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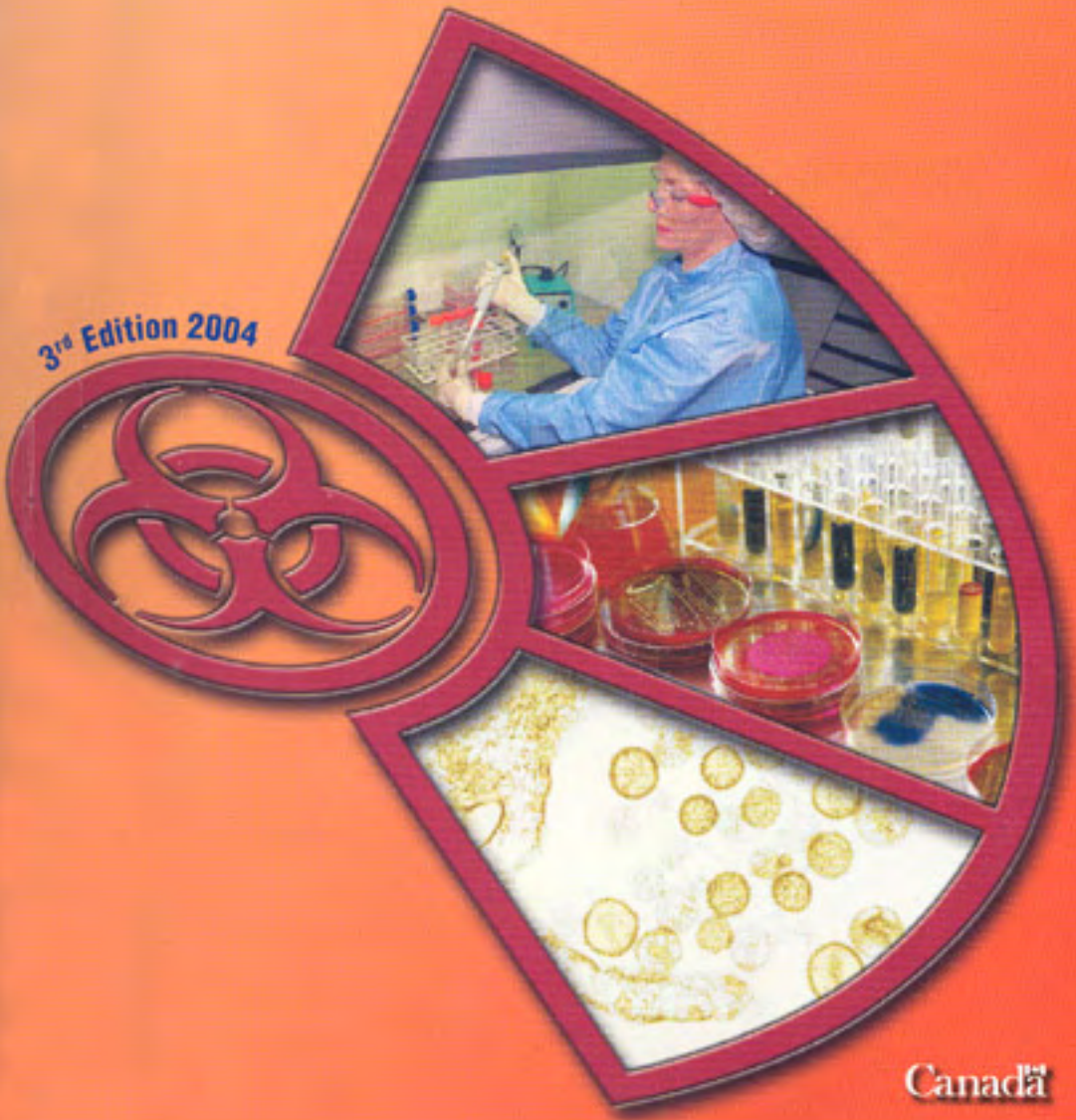
Laboratory Biosafety Guidelines



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Canada

The Laboratory Biosafety Guidelines

3rd Edition

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Our mission is to help the people of Canada
maintain and improve their health.
Health Canada

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Abbreviations

BSC	◗ biological safety cabinet
CFIA	◗ Canadian Food Inspection Agency
CL1	◗ containment level 1
CL2	◗ containment level 2
CL3	◗ containment level 3
CSA	◗ Canadian Standards Association
HC	◗ Health Canada
HEPA	◗ high efficiency particulate air
HPIR	◗ Human Pathogen Importation Regulations
HVAC	◗ heating, ventilation and air conditioning
ICAO	◗ International Civil Aviation Authority

NSF	◗ National Sanitation Foundation
Pa	◗ pascal (unit of pressure)
SMACNA	◗ Sheet Metal and Air Conditioning Contractors National Association
TDG	◗ Transportation of Dangerous Goods
w.g.	◗ water gauge (unit of pressure)
WHMIS	◗ Workplace Hazardous Materials Information System
WHO	◗ World Health Organization

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Chapter 1
Introduction

Despite a greater awareness of biosafety and biocontainment practices, handling infectious microorganisms remains a source of infection, and even mortality, among laboratory workers[1-4]. Incidents of secondary transmission of disease to the public at large, which may be due to possible contamination of the environment or personnel, are also occurring[1,5]. There is a steady increase in both the number of laboratories handling pathogens and in the number of scientists wishing to import into Canada new or exotic strains for further study. Laboratory workers can minimize the risks associated with work involving these infectious agents through the application of appropriate biosafety and containment principles and practices. Increasing demands are also being placed on regulatory authorities to ensure that such pathogens are handled in a safe and secure manner.

The *Laboratory Biosafety Guidelines* were initially developed to guide government, industry, university, hospital, and other public health and microbiological laboratories in their development of biosafety policies and programs. The *Guidelines* also serve as a technical document providing information and recommendations on the design, construction and commissioning of containment facilities. In recognition of the *Guidelines*' impact on key stakeholders, a consultation draft of the 3rd edition was distributed widely by mail-out and Web site posting to offer stakeholders an opportunity to state their opinions and comment on the implications of the draft recommendations. All comments and feedback that stakeholders provided were reviewed and incorporated where possible.

This 3rd edition has been updated to reflect current biosafety and biocontainment principles and practices. The document has been written with a performance-based approach, which not only accommodates contemporary technologies and ever-changing approaches to achieving containment but provides simple and sensible solutions as well. The development of this document parallelled the production of the 2nd edition of the Canadian Food Inspection Agency's *Containment Standards for Veterinary Facilities*[6]

with the goal of including similar containment requirements where possible in the two documents. Additions include a section on non-human primates. Separate guidelines will be available specifically for work with mycobacteria. These will reflect an ongoing area of concern of biosafety professionals and outline a stratified approach to containment according to the type of procedures used. These will be available by accessing the office of Laboratory Security's Web site at the address below.

A significant change in the 3rd edition is the removal of the Risk Group lists of human pathogens from this document, and is to be available from Health Canada's Office of Laboratory Security and its Web site. Publishing a static list in hard copy does not allow for a dynamic and ongoing assessment of risk or for the addition of new and emerging pathogens. As new risk factors are first identified and explored and more information becomes available, the selection of appropriate containment levels for work with potentially infectious materials is subject to change. The Risk Group list of human pathogens will be available from the Office of Laboratory Security's Web site:
http://www.phac-aspc.gc.ca/ols-bsl/

Finally, emphasis must be placed on the practices and procedures used by trained laboratory staff. The World Health Organization's *Laboratory Biosafety Manual* states that "no biosafety cabinet or other facility or procedure alone guarantees safety unless the users operate safe techniques based on informed understanding."[7] It is the responsibility of everyone, including managers and laboratory workers, to use the information available in these *Guidelines* and to perform their work in a safe and secure manner.

References

1. Collins, C.H., and Kennedy, D.A. *Laboratory-acquired infections.* In: *Laboratory-acquired infections: history, incidence, causes and preventions.* Oxford, UK: Butterworth-Heinemann, 1999;1-37.

2. Harding, A.L., and Brandt Byers, K. *Epidemiology of laboratory-associated infections.* In: Fleming, D.O., and Hunt, D.L. *Biological safety: principles and practices.* Washington, DC: ASM Press, 2000;35-54.

3. Sewell, D.L. *Laboratory-associated infections and biosafety.* Clin Microbiol Rev 1995;8:389-405.

4. Gaidamonvich, S.Y., Butenko, A.M., and Leschinskaya, H.V. *Human laboratory acquired arbo-, arena-, and hantavirus infections.* J Am Biol Safety Assoc 2000;5:5-11.

5. Richmond, J.Y. and McKinney, R.W. *Biosafety in microbiological and biomedical laboratories.* Washington, DC: U.S. Government Printing Office, 1999;1-250.

6. *Containment standards for veterinary facilities*. Ottawa: Agriculture and Agri-Food Canada, Minister of Supply and Services Canada, No. 1921/E, 1996.

7. *Laboratory biosafety manual.* Geneva: World Health Organization, 1993;1-133.

Chapter 2
Biological Safety

2.1 Risk Groups

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

- pathogenicity
- infectious dose
- mode of transmission
- host range
- availability of effective preventive measures
- 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[1].

Risk Group 1 (low individual and community risk)

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

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

Any pathogen that can cause human disease but, under normal circumstances, is unlikely to be a serious hazard to laboratory workers, the community, livestock or the environment. Laboratory exposures rarely cause infection leading to serious disease; effective treatment and preventive measures are available, and the risk of spread is limited.

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

Any pathogen that usually causes serious human disease or can result in serious economic consequences but does not ordinarily spread by casual contact from one individual to another, or that causes diseases treatable by antimicrobial or antiparasitic agents.

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

Any pathogen that usually produces very serious human 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.

A list of human pathogens categorized according to Risk Group can be obtained by calling the Office of Laboratory Security directly at (613) 957-1779 or accessing their Web site: http://www.phac-aspc.gc.ca/ols-bsl/

2.2 Containment Levels

Classification of organisms according to risk group is not meant to establish the actual handling of biological hazards in the laboratory setting. For example, the risk group system does not take into account the procedures that are to be employed during the manipulation of a particular organism. Containment levels are selected to provide the end-user with a description of the minimum containment required for handling the organism safely in a laboratory setting. In addition to the inherent characteristics of each organism as described in section 2.1, the containment system includes the engineering, operational, technical and physical requirements for manipulating a particular pathogen[2]. These containment levels are applicable to facilities such as diagnostic, research, clinical, teaching and production facilities that are working at a laboratory scale (for large scale see Chapter 6). Four containment levels are described as follows:

Containment Level 1 (CL1)

This applies to the basic laboratory that handles agents requiring containment level 1. CL1 requires no special design features beyond those suitable for a well-designed and functional laboratory. Biological safety cabinets (BSCs) are not required. Work may be done on an open bench top, and containment is achieved through the use of practices normally employed in a basic microbiology laboratory.

Containment Level 2 (CL2)

This applies to the laboratory that handles agents requiring containment level 2. The primary exposure hazards associated with organisms requiring CL2 are through the ingestion, inoculation and mucous membrane route. Agents requiring CL2 facilities are not generally transmitted by airborne routes, but care must be taken to avoid the generation of aerosols (aerosols can settle on bench tops and become an ingestion hazard through contamination of the hands[3]) 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 3 (CL3)

This applies to the laboratory that handles agents requiring containment level 3. These agents may be transmitted by the airborne route, often have a low infectious dose to produce effects and can cause serious or life-threatening disease. CL3 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 CL3 organisms are appropriate respiratory protection, HEPA filtration of exhausted laboratory air and strictly controlled laboratory access.

Containment Level 4 (CL4)

This is the maximum containment available and is suitable for facilities manipulating agents requiring containment level 4. These agents have the potential for aerosol transmission, often have a low infectious dose and produce very serious and often fatal disease; there is generally no treatment or vaccine available. This level of containment represents an isolated unit, functionally and, when necessary, structurally independent of other areas. CL4 emphasizes maximum containment of the infectious agent by complete sealing of the facility perimeter with confirmation by pressure decay testing; isolation of the researcher from the pathogen by his or her containment in a positive pressure suit or containment of the pathogen in a Class III BSC line; and decontamination of air and other effluents produced in the facility.

