferi
Campylobacter spp: C. coli, C. fetus, C. jejuni
Chlamydia pneumoniae, C. psittaci (non-avian strains), C. trachomatis
Clostridium botulinum, Cl. chauvoei, Cl. difficile, Cl. haemolyticum,
Cl. histolyticum, Cl. novyi, Cl. perfringens, Cl. septicum, Cl. sordellii, Cl. tetani
Corynebacterium diphtheriae, C. haemolyticum, C. pseudotuberculosis,
C. pyogenes (A. pyogenes)
Edwardsiella tarda
Erysipelothrix rusiopathae (insidiosa)
Escherichia coli: enterotoxigenic / invasive / hemorrhagic strains
Francisella tularensis Type B, (biovar palaearctica), F. novocida
Fusobacterium necrophorum
Haemophilus influenzae, H. ducreyi
Helicobacter pylori
Legionella spp.
Leptospira interrogans: all serovars
Listeria monocytogenes
Mycobacteria: all species except M. tuberculosis and M. bovis (non-BCG strain),
which are in Risk Group 3
Mycoplasma pneumoniae, M. hominis
Neisseria gonorrhoeae, N. meningitidis
Nocardia asteroides, N. brasiliensis
Pasteurella: all species except P. multocida type B, which is in Risk Group 3
Pseudomonas aeruginosa
Salmonella enterica (S. choleraesuis)
Salmonella enterica serovar arizonae (Arizona hinshawii)
Salmonella enterica serovar gallinarum-pullorum (S. gallinarum-pullorum)
Salmonella enterica serovar meleagridis (S. meleagridis)
Salmonella enterica serovar paratyphi B (S. paratyphi B) (Schottmulleri)
Salmonella enterica serovar typhi (S. typhi)
Salmonella enterica serovar typhimurium (S. typhimurium)
Shigella boydii, S. dysenteriae, S. flexneri, S. sonnei
Staphylococcus aureus
Streptobacillus moniliformis
Streptococcus spp: Lancefield Groups A, B, C, D, G
Treponema carateum, T. pallidum (including T. pertenue), T. vincentii
Ureaplasma urealyticum
Vibrio cholerae (including El Tor), V. parahaemolyticus, V. vulnificus
Yersinia enterocolitica, Y. pseudotuberculosis

FUNGI

Cryptococcaceae
- Candida albicans
- Cryptococcus neoformans

Moniliaceae
- Aspergillus flavus
- Aspergillus fumigatus
- Epidermophyton floccosum
- Microsporum spp.
- Sporothrix schenckii
- Trichophyton spp.

VIRUSES

Arthropod-borne viruses are identified with an asterisk (*). Only those viruses which may be associated with human or animal disease have been included in this list. Agents listed in this group may be present in blood, CSF, central nervous system and other tissues, and infected arthropods, depending on the agent and the stage of infection.

Adenoviridae
- Adenoviruses: all serotypes

Arenaviridae
- Lymphocytic choriomeningitis virus: laboratory adapted strains
- Tacaribe virus complex: Tamiami, Tacaribe, Pichinde

Bornaviridae
- Borna disease virus

Bunyaviridae*
- Genus Bunyavirus
- Bunyamwera and related viruses
- California encephalitis group, including LaCrosse, Lumbo and Snowshoe hare virus
- Genus Phlebovirus: all species except Rift Valley fever virus (see Appendix C)

Caliciviridae: all isolates, including Hepatitis E and Norwalk virus

Coronaviridae
- Human coronavirus: all strains
- Genus Torovirus
- Transmissible gastroenteritis virus of swine
- Hemagglutinating encephalomyelitis virus of swine
- Mouse hepatitis virus
- Bovine coronavirus
- Feline infectious peritonitis virus
- Avian infectious bronchitis virus
- Canine, Rat and Rabbit coronaviruses

Flaviviridae*
- Yellow fever virus: 17D vaccine strain
- Dengue virus: serotypes 1, 2, 3, 4
- Kunjin virus
- Hepatitis C virus

Hepadnaviridae
- Hepatitis B virus, including Delta agent

Herpesviridae
- Alphaherpesvirinae
  - Genus Simplexvirus: all isolates including HHV 1 and HHV 2, except Herpes B virus which is in Risk Group 4

Genus Varicellavirus: all isolates including varicella / zoster virus (HHV 3) and pseudorabies virus (see Appendix C)
Betaherpesvirinae
Genus Cytomegalovirus: all isolates including CMV (HHV 5)
Genus Muromegalovirus: all isolates
Gammaherpesvirinae
Genus Lymphocryptovirus: Epstein Barr Virus (HHV 4) and EB-like isolates
Genus Rhadinovirus: all isolates except H. ateles and H. saimiri in Risk Group 3
Genus Thetalymphocryptovirus: all isolates
Unassigned Herpesviruses: includes HHV 6 (human B-lymphotrophic virus), HHV 7, HHV 8, etc.

Orthomyxoviridae
Genus Influenzavirus: Influenza virus type A: all isolates
Influenza virus type B: all isolates
Influenza virus type C: all isolates

Papovaviridae
Genus Papillomavirus: all isolates
Genus Polyomavirus: all isolates

Paramyxoviridae
Genus Morbillivirus: all isolates except Rinderpest virus (see Appendix C)
Genus Paramyxovirus: all isolates
Genus Pneumovirus: all isolates

Parvoviridae
Genus Parvovirus: all isolates

Picornaviridae
Genus Aphthovirus: (see Appendix C)
Genus Cardiovirus: all isolates
Genus Enterovirus: all isolates (see Table 1 for restrictions)
Genus Hepatovirus: all isolates (Hepatitis A)
Genus Rhinovirus: all isolates

Poxviridae (see Table 1 for restrictions)
Chordopoxvirinae (poxviruses of vertebrates)
Genus Avipoxvirus: all isolates
Genus Capripoxvirus: (see Appendix C)
Genus Leporipoxvirus: all isolates
Genus Molluscipoxvirus
Genus Orthopoxvirus: all isolates except Variola virus and Monkeypox virus which are in Risk Group 4
Genus Parapoxvirus: all isolates
Genus Suipoxvirus: Swinepox virus (see Appendix C for restrictions)
Genus Yatapoxvirus
All other ungrouped poxviruses of vertebrates

Reoviridae
Genus Orbivirus: all isolates (see Appendix C for restrictions)
Genus Orthoreovirus: types 1, 2 and 3
Genus Rotavirus: all isolates

Retroviridae
Oncovirinae
Genus Oncornavirus C
Subgenus Oncornavirus C avian: all isolates
Subgenus Oncornavirus C mammalian: all isolates except HTLV-I and HTLV-II
Genus Oncornavirus B: all isolates
Lentivirinae: all isolates except HIV-I and HIV-II
Spumavirinae: all isolates

Rhabdoviridae
Genus Vesiculovirus: all laboratory adapted strains (see Appendix C for restrictions)

Genus Lyssavirus: Rabies virus (fixed virus)
Togaviridae
Genus Alphavirus*
Semliki forest virus
Sindbis virus
Chikungunya virus: high-passage strains
O'Nyong-Nyong virus
Ross river virus
Venezuelan equine encephalitis virus: only strain TC-83, no animal inoculation (see Appendix C)
Genus Rubivirus
Rubella virus
Genus Pestivirus
Bovine diarrhoea virus
Border disease virus
Genus Arterivirus
Equine arteritis virus
Unclassified viruses
Other Hepatitis viruses
Astro viruses
Chronic infectious neuropathic agents (CHINAs): Scrapie, BSE
(except Kuru and Creutzfeldt-Jakob Disease agents in Risk Group 3)

PARASITES

Infective stages of the following parasites have caused laboratory infections by ingestion, skin or mucosal penetration or accidental injection. Preparations of these parasites known to be free of infective stages do not require this level of containment.