2.3 Risk Assessment

Risk assessment is a critical step in the selection of an appropriate containment level for the microbiological work to be carried out. A detailed local risk assessment should be conducted to determine whether work requires containment level 1, 2, 3 or 4 facilities and operational practices. Individuals with varying expertise and responsibilities should be included in the risk assessment process and can include, among others, the facility director, laboratory supervisor, principal investigator, senior microbiologist, biosafety officer and biosafety committee.

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 (see section 2.1 Risk Groups). 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

- concentration
- 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).

The containment level required for work with a particular agent is based on the manipulations generally associated with laboratory scale research and clinical procedures. If a particular procedure, such as preliminary identification, poses a lower hazard than manipulation of a live culture, then a lower containment level may be appropriate. For example, primary diagnostic tests for HIV may be done in a containment level 2 physical laboratory with the use of containment level 3 operational protocols, but growing and manipulating a culture of HIV may require both containment level 3 physical facility and operational protocols.

On the other hand, an increase in containment may be required if the local risk assessment indicates that the procedures pose a higher risk than routine laboratory scale and diagnostic manipulations. For example, *Corynebacterium diphtheriae* (aerosol transmitted) may be manipulated for diagnostic work and laboratory scale research in a containment level 2 laboratory; however, animal aerosol inhalation challenges may require increased levels of physical and operational containment.

An increase in containment may be required once a facility begins large scale production. "Large scale" generally refers to volumes manipulated in a single volume in excess of 10 L. Because of the significant quantity of infectious material being handled, special precautions relating specifically to large scale quantities have been developed and are detailed in Chapter 6. It must be noted that the 10 L cut-off is not an absolute value. A hazard analysis may indicate that, because of high pathogenicity, the route of transmission and the low infectious

dose, a particular study involving volumes of < 10 L but larger than research scale volumes may pose a greater hazard than research scale quantities and therefore may require increased levels of physical and operational containment. For example, a hazard analysis may indicate that a procedure involving production of 5 L quantities of MDRTB (multi-drug resistant *Mycobacterium tuberculosis*) is more appropriately carried out at containment level 3 large scale than at diagnostic and laboratory scale containment level 3. Therefore the 10 L cut-off between laboratory scale and large scale is to be used as a guide only, and a thorough risk assessment should be carried out on a case-by-case basis.

Further guidance on carrying out a risk assessment and related information that can be used to assist in the risk assessment procedure can be found in the Centers for Disease Control and Prevention/National Institutes of Health *Biosafety in Microbiological and Biomedical Laboratories*[4]. This information is also available by accessing the following Web site:
http://www.cdc.gov/od/ohs/

2.4 Health and Medical Surveillance

A health and medical surveillance program (including pre-employment and then periodic testing) needs to be appropriate to the agents in use and the programs in place in the laboratory. As such, the details of the health and medical surveillance program would be determined and defined by a risk assessment process based upon Canadian and International practices[1,4,5] that clearly demonstrates the reasons, indications and advantages for such a program to be in place. This program may include but is not limited to the following: a medical examination; serum screening, testing and/or storage; immunizations; and possibly other tests as determined by the risk assessment process. Risk assessment should be carried out by a multidisciplinary group including management, safety and occupational health professionals. The health and medical surveillance program risk assessment would include consideration of those people working with high

risk organisms, because knowledge of immune status is critical for decisions concerning immunizations, prophylaxis, etc.[1].

Only people meeting these identified medical entry requirements (e.g., immunizations) may enter the laboratory unless the facility has been appropriately decontaminated. Or, other specified protocols can be developed and implemented to achieve the same level of protection for other individuals entering a facility.

2.5 Management of Biological Safety

Although the responsibility for the safety of staff lies with the supervisors and directors of the microbiology laboratory, it can be advantageous to identify an individual(s) to specifically manage biological safety issues. In many laboratories, this role is either informally assigned to a qualified individual who performs these duties on a part-time basis (e.g., senior microbiologist) or the role is shared by a number of individuals. This role can also be formally assigned to a dedicated Biological Safety Officer who has a working knowledge of the laboratory practices and procedures within the facility.

The formation of an Institutional Biosafety Committee to oversee the facility's biological safety program can also be incorporated into the structure for the management of biological safety issues (in some institutions and universities, the requirement for an Institutional Biosafety Committee is mandatory). The Biological Safety Officer (or individual assigned to manage biological safety issues) should liaise with the Committee through regularly scheduled meetings and can present specific safety problems, concerns or policy/protocol improvements to be considered and addressed. The Committee is also available to the Biological Safety Officer for risk assessments, disputes about biological safety matters or other matters that may be of a biological safety nature. Careful consideration is to be given to the composition of the Institutional Biosafety Committee, which, when possible, should include several individuals with varying expertise, the Biological Safety Officer, at least one member of each of the

research staff (researcher) and technical staff (technician), and a representative from management. Consideration should also be given to representation by a medical advisor.

The structure for the management of biological safety issues within each facility should be determined locally and will vary according to the level of coordination and the associated resources necessary for implementation. Determining factors include the following:

- the size of the facility (staff and square footage)
- the concentration of multiple laboratories in the facility
- the containment levels within the facility (level 2 laboratory, multiple level 3 laboratories)
- the complexity of the processes (routine diagnostics, research, large scale, recombinant work)
- the existence of shared laboratory space within the facility (multiple investigators, various organizations)
- experimental or diagnostic animal activities within the facility (mice in containment caging, large animal housing).

Biological safety issues to be managed may include the following:

- identifying training needs and assisting with the development and delivery of biosafety training programs, such as general biosafety, BSC use, animal biosafety, staff orientation and containment suite training.
- performing risk assessments when required and developing recommendations for procedural or physical laboratory modifications.
- auditing the effectiveness of the biosafety program and its associated management system on a regular basis.
- participating in accident investigations and promoting the reporting of incidents within the facility or laboratory.
- distributing new and relevant biosafety information to laboratory staff.
- coordinating and monitoring the decontamination, disinfection and disposal procedures for infectious materials in the facility or laboratory.

- coordinating the receipt, shipment and transport within the facility of infectious material according to the Workplace Hazardous Materials Information System (WHMIS) and *Transportation of Dangerous Goods* (TDG) regulations.
- establishing a record keeping and secure storage system for all infectious material entering the facility.
- coordinating emergency response activities.
- maintaining liaison with support staff, housekeeping staff and contractors on matters related to facility biosafety.

CL 3 or 4 laboratories may have the additional biosafety activities:

- certification and recertification of the laboratory (see Chapter 5).
- investigation and remediation of containment suite physical or operational failures.
- access control to the containment suites.
- liaison with applicable regulatory bodies, such as the Nuclear Safety Commission, Transport Canada, Health Canada and the Canadian Food Inspection Agency.

2.6 Biosecurity

Today, facilities handling infectious agents need not only a biosafety program but also a biosecurity plan in place. While biosafety deals with all aspects of containment to prevent any exposure to and accidental release of pathogens, biosecurity is implemented to prevent the theft, misuse or intentional release of pathogens. Whether it be for the advancement of science or the diagnosis of agents causing disease or the misuse of these technologies, there is unfortunately a dual use potential in the nature of the work (i.e., procedures, equipment, etc.) that takes place with these agents[6]. There are many international recommendations[7-10] and position papers [11-16] which can provide further assistance with the management of biological threats.

As the planning and implementation of a biosecurity plan needs to be specific to the nature of each facility, the type of research and diagnostics conducted, and the local environment, a diverse working group needs to be involved. Consideration should be made to include scientific directors, principal investigators, laboratory workers, administrators, safety officers, security staff, maintenance staff, and law enforcement agencies where appropriate. Also, include the "Responsible Official" (RO) where one is designated. A Responsible Official is typically responsible for the development, training, and implementation of safety, security, and emergency response plans. As such, the RO is contacted with timely notice of any theft, loss or release of agents. This individual is involved in allowing only approved individuals to have access to agents and is involved in the transfer and transportation of agents from the facility. This person can assist with maintaining detailed records of information necessary to give a complete accounting of all activities related to pathogens.

A primary component to a biosecurity plan must be a detailed risk assessment (see also Chapter 2.3)[7,10]. The biosecurity risk assessment should review and list the relevant assets, define the threats, outline the vulnerabilities, and determine the countermeasures or mitigation strategies specific for each facility. The biosecurity plan should then address the following factors[8,11,15]: physical protection; personnel suitability/reliability; pathogen accountability; and related incident and emergency response.

Given the importance and timely nature of the issue of biosecurity, this section was added after the final Contributors meeting. However, the specific requirements have been included in the matrices as reviewed by the Contributors.

Physical Protection

The physical protection risk assessment should include all levels of biosecurity 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 biosecurity

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. Similarly, information security for data and electronic technology need to be addressed.

Personnel Suitability/Reliability

Background checks and security clearances may be required before employees are granted access to containment facilities. These factors should be considered as part of the local risk assessment process when developing a biosecurity plan. Photo identification badges for employees and temporary badges for escorted visitors can also be used 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. Biosecurity training needs to be provided to all personnel who are given access.

Pathogen Accountability

Pathogen accountability procedures should include inventory requirements for proper labelling, tracking of internal possession, inactivation and disposal of cultures after use, and transfers within and outside the facility. These inventory controls also assist in keeping track of pathogen storage locations and under whose responsibility the pathogens lie. Inventories must 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. 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 notification process for identifying, reporting, and remediating security problems, i.e., inventory discrepancy, equipment failure, breach of security, release of agents, etc., should be in place.

Biosecurity Incident and Emergency Response

A protocol for reporting and investigating security incidents e.g., missing infectious substances, unauthorized entry, should be addressed. A mechanism needs to be in place for the reporting and removal of unauthorized persons. Biosecurity incident and emergency plans should include response to intentional (bomb threats etc.), unintentional (accidental release) and natural events (power outages, severe weather). Training needs to be provided to all relevant personnel.

Biosecurity requirements for facilities handling infectious agents at containment levels 3 and 4 will generally be more stringent than those required in clinical and research containment level 2 laboratories. Recommendations on biosecurity practices (e.g., storage of pathogens, inventories, log books to record entry) and physical design security features (locks, restricted access) have been incorporated into the requirements for each containment level in Chapters 3 and 4.

Expert advice from security and/or law enforcement experts should be sought in the development of threat assessments and security protocols specific to each facility. The threat assessment and security practices should be regularly reviewed and updated to reflect new threats that may be identified.

References

1. *Laboratory biosafety manual.* Geneva: World Health Organization, 1993;1-133.

2. Collins, C.H., and Kennedy, D.A. *Equipment- and technique-related hazards.* In: *Laboratory-acquired infections: history, incidence, causes and preventions.* Oxford, U.K: Butterworth-Heinemann, 1999; 65-109.

3. Collins, C.H., and Kennedy, D.A. *Exposure, sources and routes of infection.* In: *Laboratory-acquired infections: history, incidence, causes and preventions.* Oxford, U.K: Butterworth-Heinemann, 1999; 38-53.

4. Richmond, J.Y. and McKinney, R.W. *Biosafety in microbiological and biomedical laboratories*. Washington, DC: U.S. Government Printing Office, 1999;1-250.

5. *The management, design and operation of microbiological containment laboratories*. Advisory Committee on Dangerous Pathogens, UK: Health and Safety Executive, 2001; 7-16.

6. *Biotechnology Research in an Age of Terrorism*: *Confronting the Dual Use Dilemma*. Washington, DC: The National Academic Press, 2004.

7. *Public Health Response to Biological and Chemical Weapons: WHO Guidance*. Geneva: World Health Organization, Draft 2003.

8. *Interim Final Rule: CDC; Possession, Use, and Transfer of Select Agents and Toxins*. Federal Register, Department of Health and Human Services, 2002; 240 (67): 76885-76905.

9. Richmond, J.Y. and McKinney, R.W. *Biosafety in microbiological and biomedical laboratories*. Washington, DC: U.S. Government Printing Office, 1999;1-250.

10. *Laboratory Security and Emergency Response Guidance for Laboratories Working with Select Agents*. MMWR, 2002; 51(RR-19):1-6.

11. *ABSA Biosecurity Task Force White Paper: Understanding Biosecurity*. American Biological Safety Association, January 2003. (http://www.absa.org/0301bstf.html).

12. *Biosafety, Biosecurity, and Biological Weapons*. Germany: The Sunshine Project, October 2003;1-22.

13. Salerno, R., and Koelm, J.G. *Biological Laboratory and Transportation Security and the Biological Weapons Convention*. Albuquerque, New Mexico: Sandia National Laboratories, SAND No.2002-1067P, February 2002; 1-9. (http://cns.miis.edu/research/cbw/biosec/pdfs/sandia.pdf).

14. Barletta, M., *Biosecurity Measures for Preventing Bioterrorism*. Center for Nonproliferation Studies, Monterey Institute of International Studies, 2002; 1-11.

15. Tucker, J.B., *Biosecurity: Limiting Terrorist Access to Deadly Pathogens*. Washington, DC: United States Institute of Peace, Peaceworks, 2003; 1-51.

16. *Controls Over Biological, Chemical, and Radioactive Materials at Institutions Funded by the U.S. Department of Agriculture*. Washington, DC: United States Department of Agriculture, Report No. 50099-14-At, September 2003; 1-43. (http://www.usda.gov/oig/webdocs/50099-14-At.pdf).

Chapter 3
Handling Infectious Substances

Individuals who work in a laboratory that handles infectious substances are at risk of exposure to the substances they handle. Laboratory-acquired infections (LAIs) are not uncommon — over 5,000 cases and 190 deaths had been reported up to 1999[1], although these figures are believed to be a significant underestimate because of underreporting[2,3]. Additionally, only about 20% of infections can be attributed to any known, single exposure event[4].

There are a number of ways in which infectious substances can enter the body and cause infection, including ingestion, inhalation, or contact with mucous membranes, including conjunctivae (transfer of microorganisms to the eyes by contaminated hands), or with non-intact skin.

The types of events that can lead to an infection include the following: exposure to infectious aerosols; spills and splashes; accidental needlestick injuries; cuts from sharp objects and broken glass; bites and scratches from animals or ectoparasites; oral pipetting (which is prohibited); centrifuge accidents; secondary spread of infectious materials to nonlaboratory areas. Exposure to aerosols may be the greatest biohazard facing laboratory workers[5]. Aerosols can present a risk in terms of inhalation, ingestion, mucous membrane contact, etc. Operational practices and techniques must be used to minimize the creation of aerosols associated with common laboratory procedures.

As assessed in Chapter 2, the following are operational practices for activities involving laboratory scale use of human pathogens at the four containment levels.

3.1 Operational Practices for Laboratories

3.1.1 General Practices

The following general practices are required for all laboratories handling infectious substances.

1. A documented procedural (safety) manual must be available for all staff, and its requirements followed; it must be reviewed and updated regularly.
2. Personnel must receive training on the potential hazards associated with the work involved and the necessary precautions to prevent exposure to infectious agents and release of contained material; personnel must show evidence that they understood the training provided; training must be documented and signed by both the employee and supervisor; retraining programs should also be implemented.
3. Eating, drinking, smoking, storing of either food, personal belongings, or utensils, applying cosmetics, and inserting or removing contact lenses are not permitted in any laboratory; the wearing of contact lenses is permitted only when other forms of corrective eyewear are not suitable; wearing jewelry is not recommended in the laboratory.
4. Oral pipetting of any substance is prohibited in any laboratory.
5. Long hair is to be tied back or restrained so that it cannot come into contact with hands, specimens, containers or equipment.
6. Access to laboratory and support areas is limited to authorized personnel.
7. Doors to laboratories must not be left open (this does not apply to an open area within a laboratory).
8. Open wounds, cuts, scratches and grazes should be covered with waterproof dressings.

9. Laboratories are to be kept clean and tidy. Storage of materials that are not pertinent to the work and cannot be easily decontaminated (e.g., journals, books, correspondence) should be minimized; paperwork and report writing should be kept separate from such biohazardous materials work areas.

10. Protective laboratory clothing, properly fastened, must be worn by all personnel, including visitors, trainees and others entering or working in the laboratory; suitable footwear with closed toes and heels must be worn in all laboratory areas.

11. Where there is a known or potential risk of exposure to splashes or flying objects, whether during routine operations or under unusual circumstances (e.g., accidents), eye and face protection must be used. Careful consideration should be given to the identification of procedures requiring eye and face protection, and selection should be appropriate to the hazard.

12. Gloves (e.g., latex, vinyl, co-polymer) must be worn for all procedures that might involve direct skin contact with biohazardous material or infected animals; gloves are to be removed when leaving the laboratory and decontaminated with other laboratory wastes before disposal; metal mesh gloves can be worn underneath the glove.

13. Protective laboratory clothing must not be worn in nonlaboratory areas; laboratory clothing must not be stored in contact with street clothing.

14. If a known or suspected exposure occurs, contaminated clothing must be decontaminated before laundering (unless laundering facilities are within the containment laboratory and have been proven to be effective in decontamination).

15. The use of needles, syringes and other sharp objects should be strictly limited; needles and syringes should be used only for parenteral injection and aspiration of fluids from laboratory animals and diaphragm bottles; caution should

be used when handling needles and syringes to avoid auto-inoculation and the generation of aerosols during use and disposal; where appropriate, procedures should be performed in a BSC; needles should not be bent, sheared, recapped or removed from the syringe; they should be promptly placed in a puncture-resistant sharps container (in accordance with Canadian Standards Association [CSA] standard Z316.6-95(R2000))[6] before disposal.

16. Hands must be washed after gloves have been removed, before leaving the laboratory and at any time after handling materials known or suspected to be contaminated.

17. Work surfaces must be cleaned and decontaminated with a suitable disinfectant at the end of the day and after any spill of potentially biohazardous material; work surfaces that have become permeable (i.e., cracked, chipped, loose) to biohazardous material must be replaced or repaired.

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

19. Efficacy monitoring of autoclaves used for decontamination with biological indicators must be done regularly (i.e., consider weekly, depending on the frequency of use of the autoclave), and the records of these results and cycle logs (i.e., time, temperature and pressure) must also be kept on file.

20. All contaminated materials, solid or liquid, must be decontaminated before disposal or reuse; the material must be contained in such a way as to prevent the release of the contaminated contents during removal; centralized autoclaving facilities are to follow the applicable containment level 2 requirements.

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

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

23. Spills, accidents or exposures to infectious materials and losses of containment must be reported immediately to the laboratory supervisor; written records of such incidents must be maintained, and the results of incident investigations should be used for continuing education.

24. An effective rodent and insect control program must be maintained.

3.1.2 Containment Level 2

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

1. Good microbiological laboratory practices intended to avoid the release of infectious agents are to be employed.

2. BSCs must be used for procedures that may produce infectious aerosols and that involve high concentrations or large volumes of biohazardous material. Laboratory supervisors, in consultation with the Biological Safety Officer/Institutional Biosafety Committee, should perform a risk assessment to determine which procedures and what concentrations and volumes necessitate the use of a BSC.

3. Appropriate signage indicating the nature of the hazard being used (e.g., biohazard sign, containment level) must be posted outside each laboratory; if infectious agents used in the laboratory require special provisions for entry, the relevant information must be included on the sign; the contact information of the laboratory supervisor or other responsible person(s) must also be listed.

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

5. All people working in the containment area must be trained in and follow the operational protocols for the project in process. Trainees must be accompanied by a trained staff member. Visitors, maintenance staff, janitorial staff and others, as deemed appropriate, must also be provided with training and/or supervision commensurate with their anticipated activities in the containment area.

6. Emergency procedures for spill clean-up, BSC failure, fire, animal escape and other emergencies must be written, easily accessible and followed. A record must be made of other people entering the facility during an emergency.

3.1.3 Containment Level 3

In addition to the operational practices for all laboratories handling infectious substances and those minimum requirements for containment level 2, the following describe the minimum operational practices required at containment level 3.

1. There must be a program for the management of biological safety issues in place with appropriate authority to oversee safety and containment practices (see Chapter 2, Section 2.5).

2. Everyone entering the containment laboratory must have completed a training course in procedures specific to the containment laboratory and must show evidence of having understood the training; training must be documented and signed by the employee and supervisor.

3. Employees 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).

4. A protocol specific to the operation of the laboratory must be developed and read by personnel; employees must certify in writing that they have understood the material in the protocol. 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.

5. Personnel must have demonstrated proficiency in microbiological practices and techniques.

6. 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 by laboratory staff 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).

7. 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).

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

9. The containment laboratory must be kept locked.

10. 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.

11. Personal items such as purses and outdoor clothing must not be brought into the containment laboratory.

12. 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).

13. 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.

14. Personnel entering the containment laboratory must remove street clothing and jewelry, and change into dedicated laboratory clothing and shoes; dedicated laboratory clothing and shoes must be removed before leaving the containment laboratory in a manner that minimizes any contamination of the skin with the potentially contaminated dedicated laboratory clothing. The use of full coverage protective clothing (i.e., completely covering all street clothing) is an acceptable alternative. When a known or suspected exposure may have occurred, all clothing, including street clothing, requires appropriate decontamination. Laboratories manipulating organisms, such as HIV, that are not infectious via inhalation, are not required to remove street clothing.

15. An additional layer of protective clothing (i.e., solid-front gowns with tight-fitting wrists, gloves, respiratory protection[7]) may be worn over laboratory clothing when infectious materials are directly handled and should be removed after completion of work (e.g., dedicated for use at the BSC).

16. Centrifugation of infectious materials must be carried out in closed containers placed in sealed safety cups or rotors that are unloaded in a BSC.

17. Animals or arthropods that have been experimentally infected must remain in the laboratory or appropriate animal containment facility.

18. 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.

19. All activities with infectious materials are conducted in a BSC; if this is not possible, other primary containment devices in combination with personal protective clothing and equipment must be used; no work with open vessels containing infectious materials is conducted on the open bench.

20. Heat-sensitive materials that cannot be autoclaved out of the containment laboratory must be decontaminated at the containment barrier (e.g., fumigated with formaldehyde, vaporized hydrogen peroxide or a suitable alternative; disinfected using liquid chemicals; or subjected to other technology proven to be effective).

21. Emergency procedures for failure of air handling systems and other containment emergencies must be written, easily accessible and followed.

22. 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).

3.1.4 Containment Level 4

In addition to the operational practices for all laboratories handling infectious substances and those minimum requirements for containment level 2 and 3, the following describe the minimum operational practices required at containment level 4.

1. Protocols must be established for emergencies, including damage to positive pressure suits, loss of breathing air, and loss of chemical shower.

2. Employees must immediately notify their supervisor of any unexplained febrile illness; supervisors must contact any employee with unexplained work absences.

3. The employer must establish liaison with the local hospital/health care facility to ensure that in the event of an employee's accidental exposure to containment level 4 agents the hospital/health care facility is fully aware of the infectious agents involved and that the appropriate procedures are in place for the treatment of the employee (patient).

4. A record of containment laboratory usage is to be maintained (i.e., log book of all entry and exits) with date and time.

5. Cultures and stocks of infectious agents must be stored in a secure area inside the containment laboratory and an inventory of pathogens maintained.

6. A daily check of containment systems (e.g., directional airflow, disinfectant level in chemical shower, critical containment points for a class III BSC line) and life support systems (e.g., back-up breathing air) must be carried out before entering the laboratory.

7. Personnel entering the laboratory must remove street clothing (including undergarments) and jewelry, and change into dedicated laboratory clothing and shoes.