PROTOZOA

Babesia microti
Babesia divergens
Balantidium coli
Cryptosporidium spp.
Entamoeba histolytica
Giardia spp. (mammalian)
Leishmania spp. (mammalian)
Naegleria fowleri
Plasmodium spp. (human or simian)
Pneumocystis carinii
Toxoplasma gondii
Trypanosoma brucei, T. cruzi

HELMINTHS

Nematodes

- Ancylostoma duodenale
- Angiostrongylus spp.
- Ascaris spp.
- Brugia spp.
- Loa loa
- Necator americanus
- Onchocerca volvulus
- Strongyloides spp.
- Toxocara canis
- Trichinella spp.
- Trichuris trichiura
- Wuchereria bancrofti

Cestodes

- Echinococcus (gravid segments)
- Hymenolepis diminuta
- Hymenolepis nana (human origin)
- Taenia saginata
- Taenia solium

Trematodes

- Clonorchis sinensis
- Fasciola hepatica
- Opisthorchis spp.
- Paragonimus westermani
- Schistosoma haematobium
- Schistosoma japonicum
- Schistosoma mansoni

RISK GROUP 3

high individual risk, low community risk

A pathogen that usually causes serious human or animal disease, or which can result in serious economic consequences but does not ordinarily spread by casual contact, from one individual to another, or that can be treated by antimicrobial or antiparasitic agents.

BACTERIA, CHLAMYDIA, RICKETTSIA

Bacillus anthracis
Brucella: all species
Burkholderia (Pseudomonas) mallei, B. pseudomallei
Chlamydia psittaci: avian strains only
Coxiella burnetti
Francisella tularensis type A (biovar tularensis)
Mycobacterium bovis: non-BCG strains
Mycobacterium tuberculosis[1]
Pasteurella multocida, type B
Rickettsia: all species (see Appendix C)
Yersinia pestis

[1] Preparation of smears and primary culture of M. tuberculosis may be performed at Level 2 physical containment using Level 3 operational procedures and conditions. All other manipulations of M. tuberculosis require Containment Level 3 physical and operational conditions.

FUNGI

Moniliaceae
Ajellomyces capsulatus (Histoplasma capsulatum, including H. capsulatum var. duboisii)
Ajellomyces dermatitidis (Blastomyces dermatitidis)
Coccidioides immitis
Paracoccidioides brasiliensis

VIRUSES

Arthropod-borne viruses are identified with an asterisk (*).

Arenaviridae
Lymphocytic choriomeningitis virus: neurotropic strains
Bunyaviridae
Unclassified Bunyavirus
Hantaan, Korean haemorrhagic fever and epidemic nephrosis viruses including Hantavirus pulmonary syndrome virus
Rift Valley fever virus
Flaviviridae*
Yellow fever virus: wild type
St. Louis encephalitis virus
Japanese encephalitis virus
Murray Valley encephalitis virus
Powassan encephalitis virus
Herpesviridae
Gammaherpesvirinae
Genus Rhadinovirus: Herpesvirus ateles, Herpesvirus saimiri
Retroviridae
Oncovirinae
Genus Oncornavirus C

Human T-cell leukemia / lymphoma virus[2]

Genus Oncornavirus D

Mason-Pfizer monkey virus

Viruses from non-human primates

Lentivirinae

Human immunodeficiency viruses (HIV): all isolates[2]

Rhabdoviridae

Genus Vesiculovirus: wild type strains (see Appendix C for restrictions)

Genus Lyssavirus

Rabies virus (street virus)

Togaviridae

Genus Alphavirus*

Eastern equine encephalitis virus

Chikungunya virus

Venezuelan equine encephalitis virus (except Strain TC-83; see Appendix C)

Western equine encephalitis virus

Unclassified Viruses

Chronic infectious neuropathic agents: Kuru, Creutzfeldt-Jakob Disease agents (level of precautions depends on the nature of the manipulations and the amount of sera, biopsy / necropsy materials handled)

[2] Laboratories engaging in primary isolation and identification of HTLV or HIV may perform these activities in Containment Level 2 laboratories (physical conditions) using Containment Level 3 operational procedures and conditions. All research and production activities require Containment Level 3 physical and operational conditions.

PARASITES

None

RISK GROUP 4

high individual risk, high community risk

A pathogen that usually produces very serious human or animal disease, 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.

NOTE: Risk Group 4 agents are not approved for use at the University of Toronto.

BACTERIA
None

FUNGI
None

VIRUSES
Arthropod-borne viruses are identified with an asterisk (*).

Arenaviridae
- Lassa, Junin, Machupo, Sabia, Guanarito viruses

Bunyaviridae*
- Genus Nairovirus
- Crimean-Congo hemorrhagic fever virus

Filoviridae
- Marburg virus
- Ebola virus

Flaviviridae*
- Tick-borne encephalitis complex including Russian Spring-Summer encephalitis virus
- Kyasanur forest virus
- Omsk hemorrhagic fever virus

Herpesviridae
- Alphaherpesvirinae
- Genus Simplexvirus: Herpes B virus (Cercopithecine herpesvirus 1)

Poxviridae
- Genus Orthopoxvirus
- Variola virus
- Monkeypox virus

PARASITES
None

APPENDIX C SAFETY EQUIPMENT AND BIOLOGICAL SAFETY CABINETS

SAFETY EQUIPMENT

An essential element in maintaining personal safety and environmental protection is the correct selection, use and maintenance of safety equipment in the laboratory. Safety equipment must be maintained and regularly serviced. There must also be a regular program of testing and inspection, and accurate records must be kept. The following is a list of safety devices appropriate to the containment laboratory:

Type	**Application**
Animal cages or boxes	partial to total containment of aerosols; provide protection from cross-contamination and personnel and environmental protection
Autoclaves	high temperature steam sterilization
Blenders and mixers	aerosol-free blenders provide containment of aerosols
Biological safety cabinet	(see end of this Appendix)
Centrifuge equipment	safety cups with sealed heads provide containment of aerosols
Face / eye wash station	device for flushing face and eyes with water in event of splash or spray of biological or chemical agents
Face and eye protection	safety glasses, goggles and full-face shields provide protection from flying objects and splashes
Fume hoods	provide personnel and environmental protection; for removal or control of gases and vapours.
Gloves	provide hand protection of varying degrees; check technical specifications to determine degree of protection
HEPA filters	high efficiency particulate air filters available in various sizes, including cartridges; disposable; provide 99.97% removal of 0.3 micrometre particulates
Incinerators - micro	electric or gas with side-arm to contain splatters when flaming inoculation and transfer loops
Laboratory clothing	head covers, shoe covers, coats, gowns or ventilated suits appropriate to hazard
Leakproof containers	variety of containers, preferably of stainless steel and autoclavable, with tight-fitting lids, and which may be used for transporting waste materials to an autoclave
Pipetting devices	variety of devices which eliminate need to pipette by mouth
Respiratory protection	partial or full-face protection; provided with variety of filters
Sharp waste containers	autoclavable, puncture-resistant containers which are used for collection and disposal of used hypodermic syringes and needles, blades and other sharp waste