8. Positive pressure suits must be worn (for level 4 suit mode); the integrity of the suit must be routinely checked for leaks.

9. A chemical shower of appropriate duration is required for personnel in suits who are leaving the containment laboratory; the disinfectant used must be effective against the agents of concern, be diluted as specified and prepared fresh as required; this is not applicable for class III BSC line level 4 containment facilities.

10. A body shower is required on exit from the containment laboratory.

11. Material can be removed from the containment laboratory only after appropriate decontamination or after specific approval from the Biological Safety Officer or other appropriate authority.

12. A competent person must be available outside the containment level 4 laboratory when work is being conducted within the laboratory, to assist in case of emergency.

13. Small laboratory animals, primates or insects infected with level 4 agents are to be housed in a partial containment system (e.g., cages placed in HEPA filtered containment enclosures).

14. All laboratory procedures are to be conducted within a BSC in conjunction with a positive pressure suit or within a class III BSC line.

15. Large animals require specialized care and handling not dealt with by these *Guidelines*. For details, please refer to the current edition of *Containment Standards for Veterinary Facilities*, by the Canadian Food Inspection Agency[8]. This office can be contacted by calling the Biohazard Containment and Safety Division directly at (613) 221-7088 or accessing their Web site:
http://www.inspection.gc.ca/english/sci/lab/bioe.shtml

References

1. Harding, A.L, and Brandt Byers, K. *Epidemiology of laboratory-associated infections*. In: Fleming, D.O., and Hunt, D.L. *Biological safety: principles and practices*. Washington, DC: ASM Press, 2000;35-54.

2. Pike, R.M. *Laboratory-associated infections: incidence, fatalities, causes and preventions*. Annu. Rev. Microbiol. 1979; 33:41-66.

3. Collins, C.H., and Kennedy, D.A. Preface. In: *Laboratory-acquired infections: history, incidence, causes and preventions.* Oxford, U.K: Butterworth-Heinemann, 1999; ix-xi.

4. Pike, R.M. *Laboratory-associated infections. Summary and analysis of 3921 cases.* Health Lab Sci 1976;105-14, volume 13.

5. Collins, C.H., and Kennedy, D.A. *Exposure, sources and routes of infection*. In: *Laboratory-acquired infections: history, incidence, causes and preventions.* Oxford, U.K: Butterworth-Heinemann, 1999; 38-53.

6. *Evaluation of single use medical sharps containers for biohazardous and cytotoxic waste*. CSA standard Z316.6-95(R2000). Toronto: Canadian Standards Association, 2000.

7. *Selection, Use, and Care of Respirators*. CSA Standard Z94.4-02. Toronto: Canadian Standards Association, 2002.

8. *Containment standards for veterinary facilities*. Ottawa: Agriculture and Agri-Food Canada, Minister of Supply ans Services Canada, No. 1921/E, 1996.

Chapter 4
Laboratory Design and Physical Requirements

This chapter is designed to provide guidance on the design and layout required to achieve the four containment levels detailed in Chapter 2.

The chapter is divided into five matrices: Laboratory Location and Access; Surface (i.e., floors, walls, ceilings, sealants) Finishes and Casework; Heating, Ventilation and Air Conditioning (HVAC); Containment Perimeter; and Laboratory Services (i.e., water, drains, gas, electricity and safety equipment). Information on commissioning, certification and recertification of the containment features detailed in the matrices can be found in Chapter 5.

Matrix Legend: ● - mandatory ○ - recommended

4.1 *Matrix 1* Laboratory Location and Access

	Containment Level				
Matrix 1	**1**	**2**	**3**	**4**	**Laboratory Location and Access**
1	●	●	●	●	Separated from public areas by door.
2		●	●	●	Access limited to authorized personnel.
3		●	●	●	Laboratory room doors to have appropriate signage (e.g., biohazard sign, containment level, contact information, entry requirements).
4	●	●	●	●	Size of door openings to allow passage of all anticipated equipment.
5		●	●	●	Doors to the containment laboratory lockable (this does not apply to areas within the containment laboratory).

Matrix 1	*Containment Level* 1	2	3	4	Laboratory Location and Access
6			●	●	Doors to provide restricted access by installation of a controlled access system (e.g., card key) or equivalent.
7			○	●	Electronic locking systems to be backed up with a physical key-lock system.
8		○	●	●	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 laboratory work areas.
9			●	●	Entry to laboratory to be provided via an anteroom.
10			●		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.)
11				●	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 only).
12			●	●	Interlocked doors, if present, to have manual overrides for emergency exit.
13			●	●	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).

Matrix 1	*Containment Level* 1	2	3	4	Laboratory Location and Access
14			●	●	Exit from laboratory to be provided with a walk-through shower on the containment barrier (i.e., between “dirty” and "clean" change anterooms). 〔CL3 laboratories manipulating organisms, such as HIV, that are not infectious via inhalation, are not required to fulfil this criterion.〕
15				●	Entry to laboratory to be provided via anteroom with airtight doors (e.g., inflatable or compression seal); for laboratories using only a Class III BSC biological safety cabinet line, airtight doors are not required.
16				●	Entry to laboratory zone to be provided with a suit change area, a chemical shower on the containment barrier (i.e., between the laboratory and suit change area) and water shower on exit from the zone (i.e., between "dirty" and "clean" change areas); for laboratories using only a Class III biological safety cabinet line, suit change area and chemical shower are not required.
17			○	●	Containment laboratories to be located in close proximity to supporting mechanical services to limit the amount of potentially contaminated services.
18			○	●	Containment laboratories to be located away from external building envelope walls.
19			○	○	A laboratory support area to be provided adjacent to the containment facility for all supporting laboratory manipulations.

4.2 *Matrix 2*
Surface (i.e., floors, walls, ceilings, sealants) Finishes and Casework

	Containment Level				
Matrix 2	**1**	**2**	**3**	**4**	**Surface (i.e., floors, walls, ceilings, sealants) Finishes and Casework**
1		○	●	●	Doors, frames, casework and bench tops to be nonabsorptive (i.e., the use of organic materials should be avoided).
2		●	●	●	Working surfaces of bench tops to be non-absorptive.
3	○	●	●	●	Surfaces to be scratch, stain, moisture, chemical and heat resistant in accordance with laboratory function.
4	○	○	●	●	Surfaces to provide impact resistance in accordance with laboratory function.
5		○	●	●	Surfaces to be continuous and compatible with adjacent and overlapping materials (i.e., to maintain adhesion and a continuous perimeter); wall and floor welded seams are acceptable in level 3 laboratories.
6			●	●	Continuity of seal to be maintained between the floor and wall (a continuous cove floor finish up the wall is recommended).
7			●	●	Interior surfaces to minimize movement of gases and liquid through perimeter membrane.
8	○	●	●	●	Interior coatings to be gas and chemical resistant in accordance with laboratory function (e.g., will withstand chemical disinfection, fumigation).
9			●	●	Interior coatings to be cleanable.

Matrix 2	*Containment Level* 1	2	3	4	**Surface (i.e., floors, walls, ceilings, sealants) Finishes and Casework**
10				●	Structural stability to withstand 1.25 times maximum design pressure under supply and exhaust fan failure conditions (i.e., no wall distortion or damage).
11	○	○	●	●	Bench tops to have no open seams.
12	○	○	○	○	Bench tops to contain spills of materials (e.g., with marine edges and drip stops).
13	○	○	○	●	Benches, doors, drawers, door handles, etc. to have rounded rims and corners.
14	○	○	○	○	Backsplashes, if installed tight to wall, to be sealed at wall-bench junction.
15	○	○	○	○	Reagent shelving to be equipped with lip edges.
16	○	○	○	○	Drawers to be equipped with catches, i.e., to prevent the drawer from being pulled out of the cabinet.
17				○	Drawers to be of one piece construction.
18	○	○	○	○	Cabinet doors not to be self-closing.

4.3 *Matrix 3*
Heating, Ventilation and Air Conditioning (HVAC)

	Containment Level				
Matrix 3	**1**	**2**	**3**	**4**	**HVAC**
1		○	●	●	100% outside air to be supplied.
2			●	●	Directional inward airflow provided such that air will always flow towards areas of higher containment (e.g., ± 25 Pa differential).
3			●	●	Visual pressure differential monitoring devices to be provided at entry to containment laboratory.
4				●	Room pressure differential monitoring lines penetrating the containment barrier to be provided with filters of efficiency equal to that of HEPA filtration.
5			●	●	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.
6			●		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).
7				●	Supply air to be HEPA filtered.

Matrix 3	*Containment Level* 1	2	3	4	HVAC
8			●	●	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.〕
9			●	●	Supply air system to be interlocked (i.e., fans, dampers, electrical) with exhaust air system, to prevent sustained laboratory positive pressurization.
10			●	●	Exhaust air to be HEPA filtered. 〔CL3 laboratories manipulating organisms, such as HIV, that are not infectious via inhalation are not required to fulfil this criterion.〕
11				●	Exhaust air to be passed through two stages of HEPA filtration.
12			●	●	HEPA filters installed into the supply and exhaust system to conform to the requirements of IEST-RP-CC001.3[1].
13				●	Supply HEPA filter housings to be designed to withstand structural change at applied pressure of 2500 Pa [10 in. w.g.].
14			●		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 [10 in. w.g.].

Matrix 3	*Containment Level* 1	2	3	4	HVAC
15			●	●	Exhaust HEPA filter housings to be designed to withstand structural change at applied pressure of 2500 Pa [10 in. w.g.] 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.〕
16			●	●	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.〕
17			○	●	Supply and exhaust systems located outside of containment to be accessible for repairs, maintenance, cleaning and inspection.
18				●	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 Sheet Metal and Air Conditioning Contractors National Association (SMACNA) Seal Class A(2).
19			●		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(2).

Matrix 3	*Containment Level* 1	2	3	4	HVAC
20			●	●	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[2]. 〔CL3 laboratories manipulating organisms, such as HIV, that are not infectious via inhalation are not required to fulfil this criterion.〕
21			●	●	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[2]. 〔CL3 laboratories manipulating organisms, such as HIV, that are not infectious via inhalation are not required to fulfil this criterion.〕
22			●	●	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 fulfil this criterion.〕

4.4 *Matrix 4*
Containment Perimeter

Matrix 4	*Containment Level* 1	2	3	4	Containment Perimeter
1	○	●			Autoclave or other acceptable means of waste treatment/disposal to be provided.
2			●	●	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.〕
3			●		Barrier autoclave to be equipped with interlocking doors, or visual or audible alarms to prevent both doors from opening at the same time.
4				●	Barrier autoclave to be equipped with interlocking doors, and visual or audible alarms to prevent both doors from opening at the same time.
5			●	●	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.
6			●	●	All penetrations to be sealed with nonshrinking sealant at containment barrier.

Matrix 4	*Containment Level* 1	2	3	4	Containment Perimeter
7			●	●	All conduit and wiring to be sealed with nonshrinking sealant at the containment barrier.
8	●	●			Windows, if they can be opened, to be protected by fly screens.
9			●	●	Windows positioned on containment barrier to be sealed in place; window glazing material to provide required level of security.
10			○	○	Observation windows to be installed on containment barrier.

4.5 *Matrix 5*
Laboratory Services (i.e., water, drains, gas, electricity, and safety equipment)

	Containment Level				
Matrix 5	**1**	**2**	**3**	**4**	**Laboratory Services (i.e., water, drains, gas, electricity, and safety equipment)**
1	●	●			Hooks to be provided for laboratory coats at laboratory exit; street and laboratory clothing areas to be separated.
2	●	●	●	●	Handwashing sinks to be located near the point of exit from the laboratory or in anteroom. Not applicable to CL4 suit laboratories.
3		○	●	●	Handwashing sinks to be provided with "hands-free" capability.
4			●	●	BSCs and other primary containment devices to be provided.
5		○			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.
6		●	●		Emergency eyewash facilities to be provided in accordance with applicable regulations (i.e., ANSI Z358.1-1998[3]).
7		●			Emergency shower equipment to be provided in accordance with applicable regulations (i.e., ANSI Z358.1-1998[3]).