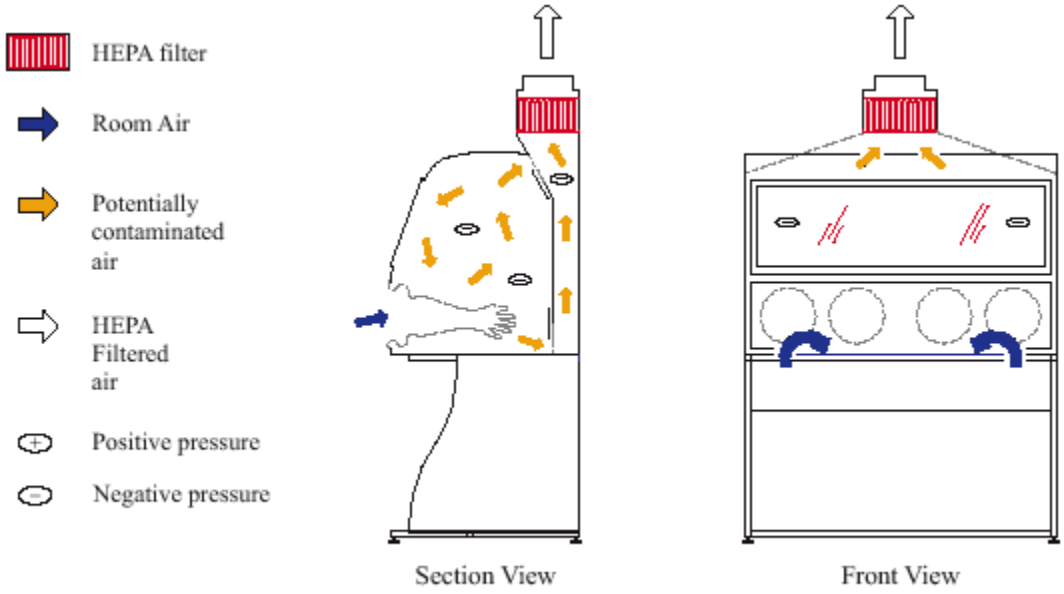
Sterility indicators	indicating devices with heat resistant bacterial spores used to determine efficacy of steam sterilization

BIOLOGICAL SAFETY CABINETS

There are three classes of BSC: Class I, Class II and Class III. Selection of the proper class of BSC requires careful evaluation of the activities to be carried out. Horizontal, clean benches that direct air towards the operator are not biological safety cabinets and must not be used for handling infectious, toxic or sensitizing materials. Only cabinets that meet the National Sanitation Foundation (NSF) Standard No. 49-2002 (independent standard for the design, manufacture and testing of BSCs) and bear an NSF 49, CSA Z316.3-95 or equivalent seal should be purchased. Those working in a BSC must be trained in its correct use and have a good understanding of the different types of cabinets and how they work.

Class I Cabinets

CLASS I BIOLOGICAL SAFETY CABINET



These cabinets have unrecirculated airflow away from the operator that is discharged to the atmosphere after filtration through a HEPA filter. They provide good operator protection but do not protect the material within the cabinet (the product) from contamination.

(Used in conjunction with the building system. Optional glove ports are shown.)

Class II Cabinets

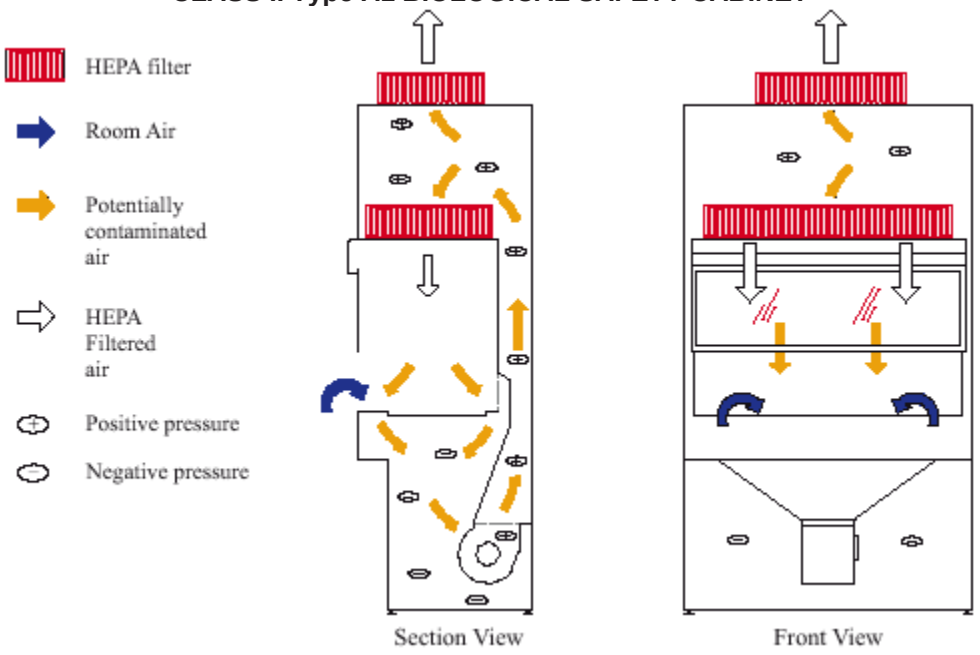
Class II cabinets are designed for personnel, product and environmental protection. They are designed for work involving microorganisms in containment levels 2, 3 and 4 laboratories and are divided into two types (A and B) on the basis of construction type, airflow velocities and patterns, and exhaust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

Class II, Type A1 Cabinets (formerly Type A)

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

- Maintain a minimum average face velocity of 0.38 m/s (75ft/min).
- May have positive pressure contaminated ducts and plenums.
- Are not suitable for work with low levels of volatile toxic chemicals and volatile radionuclides.

CLASS II Type A1 BIOLOGICAL SAFETY CABINET



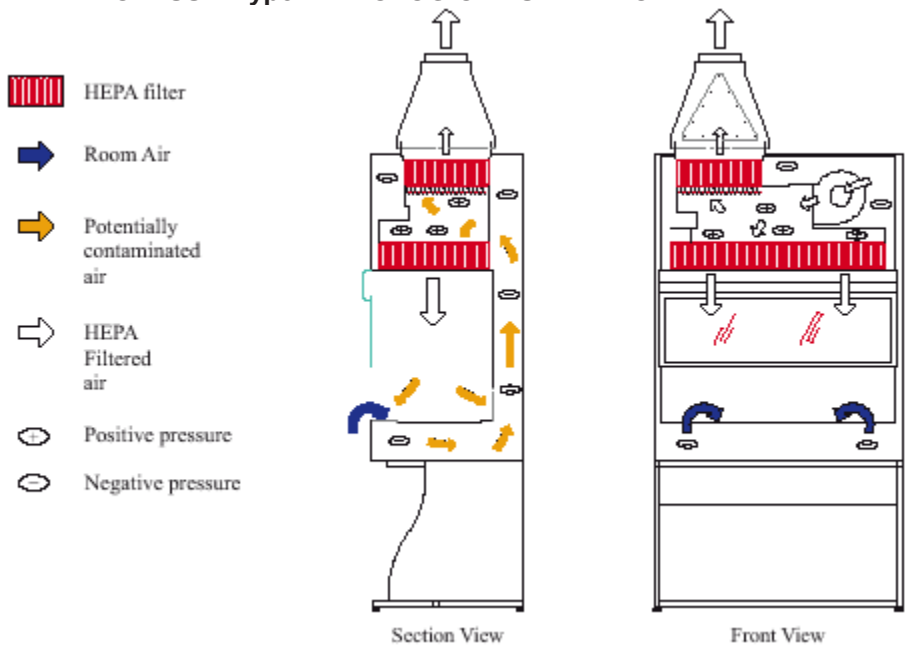
(Can be room re-circulated or air gap type (thimble).)

Class II, Type A2 Cabinets (formerly Type A/B3)

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

- Maintain a minimum average face velocity of 0.5 m/s (100 ft/min).
- Have ducts and plenums under negative pressure.
- Is suitable for work with minute quantities of volatile toxic chemicals and trace amounts of radionuclides.

CLASS II Type A2 BIOLOGICAL SAFETY CABINET

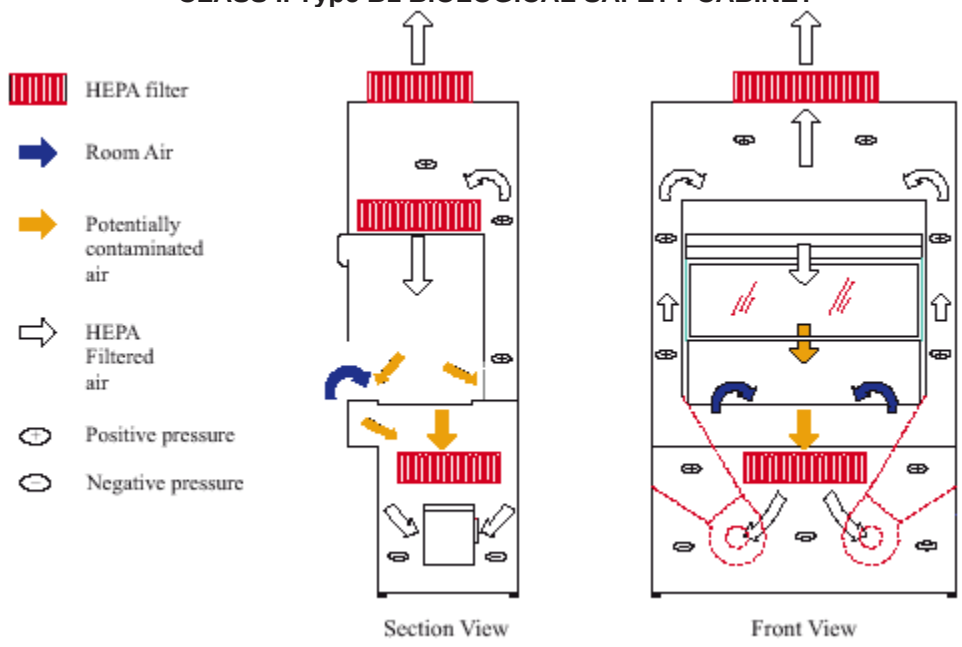


(Can be room re-circulated or air gap type (thimble).)

Class II, Type B1 Cabinets

- Hard-ducted through a dedicated duct exhausted to the atmosphere after passage through a HEPA filter; contain negative pressure plena.
- Maintain a minimum average face velocity of 0.5 m/s (100 ft/min).
- Recirculate 30% of the air within the cabinet.
- Suitable for work with low levels of volatile toxic chemicals and trace amounts of radionuclides.

CLASS II Type B1 BIOLOGICAL SAFETY CABINET

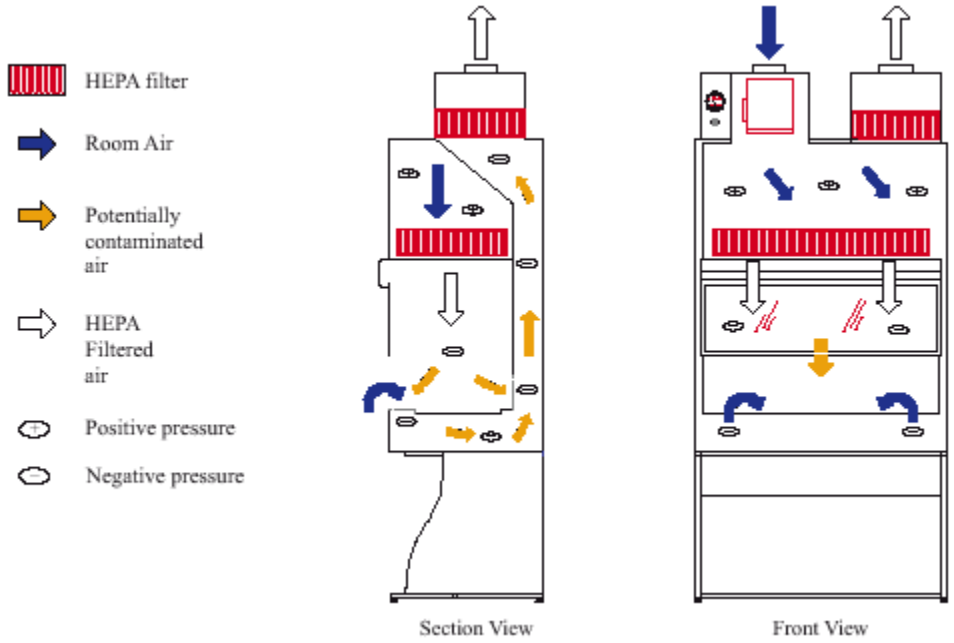


(Hard-ducted.)

Class II, Type B2 Cabinets

- Does not recirculate air within the cabinet.
- Maintain a minimum average face velocity of 0.5 m/s (100 ft/min).
- Hard-ducted through a dedicated duct exhausted to the atmosphere, 100% of cabinet air, after passage through a HEPA filter; contain negative pressure plena.
- Suitable for work with volatile toxic chemicals and radionuclides.