Matrix 5	*Containment Level* 1	2	3	4	**Laboratory Services (i.e., water, drains, gas, electricity, and safety equipment)**
8			●		When it is not possible to limit the quantities of hazardous chemicals within the laboratory, emergency shower equipment to be provided in accordance with applicable regulations (i.e., ANSI Z358.1-1998[3]).
9			●	●	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[4], and isolation valve, to be located in close proximity to the containment barrier.
10			●		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).
11				●	Drain lines and associated piping (including autoclave condensate) to be separated from areas of lower containment and to be connected to an effluent sterilization system.
12				●	Drains connected to effluent sterilization to be sloped towards sterilization system to ensure gravity flow; consideration should be given to the installation of valves to isolate sections of piping for *in situ* decontamination; the effluent sterilization system (e.g., piping, valves, tank) to be heat and chemical resistant consistent with application.
13			●	●	Autoclave condensate drain to have a closed connection. For CL3, open connection is allowable if located within containment barrier.

Matrix 5	*Containment Level* 1	2	3	4	**Laboratory Services (i.e., water, drains, gas, electricity, and safety equipment)**
14			●	●	Drainage traps to be provided to required deep seal depth in consideration of air pressure differentials.
15			○	●	Floor drains not to be provided, except when essential (e.g., body shower and animal rooms).
16				●	Plumbing vent lines (including effluent sterilization system) to be provided with filter of efficiency equivalent to that of HEPA and provided with a means of isolation and decontamination.
17			●		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.〕
18			○	●	Compressed gas cylinder(s) to be located outside the laboratory.
19				●	Laboratory supply gas piping (e.g., carbon dioxide, compressed air) to be provided with backflow prevention.
20			●	●	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 traps).

Matrix 5	*Containment Level* 1	2	3	4	**Laboratory Services (i.e., water, drains, gas, electricity, and safety equipment)**
21				●	Compressed breathing air to be provided to positive-pressure personal protective equipment (i.e., for connection to the air hose of suits), equipped with breathing air compressors and back-up cylinders (sufficient for 30 minutes per person); air hose connections to be provided in all areas where suits are worn, including chemical shower and suit change room.
22			●	●	Emergency lighting to be provided.
23			●	●	Life safety systems, lighting, HVAC systems, BSCs, security systems and other essential equipment to be supported with emergency back-up power.
24			●	●	Circuit breakers to be located outside biocontainment area.
25			○	○	Fluorescent light ballasts and starters to be located outside containment area.
26			●	●	Laboratory to be equipped with a communication system between containment area and outside support area.
27			●	●	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).
28				●	Work area to be monitored (e.g., closed circuit TV) from outside laboratory perimeter (e.g., security/biosafety office).

References

1. *HEPA and ULPA filters*. IEST-RP-CC001.3. Rolling Meadows, IL: The Institute of Environmental Science and Technology, 1993.
2. *HVAC air duct leakage test manual*. Chantilly, Virginia: Sheet Metal and Air Conditioning Contractors National Association, Inc., 1985.
3. *American national standard for emergency eyewash and shower equipment*. ANSI Z358.1-1998. Arlington, Virginia: American National Standards Institute, Inc., 1998.
4. *Manual for the selection and installation of backflow prevention devices/manual for the maintenance and field testing of backflow prevention devices*. CAN/CSA-B64.10-01/B64.10.1-01. Toronto, ON: Canadian Standards Association, 2001.

Chapter 5
Commissioning, Certification and Recertification for CL3 and CL4 Laboratories

5.1 Introduction

For the purposes of this document, "commissioning" is defined as the verification of the physical construction and performance of critical containment components and is one part of the overall certification process. "Certification" is defined as the successful completion of commissioning and verification that the facility and operational protocols meet the requirements outlined in the current edition of the *Laboratory Biosafety Guidelines*. "Recertification" is verification that the facility continues to comply with the current edition of the *Laboratory Biosafety Guidelines* and has undergone a recommissioning process as outlined below.

5.1.1 Commissioning

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. Commissioning is a requirement for the certification of containment levels 3 and 4 laboratories.

5.1.2 Certification

A matrix of critical containment components to be verified during initial certification is provided below. 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.

5.1.3 Recertification

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 (i.e., 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.

5.2 Room Integrity

Smoke testing the integrity of a containment room can be done to detect leaks in the room perimeter. All joints, corners and sealed penetrations should be surveyed for leaks. Pressure decay testing the integrity of the containment room provides an indication of the tightness of the room perimeter (i.e., the ability of gases and liquids to move through the perimeter membrane and service penetrations).

Matrix Legend: ● - mandatory ○ - recommended

5.2.1 *Matrix 6* Room Integrity

	Containment Level		
Matrix 6	**3**	**4**	**Room Integrity**
1	●	●	Integrity of containment surfaces to be tested visually and with a smoke pencil or other visual aid. Inspect floors, walls, and ceiling for cracks, chips and wear. Verify integrity of wall/floor and wall/ceiling joints. Acceptance criteria: to confirm the integrity of all penetrations (i.e., equipment, services, etc.) and seals (i.e., around doors, windows, autoclaves, etc.) on the containment barrier.

	Containment Level		
Matrix 6	**3**	**4**	**Room Integrity**
2		●	Integrity of containment to be tested by pressure decay testing. Acceptance criteria: two consecutive tests with a minimum of 250 Pa (1 in. w.g.) loss of pressure from an initial 500 Pa (2 in. w.g.) over a 20 minute period[1]. This test is not a mandatory requirement for recertification if no modification or changes have been made that will affect the integrity of the laboratory, and if a visual inspection of the containment barrier membrane indicates that the integrity has not been compromised; if the perimeter integrity is suspect upon visual inspection, the requirement for repeating the pressure decay test should be determined in consultation with the laboratory supervisor, Biological Safety Officer/Institutional Biosafety Committee.

5.2.2 Room Pressure Decay Testing

The basic procedure for room pressure decay testing under negative pressure is as follows:

- Isolate the area by closing and securing all doors, valves and bubble tight dampers at the containment barrier (avoid temporary sealing measures in doors, windows and services that would cover permanent seals and not permit their testing for leakage); plug all pressure sensor lines, e.g., magnehelic gauges.
- Install a calibrated inclined manometer across the containment barrier such that it is not affected by air distribution. Manometer to have minimum accuracy of 10 Pa (0.05 in. w.g.) and capable of reading pressure up to 750 Pa (3 in. w.g.)[1].
- Install a ball valve in the piping between the vacuum pump/fan and the room to allow the room to be sealed once the test pressure has been attained.
- Connect a vacuum source to the room and create a 500 Pa (2 in. w.g.) negative pressure differential; allow room to

stabilize and close the valve between the vacuum pump/fan and the room to seal room at 500 Pa (2 in. w.g.).

- Dynamically trend pressure loss starting at 500 Pa (2 in. w.g.) negative pressure differential; record the differential pressure at 1 minute intervals for 20 minutes.
- If repeat test is required, allow 20 minute wait period.
- Disconnect the vacuum pump/fan and open the ball valve slowly to allow room pressure to return to normal condition.
- If leak rate exceeds the acceptance value:
  - pressurize the room to a pressure adequate to locate leaks;
  - with the room under continuous pressure, apply bubble solution to areas to be tested (joints, corners, sealed penetrations, etc.) or, by using audible leak location method, locate audible leaks (electronic sound detection equipment option);
  - identify places where bubbles are found;
  - after repair of leak, retest as required.

5.3 Air Handling Systems

Various components of a containment room's air handling system require commissioning. Manufacturers' requirements for airflows for BSCs must be met. Integrity testing of HEPA filters must be performed to ensure that they do not contain leaks in the filter media, the gasketing or the seal to the filter housing. This filter housing test is performed by challenging with a known particulate concentration and scanning for percentage of penetration downstream of the filter. Ductwork systems should be pressure decay tested to confirm that specified leakage rates are not exceeded. The American Society of Mechanical Engineers (ASME) Standard N510 *Testing of Nuclear Air Treatment Systems*, 1989, reaffirmed 1995[2], gives procedures for testing the leak-tightness of ducts and plenums. The performance of room pressure control systems must meet the design intent (e.g., negative pressures must be maintained).

The following testing requirements and acceptance criteria must be satisfied for certification of laboratories.

5.3.1 *Matrix 7*
Air Handling Systems

	Containment Level		
Matrix 7	**3**	**4**	**Air Handling Systems**
1	●	●	Classes I and II BSCs to be tested in situ in accordance with NSF/ANSI 49-2002[3] or CSA Z316.3-95[4].
2	●	●	Class III BSCs to be tested in situ in accordance with the Laboratory Safety Monograph, NIH 1979[5] and BS EN 12469-2000[6].
3	●	●	Interlocks (i.e., Class II Type B2 BSC internal cabinet supply fan and exhaust fan) to be tested in accordance with NSF/ANSI 49:2002[3] to ensure that internal supply fan shuts off whenever exhaust fan fails.
4	●	●	Alarms to be tested for detection of BSC and/or exhaust fan failure by simulation of alarm conditions.
5	●	●	Integrity of HEPA filters installed into supply as method of backdraft protection and exhaust ductwork to be tested *in situ* by particle challenge testing using the scanning method according to IEST-RP-CC-006.2 (section 6.2)[7]. Acceptance criteria: particle penetration not to exceed 0.01%. Small in-line filters need not be *in situ* scan tested – maintenance program to include visual inspection and regular replacement.

	Containment Level		
Matrix 7	**3**	**4**	**Air Handling Systems**
6	●	●	Integrity of HEPA filter housings with inlet and outlet bubble tight dampers installed into supply ductwork, where HEPA filters are used as backdraft protection, and exhaust ductwork to be tested in situ by pressure decay testing in accordance with ASME N510[(2)]. Acceptance criteria: rate of air leakage not to exceed 0.1% of housing vol/min at 1000 Pa (4 in. w.g.) minimum test pressure. This test is not a mandatory requirement for recertification if no physical modification or changes have been made. If modifications have been performed then the laboratory supervisor, in consultation with the Biological Safety Officer/ Institutional Biosafety Committee, shall determine the degree of change and whether this test is subsequently required.
7	●		Supply ductwork, where backdraft protection is required on supply, and exhaust air ductwork located between containment perimeter and HEPA filter or bubble tight backdraft damper to be tested *in situ* by pressure decay method in accordance with ASME N510[(2)]. Acceptance criteria: rate of air leakage not to exceed 0.1% duct vol/min at 1000 Pa (4 in. w.g.) minimum test pressure.
8		●	Supply and exhaust air ductwork between containment perimeter and HEPA filter or bubble tight backdraft damper to be tested *in situ* by pressure decay method in accordance with ASME N510[(2)]. Acceptance criteria: rate of air leakage not to exceed 0.1% duct vol/min at 1000 Pa (4 in. w.g.) minimum test pressure.

	Containment Level		
Matrix 7	**3**	**4**	**Air Handling Systems**
9	○	○	All supply and exhaust air ductwork is not required to be tested for pressure decay for recertification if no physical modifications have been done. If modifications have been performed then the laboratory supervisor, in consultation with the Biological Safety Officer/Institutional Biosafety Committee, shall determine the degree of change and whether this test is subsequently required.
10	●	●	Pressurization relationships across adjacent areas to be verified (i.e., clean change to dirty change, dirty change to laboratory). Acceptance criteria: inward directional airflow (under normal operations) to be visually demonstrated (e.g., by holding a smoke pencil at each door leading to adjacent areas).
11	●	●	Control systems to be tested for fail-safe operation by failure of system components, (i.e., exhaust fan failure, supply fan failure, power failure [where possible], Class II B2 BSC exhaust failure). This is to include audible/visual alarm testing. Acceptance criteria: inward directional airflow. The sustained reversal of airflow across containment barrier is to be prevented. This test is not a mandatory requirement for recertification if no control system hardware or logic changes or upgrades have been done; if modifications have been performed, or if frequent control system problems or failures have been encountered, then the requirement for retesting should be determined in consultation with the Biological Safety Officer/ Institutional Biosafety Committee. Note, this may necessitate the need for decontamination. While not required annually, control systems should be retested periodically. Consult Manufacturer's specifications.