CLASS II Type B2 BIOLOGICAL SAFETY CABINET



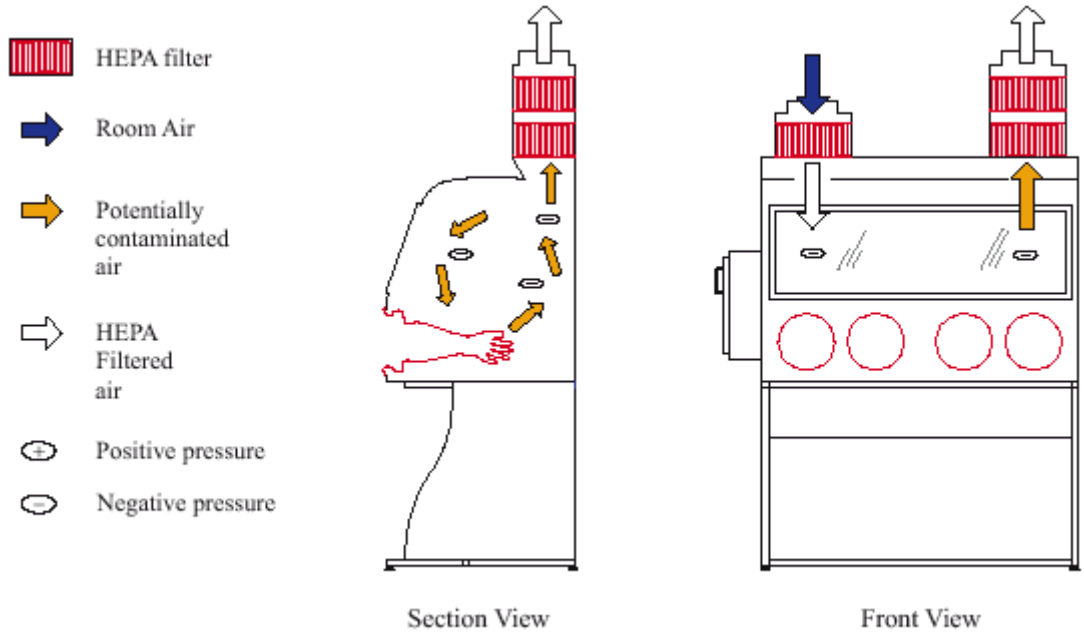
(Hard-ducted.)

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

Class III Cabinets

Class III cabinets are totally enclosed and gas-tight with HEPA filtered supply and exhaust air. Work is performed with attached long-sleeved gloves. The cabinet is kept under negative pressure of at least 120 Pa (0.5 in. w.g.), and airflow is maintained by a dedicated exterior exhaust system. Class III cabinets protect the worker and the product. They are designed for work with risk group 4 pathogens and provide an alternative to the positive-pressure suit made for maximum containment laboratories. Cabinet lines consisting of several Class III cabinets (e.g., for centrifuges, animal cages, incubators, refrigerators) and transfer devices joined together are traditionally custom built. The exhaust air is double HEPA filtered or treated by HEPA filter and incineration. Removal of materials from the cabinet must be through a dunk tank, double door autoclave or air-lock pass-through for decontamination. Interlock or protocols must be used for the autoclave and pass-through doors to prevent both doors from being open at the same time.

CLASS III BIOLOGICAL SAFETY CABINET



(Hard-ducted.)

E.3 BIOLOGICAL SAFETY CABINET SELECTION

The primary consideration when selecting a biological safety cabinet (BSC) is safety; though cost and ergonomics are often also involved in the selection process. Factors related to safety that should be considered include the following:

- The type of protection required
  - Product protection only

    - Personal and environmental protection only
    - Product, personal and environmental protection
- The different types of work that will be done in the cabinet
- Types and quantities of toxic chemicals that will be used in procedures
- The type of exhaust system required

SUMMARY OF BIOLOGICAL SAFETY CABINETS

CLASS	**I**	**II**						**III**
Type		***A1***		***A2***		***B1***	***B2***	
Minimum average velocity (fpm)	75	75		100		100	100	NA
Air recirculated (%)	0	70		70		30-50	0	0
Exhausts to	Outside	Room	Outside	Room	Outside	Outside	Outside	Outside
Exhaust duct connection		None	Canopy	None	Canopy	Hard duct	Hard duct	
Requires dedicated exhaust connection		No	No	No	No	Yes	Yes	
Requires face velocity alarm		No	No	No	No	Yes	Yes	
Suitable for work with odorous materials		No	Yes	No	Yes	Yes	Yes	
Suitable for work with toxic chemicals and radionuclides	No	No	No	No	Minute quantities	Minute quantities	Minute quantities	
Product protection	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Capital cost		Low	Moderate	Low	Moderate	High	High	
Installation cost		Low	Moderate	Low	Moderate	High	High	
Energy Loss		Low	Moderate	Low	Moderate	High	High	

USE OF THE BIOLOGICAL SAFETY CABINET

START-UP:

1. Turn off UV lights if in use and ensure that the sash is in the appropriate position.
2. Turn on fluorescent light and cabinet blower, if off.
3. Check the air intake and exhaust grilles for obstructions.
4. If the cabinet is equipped with an alarm, test the alarm and switch it to the "on" position.
5. Confirm inward airflow by holding a tissue at the middle of the edge of the viewing panel and ensuring that it is drawn in.
6. Disinfect the interior surfaces with a suitable, non-corrosive disinfectant.
7. Assemble all materials required for the procedure and load them into the cabinet; do not obstruct the air grilles; the working surface may be lined with absorbent paper with plastic backing; segregate

"clean" items from "contaminated" items.
8. Wait 5 minutes to purge airborne contaminants from the work area.

WORKING IN THE CABINET:

1. Don protective clothing and gloves as appropriate.
2. Perform operations as far to the rear of the work area as possible.
3. Avoid movement of materials or excessive movement of hands and arms through the front access opening during use; when you do enter or exit the cabinet, do so from straight on; allow the cabinet to stabilize before resuming work.
4. Keep discarded, contaminated material to the rear of the cabinet; do not discard materials in containers outside of the cabinet.
5. Do not work with open flames inside the cabinet.
6. If there is a spill during use, surface decontaminate all objects in the cabinet; disinfect the working area of the cabinet while it is still in operation (do not turn the cabinet off).

CLEAN-UP/SHUTDOWN:

1. Allow the cabinet to run for 5 minutes with no activity.
2. Close or cover open containers before removing them from the cabinet.
3. Surface disinfect objects in contact with contaminated material before removal from the cabinet.
4. Remove contaminated gloves and dispose of them as appropriate; wash hands.
5. Don clean gloves, and ensure that all materials are placed into biohazard bags within the cabinet.
6. Using a suitable non-corrosive disinfectant (e.g., 70% ethanol), disinfect interior surfaces of cabinet; periodically remove the work surface and disinfect the area beneath it (including the catch pan) and wipe the surface of the UV light with disinfectant.
7. Turn off the fluorescent light and cabinet blower when appropriate (some cabinets must be left on at all times; if you are unsure, check with your cabinet certifier, safety officer or building maintenance personnel).
8. Turn on the UV light if appropriate (do not turn on when people are working close by);UVmust be tested to ensure that it is emitting a germicidal wavelength (ask your cabinet certifier to perform this test).

APPENDIX D LABORATORY DESIGN

This appendix is intended to inform building operators, physical plant departments, laboratory management, designers and cost estimators of the construction features required to achieve the levels of containment outlined in Section 7.3.

A matrix system is used to present detailed information on the variety of design elements necessary for required biosafety standards, and to allow rapid comparison of the features required to design or upgrade existing facilities. ***Within the matrices, M stands for Mandatory and R for Recommended***.

Laboratory Location and Access

The location of laboratories relative to other facilities in a building is one factor in containment. Proper location can help to control entry by uninformed people. Lower and higher containment areas can be integrated to concentrate the necessary support services close to sterilizing, waste disposal, and exhaust air treatment facilities.