5.4 *Matrix 8*
Laboratory Equipment and Services

	Containment Level		
Matrix 8	**3**	**4**	**Laboratory Equipment and Services**
1	●	●	Operation of water supply backflow preventers to be verified in accordance with CAN/CSA-B64.10-01/ B64.10.1-01[8].
2	●	●	Backflow prevention for other services (e.g., gases) to be verified to ensure that system will operate as specified.
3		●	Compressed breathing air and systems to be verified in accordance with CAN/CSA-Z180.1-00[9]. Systems to be verified for switchover to backup system and to test the response of the alarm.
4		●	Operation of positive-pressure personal protective equipment (i.e., suit) to be tested to ensure that the suit will operate as specified.
5	●	●	Water and chemical shower systems to be tested to ensure that systems will operate as specified and to test the response of the (CL4) disinfectant tank low level alarm.
6	●	●	Standby power and UPS systems to be tested under appropriate load conditions to ensure that the systems will operate as specified.
7	●	●	Operation of interlocking doors to be verified to ensure that doors cannot be opened at the same time.
8	●	●	Operation of security systems (e.g., controlled access, closed circuit TV) to be verified to ensure that the system will operate as specified.
9	●	●	Operation of communication and electronic paper transfer systems (e.g., intercom, telephone, fax) to be verified to ensure that the system will operate as specified.

	Containment Level		
Matrix 8	**3**	**4**	**Laboratory Equipment and Services**
10	●	●	Operation of decontamination systems (e.g., autoclaves, fumigation chambers, liquid effluent) to be verified for operation as specified and microbiologically tested using representative loads; resistance of test organism to be representative of organisms likely to be encountered.
11		●	For containment level 4 laboratories, drains and associated piping leading to liquid effluent treatment systems (including associated vent lines) to be tested in accordance with Section 3.6 of the National Plumbing Code of Canada (1995)[10]; pressure for air test on drainage system shall be at standard code requirements of 35 kPa (14 in. w.g.).

References

1. *ARS facilities design standards.* 242.1M-ARS. Facilities Division, Facilities Engineering Branch AFM/ARS, United States Department of Agriculture, 2002.
2. *Testing of nuclear air treatment systems.* ASME N510. New York, NY: American Society of Mechanical Engineers, 1989 (reaffirmed 1995).
3. *Class II (laminar flow) biohazard cabinetry.* Standard 49. Ann Arbor, Michigan: NSF International, 2002.
4. *Biological containment cabinets: installation and field testing.* CSA Z316.3-95. Toronto, ON: Canadian Standards Association, 1995.
5. National Cancer Institute Office of Research Safety and the Special Committee of Safety and Health Experts. *Laboratory safety monograph: a supplement to the NIH guidelines for recombinant DNA research.* Bethesda, MD: National Institutes of Health, 1979.

6. *Biotechnology – performance criteria for microbiological safety cabinets*. BS EN 12469:2000. European Committee for Standardization (CEN), 2000.

7. *Testing cleanrooms*. IEST-RP-CC006.2. Rolling Meadows, IL: Institute of Environmental Sciences and Testing (IEST), 1997.

8. *Manual for the selection and installation of backflow prevention devices/manual for the maintenance and field testing of backflow prevention devices*. CAN/CSA-B64.10-01/B64.10.1-01. Toronto, ON: Canadian Standards Association, 2001.

9. *Compressed breathing air and systems*. CAN/CSA-Z180.1-00. Toronto, ON: Canadian Standards Association (approved 2001).

10. *National plumbing code of Canada*. Ottawa, ON: Canadian Commission on Building and Fire Codes, National Research Council, 1995.

Chapter 6
Large Scale Production of Microorganisms

6.1 Introduction

Canada has been steadily increasing its industrial base in the area of biotechnology. It is important that industrial fermentation and large scale manipulation of microorganisms be addressed in these *Guidelines* to minimize the risk to workers and the surrounding environment. Large scale work is not necessarily more hazardous than research/laboratory scale work, as many procedures and processes are conducted in closed systems, which thereby reduces the probability of exposure of the operator and the environment to the infectious material[1,2]. This has been adequately demonstrated with many highly infectious agents used for vaccine production[3].

Fermentation processes have aerosol generating capabilities, and thus aerosols are likely to be the highest risk of exposure to pathogenic organisms and their products[4]. The scale of operations also poses a hazard for the potential release of large volumes of pathogenic organisms into the facility and/or the environment[1]. Therefore, it is essential for facilities working with large scale fermentation to ensure that appropriate containment equipment is in place (both physical and operational) and that a detailed contingency plan is available for activation, so that potential exposures will be minimized if a malfunction in the fermentation process occurs.

6.2 Scope

Large scale processes involve fermenters and equipment that cannot be easily moved and sterilized in an autoclave and therefore require *in situ* sterilization and decontamination. However, as is discussed in detail in Chapter 2, Section 2.3 Risk Assessment, the volumes that define "large scale" and therefore require special consideration are based upon a detailed local risk assessment of the work being done and the organisms being manipulated. The volume of 10 L as the cut-off between laboratory scale and large scale is to be used as a guide only.

This chapter details the technical requirements for large scale processes involving organisms that require containment levels 1 to 3. Specific requirements have not been outlined for large scale research or production of viable organisms requiring containment level 4. These requirements should be established on a case-by-case basis, and assistance can be provided by the Office of Laboratory Security, Health Canada.

6.3 Operational Practices and Physical Requirements

The containment requirements listed here are the minimal requirements for large scale process areas and are to be used in addition to the corresponding laboratory scale containment facility operational procedures (see Chapter 3) and physical requirements (see Chapter 4).

6.3.1 Containment Level 1 Large Scale

1. Visual inspections of the integrity of the containment systems are important to detect small leaks.
2. 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.

3. 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.

4. 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.

5. 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.

6. 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.

7. 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.

8. 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.

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

6.3.2 Containment Level 2 Large Scale

In addition to the requirements for containment level 1 large scale, the following describes additional requirements at containment level 2 large scale.

1. Cultures of viable organisms to be contained within a closed system or other primary containment equipment designed to prevent the release of aerosols.
2. Process equipment seals and other directly associated mechanical devices should prevent leakage or be fully enclosed in ventilated housings that are exhausted through HEPA or equivalent filters, or through other equivalent treatment technologies.
3. 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.
4. Process equipment to be tested for integrity of containment capability before initial use and after modifications or changes to the system that could affect the containment characteristics of the equipment; testing procedures and acceptance criteria to be appropriate for the process equipment and closed system design; records to be maintained of such testing.
5. 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.
6. Personal protective equipment requirements to be posted at entry.
7. Entry to process area to be restricted to authorized personnel while production is in progress.

6.3.3 Containment Level 3 Large Scale

In addition to the requirements for containment level 1 and level 2 large scale, the following describes additional requirements at containment level 3 large scale.

1. 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); reusable 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.
2. Hazard warning signs and identification to be used in all records relevant to the history of the equipment (e.g., testing, operation, maintenance).
3. Provision to be made in the process area to contain the full volume of a release of process fluids (e.g., process equipment in diked area).

References

1. Grinsted, J. *Risk assessment and contained use of genetically modified organisms*. In: Tzotzos, G.T. *Genetically modified organisms: a guide to biosafety*. Wallingford, UK: Centre for Agriculture and Biosciences (CAB) International, 1995;17-35.

2. Cipriano, M.L. *Biosafety considerations for large-scale production of microorganisms*. In: Fleming, D.O., and Hunt, D.L. *Biological safety principles and practices*. Washington, D.C.: ASM Press, 2000; 541-55.

3. Collins, C.H. *Safety in microbiology: an overview*. In: Collins, C.H., and Beale, A.J. *Safety in industrial microbiology and biotechnology*. Oxford, UK: Butterworth-Heinemann Ltd, 1992;1-5.

4. Brunius, N.G.F. In: Collins, C.H., and Beale, A.J. *Safety in industrial microbiology and biotechnology*. Oxford, UK: Butterworth-Heinemann Ltd., 1992;239-42.

Chapter 7
Program Specific Guidelines

7.1 Laboratory Animals

7.1.1 General Requirements

Work with animals poses a variety of unique hazards, including exposure to infectious agents (naturally occurring or experimentally produced), animal bites and scratches, kicks and crushing injuries, allergies and physical hazards (noise, temperature). In addition to keeping infectious agents from spreading to laboratory workers there is 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). Animal facilities for work with small and large animals should be designed and operated in accordance with the *Containment Standards for Veterinary Facilities*[1], published by the Canadian Food Inspection Agency, the *Guide to the Care and Use of Experimental Animals*, published by the Canadian Council on Animal Care[2] and other CCAC guidelines and policies (as revised from time to time). 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[3-5].

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. 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.

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 handwashing 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. Recent technological improvements have been incorporated into a wide variety of housing systems to provide control of micro-environmental factors such as temperature, air exchange and humidity. Descriptions of currently used caging and bedding disposal systems have been provided elsewhere[6].

At least one-fifth of people who work with laboratory rodents, guinea pigs and rabbits develop allergies[7]. 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[3,4].

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. 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. 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.

7.1.2 Non-human Primates

Working with non-human primates presents unique hazards related to naturally occurring pathogenic organisms and to the animals themselves. Their long canine teeth and powerful jaws can inflict serious and painful lacerations. The animals also have sharp fingernails and toenails 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. For a more comprehensive list of infectious hazards see reference 5.

CHV-1 is an enzootic virus present in up to 70% of captive macaques, including rhesus and cynomolgus non-human primates[8]. 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. Human infection has been documented in at least 50 instances, resulting in either severe disease or death[9]. Except for one case of person-to-person transmission, all have occurred in people exposed to non-human primates or non-human primate tissues. Transmission to humans is believed to occur primarily by exposure to contaminated non-human primate saliva through bites and scratches, although one fatal case following mucocutaneous exposure without injury has been reported[8]. 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[5,8,10-12]. Risk of exposure to pathogenic

agents can also be reduced through an adequate animal health surveillance program, with emphasis on identification and treatment of diseased animals. Further information on hazard identification and risk assessment can be found in reference 5. Animal handlers should be enrolled in a health and medical surveillance program (see Chapter 2.4 and reference 5).

Everyone who handles non-human primates must be trained in proper methods of restraint and in the use of protective clothing to help prevent bites, scratches and splash exposures. Such methods include the use of squeeze-back cages, where feasible, transfer boxes, chutes, tunnels and squeeze mechanisms for non-human primates housed in groups. Cages and other equipment should be free of sharp edges and corners that may cause scratches or wounds. When feasible, chemical restraint may be used before removing animals from cages, especially in the case of macaques and other larger non-human primates. Behavioural conditioning can also be effectively used in combination with restraint procedures. Handlers are to be protected with arm-length reinforced leather gloves and long-sleeved gowns/coveralls to prevent scratches. Protection against aerosol exposure and splashes of mucous membranes (e.g., with surgical mask, face shield, eye goggles) should be provided to handlers and everyone 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 laundry. Animal handlers must be instructed to cleanse immediately and thoroughly all bites, scratches and abraded skin and to report these exposures at once. Postexposure procedures should also be instituted[5,12].

Facilities for housing non-human primates should conform to the recommendations for small animal containment facilities in the *Containment Standards for Veterinary Facilities*[1]. 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[5,9]. The *Guide to the Care and Use of Experimental Animals* also provides information on housing and handling requirements specific to non-human primates[2]. Additional information on non-human primate facilities, equipment and special practices can be found elsewhere[13]. 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.

7.2 Recombinant DNA and Genetic Manipulation

Genetic methods, such as natural selection, cross breeding, conjugation and transformation, have been used for many years to change biological species and organisms. These methods have been supplemented by 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; and the production of full-length infectious viral clones, including the reconstruction of infectious virions from recombinant constructs (reverse genetic engineering).

The initial fear of possible risks arising from organisms altered by this technology led Canada, the United States and Great Britain, among other countries, to develop stringent biosafety guidelines. Experience rapidly showed that the initial fears

were not justified and that most recombinant DNA research in itself does not pose any specific risks to biological safety[14].

Guidance in how to assess potential risks in recombinant DNA research is available[15,16] but can only be very general. Factors to consider when determining the containment level of a recombinant organism should include:

1. the containment level of the recipient organism;
2. the containment level of the donor organism;
3. the replication competency of the recombinant organism;
4. the property of the donor protein to become incorporated into the recombinant particle; and
5. potential pathogenic factors associated with the donor protein.

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. In general, if none of the components of the genetic manipulation presents any known hazard and none can be reasonably foreseen to result from their combination, then no biohazard restrictions are needed. If one of the components of the reaction is hazardous, then, in general, discussion of the containment level required should start at the level appropriate to the known hazard. Its containment level might be increased or decreased according to such considerations as the particular gene being transferred; the expression of the gene in the recombinant organism; the biological containment offered by the host vector systems; the envisaged interactions between the gene being transferred and the host vector systems; and the viability of the host vector

systems. In any research with genes coding for hazardous products, host vector systems with limited ability to survive outside the laboratory should be used; their use will reduce the level of containment required.

Examples of such considerations follow:

1. A recombinant vesicular stomatitis pseudotype virus expressing a different viral glycoprotein would be at level 2 because the virus is replication-deficient.

2. A recombinant vesicular stomatitis virus expressing a different viral glycoprotein would be at least at the level of vesicular stomatitis virus since the virus is replication-competent and could have an altered tropism.

3. A recombinant vaccinia virus expressing a different viral glycoprotein would be at the containment level of wild type vaccinia virus since the protein does not get incorporated into the virus particle, and it is unlikely that this manipulation will change the biological properties of the recombinant virus.

7.3 Cell Lines

Cell lines (cell cultures) are commonly used in diagnostic and microbiology laboratories, and in industry for the production of pharmaceuticals. There have been cases of laboratory-acquired infections reported as a result of manipulation of primary cell cultures[17,18]. Although cell lines do not inherently pose a risk to individuals manipulating them in the laboratory[19], because of their potential to contain pathogenic organisms – either naturally or through contamination by adventitious agents, transformation or recombination – an assessment must be made as to the level of hazard associated with a particular line[20]. Cell lines can be contaminated with bacteria, fungi, mycoplasma, viruses and prions.

7.3.1 Risk Assessment

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[21]);
- 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.

7.3.2 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[20].

Viral contamination

Unlike bacteria and fungi, viruses are not readily identified and so can pose a significant hazard to those manipulating primary cell lines. A documented laboratory-acquired hantavirus infection has been linked to the manipulation of rat tumour material[22]. 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[23].

- *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[21].

- 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[20]. Cell lines can be grown in an altered manner by applying various treatments (e.g., change in pH, serum level, temperature, medium supplements, co-cultivation). These treatments may cause altered expression of oncogenes, expression of latent viruses, interactions between recombinant genomic segments or altered expression of cell surface proteins[21].

- 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* (Cercopithe- cine 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)[24,25].

- 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/lab/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[21] 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.

7.3.3 Self-self Experiments

Procedures or experiments with transformed human cells derived from the individual (human autologous) manipulating the cells is prohibited . Such experiments put the individual at risk, since any immune protection that is normally available to destroy foreign cells is now bypassed[20,21].

References

1. *Containment standards for veterinary facilities*. Ottawa, ON: Agriculture and Agri-Food Canada, Minister of Supply and Services Canada, No. 1921/E, 1996.
2. Canadian Council on Animal Care. *Guide to the care and use of experimental animals.* Ottawa, ON: CCAC, 1984.
3. National Research Council. *Occupational Health and Safety in the Care and Use of Research Animals*. Washington, DC: National Academy Press, 1997.
4. National Research Council. *Occupational Health and Safety in Biomedical Research*. ILAR Journal ISSN 1084-2020, 2003;44(1).
5. National Research Council. *Occupational Health and Safety in the Care and Use of Non-human Primates*. Washington, DC: The National Academies Press, 2003.
6. Hessler, J.R., Broderson, J.R., and King, C.S. *Small animal research facilities and equipment*. In: Richmond, J.Y., and McKinney, R.W. *Biosafety in microbiological and biomedical laboratories.* Washington, DC: U.S. Government Printing Office, 1999;191-218.
7. Phipatanakul, W., and Wood, R.A. *Allergens of animal and biological systems*. In: Fleming, D.O., and Hunt, D.L. *Biological safety principles and practices*. Washington, DC: ASM Press, 2000; 249-59.
8. Centers for Disease Control. *Fatal Cercopithecine herpesvirus 1 (B virus) infection following a mucocutaneous exposure and interim recommendations for worker protection*. MMWR 1998; 47:1073-6,1083.

9. Richmond, J.Y., and McKinney, R.W. *Biosafety in microbiological and biomedical laboratories*. Washington, DC: U.S. Government Printing Office, 1999.

10. Centers for Disease Control. *Guidelines for the prevention of herpesviurs simiae (B virus) infection in monkey handlers*. MMWR 1987;36:680-2, 687-9.

11. Centers for Disease Control. *Update: Ebola-related filovirus infection in non-human primates and interim guidelines for handling non-human primates during transit and quarantine*. MMWR 1990;39(2):22-24, 29-30.

12. Holmes, G.P., Chapman, L.E., Stewart, J.A., et al. *Guidelines for the prevention and treatment of B-virus infections in exposed persons.* Clin Infect Dis 1995; 20:421-39.

13. Bennet, B.T., Abee, C.R., and Henrickson, R. *Non-human primates in biomedical research*. San Diego, CA: Academic Press, 1995.

14. Health Canada. *Laboratory biosafety guidelines*, 2nd edition. Ottawa: Minister of Supply and Services Canada, 1996.

15. National Institutes of Health. *Guidelines for research involving recombinant DNA molecules.* Federal Register (with subsequent amendments) 59 Federal Register 34496, July 5, 1995.

16. National Institutes of Health. *NIH guidelines for research involving recombinant DNA molecules.* 66 Federal Register 1146, January 2001.

17. Davidson,W.L., and Hummeler, K. *B virus infection in man.* Ann NY Acad Sci 1961;85:970-79.

18. Gandsman, E.J., Aaslestad, H.G., Ouimet, T.C., and Rupp, W.D. *Sabia virus incident at Yale University*, American Ind Hyg Assoc J, 1997;58(1):51-3.

19. National Research Council. *Safe handling of infectious agents*. In: *Biosafety in the laboratory: prudent practices for the handling and disposal of infectious materials.* Washington, DC: National Academy Press, 1989;13-33.

20. Frommer, W. *Safe biotechnology (5): recommendations for safe work with animal and human cell cultures concerning potential human pathogens*. Appl Microbiol Biotech 1993;39:141-7.

21. Doblhoff-Dier, O., and Stacey, G. *Cell lines: applications and biosafety*. In: Fleming, D.O., and Hunt, D.L. *Biological safety principles and practices*. Washington, DC: ASM Press, 2000;221-39.

22. Lloyd, G., and Jones, N. *Infection of laboratory workers with hantavirus acquired from immunocytomas propagated in laboratory rats*. J Infect 1986;12:117-25.

23. World Health Organization. *Laboratory biosafety manual*. Geneva: WHO, 1993.

24. Griffith, J.S. *Self-replication and scrapie*. Nature 1967; 215 (105): 1043-44.

25. Prusiner, S.B. *Novel proteinaceous infectious particles cause scrapie*. Science 1982;1365-144.

Chapter 8
Decontamination

8.1 Introduction

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). A list of various decontaminants, their effectiveness against different microbial groups, their important characteristics and their most appropriate application in research and clinical laboratories have been amply summarized by others[1-4]. 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. Employee infection with *M. tuberculosis* as a result of exposure to contaminated waste has been documented[5]. Reports of infections with *Coxiella burnetii* in commercial laundry workers, presumably due to improper decontamination of laboratory coats and gowns prior to laundering, also exist in the literature[6,7]. Employees should be responsible for leaving their laboratory clothing for laundering in a designated spot or area.

Most Canadian jurisdictions have prepared or are preparing guidelines or regulations concerning the management of biomedical waste. The treatment procedures used at each laboratory are subject to the standards in place for that province or territory. The Canadian Council of Ministers of the Environment (CCME) has also developed minimum national

guidelines for defining, handling, treating and disposing of biomedical wastes[8]. The intent of the guidelines is to promote uniform practices and set minimum national standards for managing biomedical waste in Canada. Provincial, territorial and municipal regulatory authorities should also be consulted as they may specify more stringent requirements.

As mentioned in the operational requirements elsewhere in this document, all contaminated materials must be decontaminated before disposal or cleaning for reuse. 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 must be developed and followed for each process. 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.

8.2 Autoclaves

Infectious laboratory wastes (petri dishes, pipettes, culture tubes, glassware, etc.) can be effectively decontaminated in either a gravity displacement or prevacuum autoclave. Prevacuum autoclaves remove air from the chamber by pulling a vacuum (except for liquid cycles) before the saturated steam enters the autoclave chamber, resolving problems with air entrapment during removal of air by gravity displacement. The effectiveness of decontamination by steam autoclaving depends upon various loading factors that influence the temperature to which the material is subjected and the contact time. Particular attention must be given to packaging, including the size of containers and their

distribution in the autoclave. Containers of waste must allow steam penetration and must be arranged in the autoclave in a manner that permits free circulation of steam. Tight-fitting containers do not permit steam penetration. Piling containers above one another and overloading can result in decontamination failure.

Effective operating parameters for autoclaves should be established by developing standard loads and their processing times through the use of thermocouples and biological indicators placed at the centre of the load (i.e., the most difficult areas of the load to decontaminate). Biological indicators are also used for routine monitoring (e.g., weekly, based on the frequency of use) of the sterilization process. A biological indicator is a standardized population of bacterial spores intended to demonstrate favourable sterilization conditions in the load. Attention must be paid to appropriate selection of the indicator, as the design and construction vary depending on its intended use (e.g., liquid versus dry load, self-contained system, enzyme-based rapid method). Chemical indicators are meant to be used in conjunction with biological indicators and physical monitors (i.e., pressure and temperature readings). They provide instant results for day-to-day monitoring that the load has been processed; however, they must not be used as the sole indicator of sterility. Favero presents a comprehensive overview of the types of biological and chemical/physical indicators and their recommended use[9]. The results of biological indicator testing should be kept on file.

8.3 Chemical Disinfection

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[2,3]. Mycobacteria and nonenveloped viruses are less susceptible; bacterial spores and protozoan cysts are generally the most resistant[2-4]. Consideration should also be given to practicability, stability, compatibility with materials and health hazards[1].

There are usually striking differences between the activity of disinfectants when employed under actual laboratory conditions as opposed to the controlled, standardized testing methods used to generate efficacy data for product registration. A review of the official protocols used to assess disinfectant activity is under way[10]. 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[4]; temperature; relative humidity; concentration; and contact time[2,4]. 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[11]. 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[11].

Selection of an appropriate disinfectant can become a dismaying task given the numerous products on the market. There are increasing numbers of disinfectants being developed and new disinfectant options being explored[12]. However, 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.

8.4 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. The recommended protocol involves the depolymerization of paraformaldehyde into a well-sealed room to provide a concentration in air of 10.6 g/m^3 (0.3 g/ft^3)[1]. After a contact time of at least 6 hours, the formaldehyde is neutralized with ammonium carbonate (using 1.1 times the weight of formaldehyde) before venting and aeration[1]. The room and surrounding space should be monitored for airborne levels of formaldehyde, and only when levels are below the exposure limits can the area be considered safe for re-entry without protective clothing. Successful gaseous decontamination requires an ambient temperature of at least 21° C and a relative humidity of 70%[1]. Biological indicators should be used to monitor the effectiveness of the gaseous decontamination procedure[13].