Containment Levels			
1	**2**	**3**	
M	**M**	**M**	Separated from public areas by door.
	M	**M**	Access limited to authorized personnel.
	M	**M**	Laboratory room doors to have appropriate signage (e.g., biohazard sign, containment level, contact information, entry requirements).
M	**M**	**M**	Size of door openings to allow passage of anticipated equipment.
	M	**M**	Doors to the containment laboratory lockable (not applicable to areas within the containment laboratory).
		M	Doors to provide restricted access by installation of a controlled access system (e.g., key card) or equivalent.
		R	Electronic locking systems to be backed up with a physical key-lock system.
	R	**M**	Office areas to be located outside of containment laboratory. Paperwork stations for data collection can be within containment laboratory provided they are located away from the laboratory work areas.
		M	Entry to laboratory to be provided via an anteroom.
		M	Anteroom door(s) located between the clean and dirty change rooms not to be opened simultaneously with either the containment laboratory door or the clean change entry door. (Interlock, visual or audible alarms, or protocols are all acceptable means.)
		M	Interlocked doors, if present, to have manual overrides for emergency exit.
		M	Entry to laboratory zone to be provided with clothing change areas separating personal and laboratory clothing dedicated to that zone (i.e., “clean” change area separated from “dirty” change area).
		M	Exit from laboratory to be provided with a walk-through shower on the containment barrier (i.e., between “dirty” and “clean” change anterooms). (Not required for CL3 laboratories handling organisms like HIV, that are not infectious via inhalation)
		R	Containment laboratories to be located in close proximity to supporting mechanical services to limit the amount of potentially contaminated services.
		R	Containment laboratories to be located away from external building envelope walls.
		R	A laboratory support area to be provided adjacent to the containment facility for all supporting laboratory manipulations.

Physical Construction of Containment Barriers

In addition to providing physical separation, the construction of the laboratory perimeter can also contribute to containment by providing surfaces that are easy to clean and disinfect. Chemical resistance, impermeability, durability and compatibility with other construction materials must be considered in choosing finishes and sealants of the containment perimeter. Careful consideration of long-term uses at the time of design and construction can allow for cost-effective upgrading at minimal cost.

For Containment Level 3 laboratories, the requirement for negative air pressure must be considered in the construction of walls and ceilings. Designers, builders and users of these laboratories should be aware of the high costs involved in the construction and long-term maintenance of these facilities.

Laboratory Containment Perimeter

Containment Levels			
1	**2**	**3**	
WALLS			
		R	Reinforced structural masonry.
		R	Reinforced non-load-bearing masonry.
		R	Steel frame reinforced non-load-bearing masonry.
		R	Reinforced concrete.
CEILINGS			
M	**M**		Steel frame and gypsum partition or impervious ceiling acoustic tile.
		M	Reinforced steel frame and gypsum ceiling, filler, primer and paint Finish.
COATINGS & SEALANTS			
	R	**M**	Seamless, gas and chemical-resistant wall and ceiling coatings.
	R	**M**	Chemical, gas and disinfectant-resistant, non-hardening sealants.
	R	**M**	Containment seals for mechanical / electrical service openings.
DOORS			
	R	**M**	Doors lockable.
	R	**M**	Doors self-closing.
		R	Doors to provide restricted access via cardkey system or equivalent.
	R	**M**	Doors and frames of solid construction.
M	**M**	**M**	Door openings should be sized to allow passage of all anticipated equipment.
M	**M**	**M**	Doors to have fire ratings as required and be located as per fire safety standards.
		R	Entrance doors to be interlocked with manual overrides.
M	**M**	**M**	All exits marked and illuminated.
M	**M**	**M**	Egress to fire exits set out so that travel through any high hazard areas is minimized or to conform to applicable codes.
WINDOWS			
M	**M**		Windows, if openable, protected by fly screens.
		M	Windows of safety glass, of proven performance, solid stops sealed in place.
FLOORS			
M	**M**	**M**	Slip-resistant flooring.
R	**R**	**M**	Seamless, gas and chemical-resistant coating (e.g. epoxy) with integral coved base.
M	**M**		Seamless, rolled or resilient tile flooring (e.g. vinyl).

Surface (i.e., Floors, Walls, Ceilings, Sealants) Finishes and Casework

Containment Levels			
1	**2**	**3**	

	R	**M**	Doors, frames, casework and bench tops to be non-absorptive (i.e., the use of organic materials should be avoided).
	M	**M**	Working surfaces of bench tops to be non-absorptive.
R	**M**	**M**	Surfaces to be scratch, stain, moisture, chemical and heat resistant in accordance with laboratory function
R	**R**	**M**	Surface to provide impact resistance in accordance with laboratory function.
	R	**M**	Surfaces to be continuous and compatible with adjacent and overlapping materials (i.e., to main adhesion and a continuous perimeter); wall and floor welded seams are acceptable in level 3 laboratories.
		M	Continuity of seal to be maintained between the floor and wall (a continuous cove floor finish up the wall is recommended).
		M	Interior surface to minimize movement of gases and liquid through perimeter membrane.
R	**M**	**M**	Interior coatings to be gas and chemical resistant in accordance with laboratory function (e.g., will withstand chemical disinfection, fumigation).
		M	Interior coatings to be cleanable.
R	**R**	**M**	Bench tops to have no open seams.
R	**R**	**R**	Bench tops to contain spills of materials (e.g. with marine edges and drip stops).
R	**R**	**R**	Benches, doors, drawers, door handles, etc. to have rounded rims and corners.
R	**R**	**R**	Backsplashes, if installed tight to wall, to be sealed at wall-bench junction.
R	**R**	**R**	Reagent shelving to be equipped with lip edges.
R	**R**	**R**	Drawers to be equipped with catches, i.e., to prevent the drawer from being pulled out of the cabinet.
			Drawers to be of one piece construction.
R	**R**	**R**	Cabinet doors not to be self-closing.

Air Handling Systems

Due to their insidious, pervasive nature, aerosols are a major factor in the dissemination of hazardous agents. They pose a risk to both the laboratory worker and the environment. Contamination by aerosols can be minimized by the use of proper laboratory techniques, biological safety cabinets, primary containment devices, setting and balancing the room air supply and exhaust systems and the use of HEPA filters.

It is of particular importance that containment equipment (e.g. biological safety cabinets) be tested to meet specified standards after installation and that the connections between such equipment and room air handling systems should meet the standards required.

The following matrix indicates the HEPA filtration requirements for each containment level for laboratory supply and exhaust air and for biological safety cabinets.

Supply and Exhaust Ventilation

Containment Levels			
1	**2**	**3**	
	R	**M**	100% outside air to be supplied
		M	Directional inward airflow provided such that air will always flow towards areas of higher containment.
		M	Visual pressure differential monitoring devices to be provided at entry to containment laboratory.
		M	Alarm (visual or audible) to be provided in the laboratory and outside laboratory area (i.e., to warn others and maintenance personnel) to signal air handling systems failure.
		M	Where determined necessary by a local risk assessment, supply air duct to be

			provided with backdraft protection (i.e., HEPA filter; bubble tight backdraft damper).
		M	Supply air system to be independent of other laboratory areas. CL3 supply can be combined with areas of lower containment when provided with backdraft protection (i.e., HEPA filter, bubble tight backdraft damper) downstream from the connection. (For CL3 laboratories manipulating organisms, such as HIV, that are not infectious via inhalation, this criterion is only recommended)
		M	Supply air system to be interlocked (i.e., fans, dampers, electrical) with exhaust air system, to prevent sustained laboratory positive pressurization.
		M	Exhaust air to be HEPA filtered. (For CL3 laboratories manipulating organisms, such as HIV, that are not infectious via inhalation are not required t fulfil this criterion).
		M	HEPA filters installed into the supply and exhaust system to conform to the requirements of IEST-RP-CC001.3.
		M	Where HEPA filters are used for backdraft protection in accordance with local risk assessment, supply HEPA filter housings to be designed to withstand structural change at applied pressure of 2500 Pa.
		M	Exhaust HEPA filter housings to be designed to withstand structural change at applied pressure of 2500 Pa and to be provided with a method of isolation and decontamination. (For CL3 laboratories manipulating organisms, such as HIV, that are not infectious via inhalation, this criterion is only recommended).
		M	Exhaust air system to be independent of other laboratory areas. CL3 exhaust can be combined with areas of lower containment when provided with a HEPA filter upstream from the connection. (For CL3 laboratories manipulating organisms, such as HIV, that are not infectious via inhalation, this criterion is only recommended).
		R	Supply and exhaust systems located outside of containment to be accessible for repairs, maintenance, cleaning and inspection.
		M	When backdraft protection is required in accordance with local risk assessment, supply air ductwork that is outside the containment perimeter (e.g., between containment perimeter and HEPA filter or bubble tight backdraft damper) to be sealed air tight in accordance with SMACNA Seal Class A.
		M	Exhaust air duct work that is outside the containment perimeter (e.g., between containment perimeter and HEPA filter or bubble tight backdraft damper) to be sealed air tight in accordance with SMACNA Seal Class A. (CL3 laboratories manipulating organisms, such as HIV, that are not infectious via inhalation are not required to fulfill this criterion)
		M	Airflow control devices and duct sensors to be located downstream of the exhaust HEPA filter and upstream of the supply bubble tight backdraft damper or HEPA filter, or if located upstream, duct penetrations to be sealed in accordance with SMACNA Seal Class A. (CL3 laboratories manipulating organisms, such as HIV, that are not infectious via inhalation are not required to fulfill this criterion)
		M	Bubble tight backdraft dampers and HEPA filters to be located in close proximity to the containment perimeter. (CL3 laboratories manipulating organisms, such as HIV, that are not infectious via inhalation are not required to fulfill this criterion)
		R	Lab equipped with magnehelic gauges or pressure devices at entry for room exhaust.
	R	**M**	Exhaust from laboratory at a minimum of 10 room volumes per hour.
R	**R**	**R**	Air vertically discharges to the outside, clear of buildings or supply air intakes, at 12 metres/second.
R	**R**	**M**	Minimization of dead spaces where contaminated air can linger.
M	**M**	**M**	Ventilation sufficient to remove vapours of flammable liquids and dangerous chemicals before they reach hazardous concentrations.

Biological Safety Cabinets

Containment Levels			
1	**2**	**3**	
	R	**M**	Class I
	R	**M**	Class II
	M	**M**	Cabinet air can be recirculated in laboratory if HEPA filtered.

Fume Hoods

Containment Levels			
1	**2**	**3**	
R	**R**	**R**	Recommended when necessary
		R	HEPA and charcoal filters (if required).
R	**R**	**R**	Air flow alarms.

Containment Perimeter

Containment Levels			
1	**2**	**3**	
R	**M**		Autoclave or other acceptable means of waste treatment/disposal to be provided.
		M	Double-door barrier autoclave with bioseal to be located on containment barrier; body of autoclave to be preferably located outside of containment for ease of maintenance. (For CL3 laboratories manipulating organisms, such as HIV, that are not infectious via inhalation it is not mandatory that the autoclave be a double-door barrier model).
		M	Barrier autoclave to be equipped with interlocking doors, or visual or audible alarms to prevent both doors from opening at the same time.
		M	For materials that cannot be autoclaved (e.g., heat sensitive equipment, samples, film) other proven technologies for waste treatment (e.g., incineration, chemical or gas) to be provided at containment barrier.
		M	All penetrations to be sealed with non-shrinking sealant at containment barrier.
		M	All conduit and wiring to be sealed with non-shrinking sealant at containment barrier.
M	**M**		Windows, if they can be opened, to be protected by fly screens.
		M	Windows positioned on containment barrier to be sealed in place; window glazing material to provide required level of security.
		R	Observation windows to be installed on containment barrier.

Laboratory Services (i.e., Water, Drains, Gas, Electricity, and Safety Equipment

Containment Levels				
1	**2**	**3**	**4**	
M	**M**			Hooks to be provided for laboratory coats at laboratory exit; street and laboratory clothing areas to be separated.
M	**M**	**M**	**M**	Handwashing sinks to be located near the point of exit from the laboratory or in the anteroom.
	R	**M**	**M**	Handwashing sinks to be provided with “hands-free” capability.
		M	**M**	BSCs and other primary containment devices to be provided.
	R			BSCs and other primary containment devices to be provided. Examples for use

				include procedures with the potential for producing aerosols and those involving high concentrations, large volumes or particular types of agents.
	M	**M**		Emergency eye wash facilities to be provided in accordance with applicable regulations.
	M			Emergency shower facilities to be provided in accordance with applicable regulations.
		M		When it is not possible to limit the quantities of hazardous materials within the laboratory, emergency shower equipment to be provided in accordance with applicable regulations.
		M	**M**	Domestic water branch piping serving laboratory area(s) to be provided with backflow prevention, in accordance with CAN/CSA-B64.10-01/B64.10.1-01, and isolation valve to be located in close proximity to the containment barrier.
		M		Drain lines and associated piping (including autoclave condensate) to be separated from lower containment laboratory areas and to go directly to main building sanitary sewer at point of exit from building (downstream of all other connections).
		M	**M**	Autoclave condensate drain to have a closed connection. For CL3, open connection is allowable if located within containment barrier.
		M	**M**	Drainage traps to be provided to required deep seal depth in consideration of air pressure differentials.
		R	**M**	Floor drains not to be provided, except when essential (e.g., body shower and animal rooms).
		M		Plumbing vent lines to be independent of lower containment plumbing vent lines, or combined with lines from lower containment when provided with a filter of efficiency equivalent to that of HEPA upstream from the connection. (CL3 laboratories manipulating organisms, such as HIV, that are not infectious via inhalation are not required to fulfil this criterion)
		R	**M**	Compressed gas cylinder(s) to be located outside the laboratory.
		M	**M**	Portable vacuum pump to be provided in the laboratory. Internal contamination of vacuum pump to be minimized (e.g., HEPA filtration of vacuum line, use of disinfectant tracks).
		M	**M**	Emergency lighting to be provided.
		M	**M**	Life safety systems, lighting, HVAC systems, BSCs, security systems and other essential equipment to be supported with emergency back-up power.
		M	**M**	Circuit breakers to be located outside by containment area.
		R	**R**	Fluorescent light ballasts and starters to be located outside containment area.
		M	**M**	Laboratory to be equipped with a communication system between containment area and outside support area.
		M	**M**	System to be provided for electronic transfer of information and data from laboratory area to outside laboratory perimeter. (Note: paperwork from the containment laboratory may be removed after appropriate decontamination, i.e., autoclaving, irradiation, microwaving; such practices are generally not recommended for use on a routine basis).

APPENDIX E LARGE SCALE PRODUCTION

This section provides information for those who design, build, operate or work in research and industrial facilities where large scale manipulation of micro-organisms is performed with fermenters and equipment that cannot be easily moved and sterilized in an autoclave and therefore requiring *in situ* sterilization. This would normally apply to volumes greater than 10 litres.

It is imperative that all industrial fermentation and large scale manipulations of micro-organisms be conducted under conditions of containment that will ensure minimal risk to the workers and the environment. Large scale work is not necessarily more hazardous than laboratory scale work. In fact, it is often less of a concern since most procedures in large scale fermentation are automated and often conducted in closed systems, thereby reducing the probability of exposure of the operator. This has been demonstrated with infectious agents used for vaccine production. However, all organizations engaging in large scale fermentation must ensure that the appropriate equipment for such procedures, and contingency plans, are in place to minimize risks should there be a malfunction in the process.

A production facility engaged in activities with micro-organisms in Risk Groups 2 and 3 must designate an individual to serve as the facility's co-ordinator. This individual should be experienced and knowledgeable in the handling of micro-organisms and eukaryotic cells, and in large scale containment.

Containment Level Requirements

Containment Level (LS1) Large Scale 1

Containment Level LS1 is suitable for large scale work with Risk Group 1 agents. In addition to the general laboratory safety practices and the requirements of Containment Level 1, the following is required.

Physical and Operational Requirements

- Visual inspections of the integrity of the containment systems are important to detect small leaks.
- Spills and accidents that result in exposures to organisms to be immediately reported to the facility director and facility Biological Safety Officer; medical attention and surveillance to be provided as appropriate; written records to be maintained.
- Emergency plans and procedures to be readily available and to include appropriate equipment and training for emergency response to spills or accidental release of organisms (i.e., personal protective equipment, disinfectants); training to be documented.
- Cultures of viable organisms to be contained within a closed system or other primary containment equipment (e.g., BSC) that is designed to reduce the potential for release of aerosols.
- Culture fluids, except as allowed below, are not to be removed from a closed system or other primary containment equipment without prior inactivation of the organisms by a validated procedure. A validated inactivation procedure is one that has been demonstrated to be effective against the organism in use. Culture fluids that contain viable organisms intended as the final product may be removed from the primary containment equipment by way of closed systems for sample analysis, further processing or final fill.
- Sample collection, the addition of materials and the transfer of culture fluids from one closed system to another to be performed in a manner that prevents the release of aerosols or contamination of exposed surfaces.

- Process equipment, closed systems or other primary containment equipment to be provided with treatments (i.e., HEPA or equivalent filters, incineration, or gaseous decontamination through chemical disinfectants) to prevent the release of the viable organisms.

- A closed system or other primary containment equipment that has contained viable organisms is not be opened for maintenance or other purposes without prior inactivation of the organisms by a validated procedure; a validated inactivation procedure is one that has been demonstrated to be effective against the organism in use.

- Facilities to be designed to prevent the release of viable organisms to sanitary sewer (e.g., capping or raising of floor drains).

Containment Level (LS2) Large Scale 2

Containment Level LS2 is suitable for large scale work with Risk Group 2 agents. In addition to the requirements of Containment Level LS1 and Containment Level 2, the following is required.

Physical and Operational Requirements

- Process equipment must contain the organisms within a closed system and must be provided with HEPA filters, or equivalent, which have been integrity tested to prevent release of aerosols. Equivalent, alternative procedures (e.g., incineration, off gassing through chemical disinfectants) may be used to prevent escape of micro-organisms.

- Process equipment must be capable of being decontaminated with a validated inactivation procedure.

- Process equipment to contain sensing devices (or equivalent), where possible, to monitor the integrity of containment during operations and alarm conditions leading to containment failure.

- Unit operations and transfers between operations must be designed to prevent the release of aerosols.

- Seals and mechanical devices associated with the process equipment shall be designed to prevent leakage or shall be enclosed in housings from which air is extracted through a HEPA filter, or equivalent.

- Hazard warning signs (e.g., biohazard sign, containment level, contact information, entry requirements) must be posted at the entry to the process area. Consideration should be given to the addition of this signage to relevant process and primary containment equipment used to contain viable organisms.

- Decontamination of process equipment and process effluent must be performed with a validated inactivation procedure.

- Decontamination must precede breach of containment.

- Sampling is to be performed in a controlled manner which prevents the release of infectious materials.

- Process equipment should be tested regularly (i.e., after each use or operation) for integrity of containment capability, and records of such testing maintained.

- Unauthorized personnel are not permitted to enter process areas.

- Staff must be acquainted with emergency procedures to deal with spills or accidental release of viable organisms. Procedures should be posted and training documented.

- Personal protective equipment requirements to be posted at entry.

- Equipment for decontamination and emergency response must be readily available in the process area and be maintained for immediate and effective use.

Containment Level (LS3) Large Scale 3

Large scale production and processing of Risk Group 3 agents may result in serious hazard to humans, animals and the environment. Containment Level LS3 is characterized by design features which involve primary and secondary levels of physical containment.

Containment Level LS3 is suitable for large scale work with Risk Group 3 agents. In addition to the requirements of Containment Level LS2 and Containment Level 3, the following is required.

Physical and Operational Requirements

- Air leaving the containment area must be HEPA filtered.

- Provision must be made to contain the full volume of a complete release of all process fluids in the process area.

- All potentially contaminated liquids must be transferred in closed piping.

- Effluent must be transferred to a decontamination system which is regularly validated to ensure efficacy of the process.

- Showers and change rooms must be provided.

- Entry to the process area should be restricted to essential personnel while production is in progress.

- Personal protective equipment to include full change out of street clothes into dedicated process area garments (dedicated pants, shirts, shoes, socks, head covers, gloves) or complete coverage of street clothing with process area garments (dedicated jumpsuits, shoe covers, head covers, gloves); reuseable garments to be removed upon exit, decontaminated and laundered after each use; single use garments to be removed upon exit, decontaminated and discarded after each use; respiratory protection may be appropriate depending on the organism being processed.

- Hazard warning signs and identification to be used in all records relevant to the history of the equipment (e.g., testing, operation, maintenance).

- A personal shower should be used on egress.

- It is recommended that provision should be made for electronic transfer of all data from the containment area (e.g., computer networking).

Transportation of Large Scale Products

External Transport

Requirements for the shipment of biological agents are outlined in Section 2. All micro-organisms in Risk Groups 2, 3 and 4 must be packaged, labelled and transported in accordance with the Transportation of Dangerous Goods Act and Regulations.

Further information may be obtained from:

Transport of Dangerous Goods Directorate
Transport Canada
344 Slater Street
14th Floor
Ottawa, Ontario
K1A 0N3
(613) 958-0517

On-Site Transportation between Containment Areas

Transportation of Risk Group 2, or higher, micro-organisms within a facility must be accomplished within a closed system. Process piping or a secure, unbreakable, sealed container must be used. Containers or transfer lines must be capable of being decontaminated.

The facility's emergency response plan must include provision for response to a spill, leak or incident during transportation between containment areas. All emergency response materials and equipment must be ready for use whenever needed.

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