Vaporized hydrogen peroxide has been proposed as a safer alternative to gaseous decontamination with formaldehyde. In the sterilization process, 30% liquid hydrogen peroxide is vaporized to yield approximately 1200 ppm. The vapour breaks down into nontoxic oxygen and water. Vaporized hydrogen peroxide has been successfully used as a nondestructive sterilant for the decontamination and removal of laboratory equipment and materials (e.g., telephone, camera, computer, pipette, electric drill) from a containment laboratory[14]. Future commercial applications may address today's constraints associated with the cumbersome size of the vaporized hydrogen peroxide generator and limitations in the

space capable of being decontaminated (e.g., pass-through boxes, small rooms).

8.5 Liquid Effluent Treatment Systems

Liquid effluent treatment systems are used in containment level 4 laboratories (and containment level 3 laboratories handling nonindigenous animal pathogens) for decontaminating liquid waste streams from sinks, showers, autoclave chambers and other drains. These systems represent a secondary treatment system, as no infectious microorganisms are disposed of directly into the drain without prior treatment (i.e., the addition of chemical disinfectants). The decontamination parameters (i.e., time and temperature for heat-based systems) must be defined and must be effective against the microorganisms of concern. The internal temperature and pressure of the effluent tanks and the decontamination time should be logged throughout the cycle. Chemical-based decontamination systems may be practical on a small scale where smaller volumes of liquid effluent require treatment. Decontaminated liquids released from the treatment system must meet all applicable regulations (e.g., municipal bylaws for temperature, chemical/metal content, suspended solids, oil/grease and biochemical oxygen demand).

8.6 Irradiation

Gamma irradiation (e.g., ^{60}Co) 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[2].

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[15,16].

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[1]. 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.

8.7 Incineration

Incineration has traditionally been the chosen method for processing anatomical biomedical waste and animal carcasses. In most cases, wastes to be incinerated have to be packaged and transported off-site in accordance with provincial or territorial legislation. Materials removed from containment laboratories for off-site incineration should initially be treated at the containment barrier, preferably by autoclaving. Effective incineration depends on proper equipment design; on provision for the time, temperature, turbulence and air required for complete oxidation; and on careful feeding of the unit. Modern incinerators have two chambers with an ideal temperature in the primary chamber of at least 800° C and in the secondary chamber of at least 1000° C[2,15]. Loads with high moisture content may lower the processing temperature. There are no microbial standards for stack discharge, but there are for emission of particulate matter and selected chemical contaminants[8]. Provincial or territorial regulatory authorities should be consulted for additional requirements regarding incinerator operations and emissions.

8.8 New Technologies

Growing concern with air pollution has caused many regulatory agencies to introduce more stringent standards for incinerators with a resultant explosion in alternative waste treatment systems. Most of these systems use one or more of the following methods: heating by means of microwaves, radio waves, hot oil, hot water, steam or superheated gases; exposure to chemicals such as hypochlorite, chlorine dioxide or sodium hydroxide; subjecting the waste to heated chemicals; and exposing the medical waste to irradiation sources[15,16]. A description of many of these technologies and their advantages and disadvantages have been previously summarized[15,16]. A modified rendering process has been shown to be an effective alternative and has been successfully used to decontaminate infected animal carcasses[17]. New technologies are subject to the approval of provincial or territorial regulatory authorities, and laboratories should consult these authorities before purchasing products or implementing new approaches to decontamination.

References

1. Vesley, D., Lauer, J.L., and Hawley, R.J. *Decontamination, sterilization, disinfection and antisepsis.* In: Fleming, D.O., and Hunt, D.L. *Biological safety principles and practices.* Washington, DC: ASM Press, 2000;383-402.

2. Dychdala, G.R., Gottardi, W., Block, S.S., Wickramanayake, G.B., Larson, E.L., Morton, H.E., O'Connor, D.O., Rubino, J.R., Merianos, J.J., Lopes, J.A., Denton, G.W., Rossmoore, H.W., May, O.W., Opperman, R.A., Gitlitz, M.H., Beiter, C.B., and Yeager, C.C. *Part III: disinfectants and antiseptics (section A: by chemical type)*. In: Block, S.S. *Disinfection, sterilization and preservation.* Malvern, PA: Lea & Febiger, 1991;131-364.

3. Hugo, W.B., and Russell, A.D. *Types of antimicrobial agents*. In: Russell, A.D., Hugo, W.B., and Ayliffe, GA.J. *Disinfection, preservation and sterilization*. Oxford, UK: Blackwell Science Ltd, 1999;5-94.

4. Russell, A.D. *Factors influencing the efficacy of antimicrobial agents*. In: Russell, A.D., Hugo, W.B., and Ayliffe, GA. J. *Disinfection, preservation and sterilization*. Oxford, UK: Blackwell Science Ltd., 1999;95-123.

5. Weber, A.M., Boudreau, U.V., and Mortimer, V.D. *A tuberculosis outbreak among medical waste workers.* J Am Biol Safety Assoc 2000; 2:570-88.

6. Collins, C.H., and Kennedy, D.A. *Laboratory-acquired infections.* In: *Laboratory-acquired infections: history, incidence, causes and preventions*. Oxford, UK Butterworth-Heinemann, 1999;1-37.

7. Richmond, J.Y., and McKinney, R.W. *Biosafety in microbiological and biomedical laboratories*. Washington, DC: U.S. Government Printing Office, 1999.

8. Canadian Council of Ministers of the Environment. *Guidelines for the management of biomedical waste in Canada.* Toronto, ON: Canadian Standards Association. CCME EPC-WM-42E, 1992.

9. Favero, M.S. *Developing indicators for monitoring sterilization*. In: Rutala, W.A. *Disinfection, sterilization and antisepsis in health care*. Washington, DC: Association for Professionals in Infection Control and Epidemiology Inc., 1998;119-32.

10. Sattar, S.A. *Microbicidal testing of germicides: an update*. In: Rutala, W.A. *Disinfection, sterilization and antisepsis in health care*. Washington, D.C.: Association for Professionals in Infection Control and Epidemiology Inc., 1998;225-240.

11. Best, M., Sattar, S.A., Springthorpe, V.S., and Kennedy, M.E. *Efficacies of selected disinfectants against Mycobacterium tuberculosis*. J Clin Microbiol 1990;28:2234-39.

12. Springthorpe, M.S. *New chemical germicides*. In: Rutala, W.A. *Disinfection, sterilization and antisepsis in health care*. Washington, DC: Association for Professionals in Infection Control and Epidemiology Inc., 1998;273-80.

13. Abraham, G., Leblanc, P.M., Smith, P.M., and Nguyen, S. *The effectiveness of gaseous formaldehyde decontamination assessed by biological monitors*. J Am Biol Safety Assoc 1997;2:30-38.

14. Best, M. *Efficacy of vaporized hydrogen peroxide against exotic animal viruses*. Appl Environ Microbiol 1997;63:3916-18.

15. Salkin, I.F., Krisiunas, E., and Thumber, W.L. *Medical and infectious waste management*. In: Richmond, J.Y. *Anthology of biosafety II: facility design considerations*. Mundelein, IL: American Biological Safety Association, 2000;140-60.

16. Turnber, W.L. *Biohazardous waste: risk assessment, policy and management*. New York, NY: John Wiley & Sons Inc., 1996.

17. Thompson, L., Best, M., and Langevin, P. *Biological efficacy testing of liquid effluent and tissue/carcass sterilization systems.* Mundelein, IL: American Biological Safety Association, 1998.

Chapter 9
Biological Safety Cabinets

9.1 Introduction

When properly maintained and used in conjunction with good laboratory techniques, BSCs provide effective primary containment for work with human pathogens. In containment level 2 facilities, BSCs are used for procedures with the potential to produce infectious aerosols and for high concentrations or large volumes of infectious material. In containment levels 3 and 4, all open vessel activities with infectious materials are conducted in a BSC. Every employee working in a BSC must be trained in its correct use and have a good understanding of the different types of cabinets and how they work. Detailed information on the selection, function, and use of BSCs can be found elsewhere in the literature[1-3].

9.2 Classes and Characteristics of 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 seal should be purchased[4].

Class I Cabinets (Figures 1a and 1b)

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.

Class II Cabinets (Figures 2-5)

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[4].

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 (Figure 2)

- 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 (75 ft/min).
- May have positive pressure contaminated ducts and plenums.
- Are not suitable for work with low levels of volatile toxic chemicals and volatile radionuclides[4].

Class II, Type A2 Cabinets (Figure 3)

- 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 B1 Cabinets (Figure 4)

- 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 B2 Cabinets (Figure 5)

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

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

Class III Cabinets (Figure 6)

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 level 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. Specific guidance on the unique requirements for constructing, installing, certifying and using Class III cabinet lines can be found elsewhere[5-7]. 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.

9.3 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*[8]. 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.

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[1]. 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 or annex F of NSF 49. 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. On-site field testing must be performed by experienced qualified individuals. The NSF accreditation program for BSC certifiers provides a list of individuals who have demonstrated their competence by means of written and practical examinations administered by the NSF[9]. Whenever possible, it is recommended that NSF-accredited field certifiers be used.

9.4 Use of the Cabinet

Follow these **start-up procedures** when preparing for work in the BSC:

1. Turn off UV lights if in use and ensure that the sash is in the appropriate position.
2. Turn on fluorescent light and cabinet blower, if off.
3. Check the air intake and exhaust grilles for obstructions.
4. If the cabinet is equipped with an alarm, test the alarm and switch it to the "on" position.
5. Confirm inward airflow by holding a tissue at the middle of the edge of the viewing panel and ensuring that it is drawn in.
6. Disinfect the interior surfaces with a suitable, noncorrosive 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.

Follow these procedures for **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).

Follow these procedures upon **completion of the work**:

1. Allow the cabinet to run for 5 minutes with no activity.
2. Close or cover open containers before removing them from the cabinet.
3. Surface 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); UV must be tested to ensure that it is emitting a germicidal wavelength (ask your cabinet certifier to perform this test).

References

1. Centers for Disease Control and Prevention. *Primary containment for biohazards: selection, installation and use of biological safety cabinets*. Washington, DC: U.S. Government Printing Office, 2000.

2. Kruse, R.H., Puckett, W.H., and Richardson, J.H. *Biological safety cabinetry*. Clin Microbiol Rev 1991;4:207-41.

3. Stuart, D.G. *Primary barriers: biological safety cabinets, fume hoods, and glove boxes*. In: Fleming, D.O., and Hunt, D.L. *Biological safety principles and practices*. Washington, DC: ASM Press, 2000;313-30.

4. NSF International. *Class II (laminar flow) biohazard cabinetry. Standard 49*. Ann Arbor, Michiganj: NSF International, 2002.

5. Stuart, D.G., Hilliard , J., Kenkel, R., Kelley, J., and Richmond, J. *Role of the class III cabinet in achieving BSL-4.* In: Richmond, J.Y. *Anthology of biosafety I: perspective on laboratory design.* Mundelein, IL: American Biological Safety Association, 1999;149-60.

6. National Cancer Institute Office of Research Safety and the Special Committee of Safety and Health Experts. *Laboratory safety monograph, a supplement to NIH guidelines for recombinant DNA research*. Bethesda, MD: NIH, 1979.

7. *Biotechnology – performance criteria for microbiological safety cabinets*. BS EN 12469:2000. European Committee for Standardization (CEN), 2000.

8. *Biological containment cabinets (class I and II): installation and field testing*. Z316.3-95. Canadian Standards Association, Toronto, ON, 1995.

9. NSF International. *NSF listings — field certifier accreditation*. Ann Arbor, Michigan: NSF International, 2000.

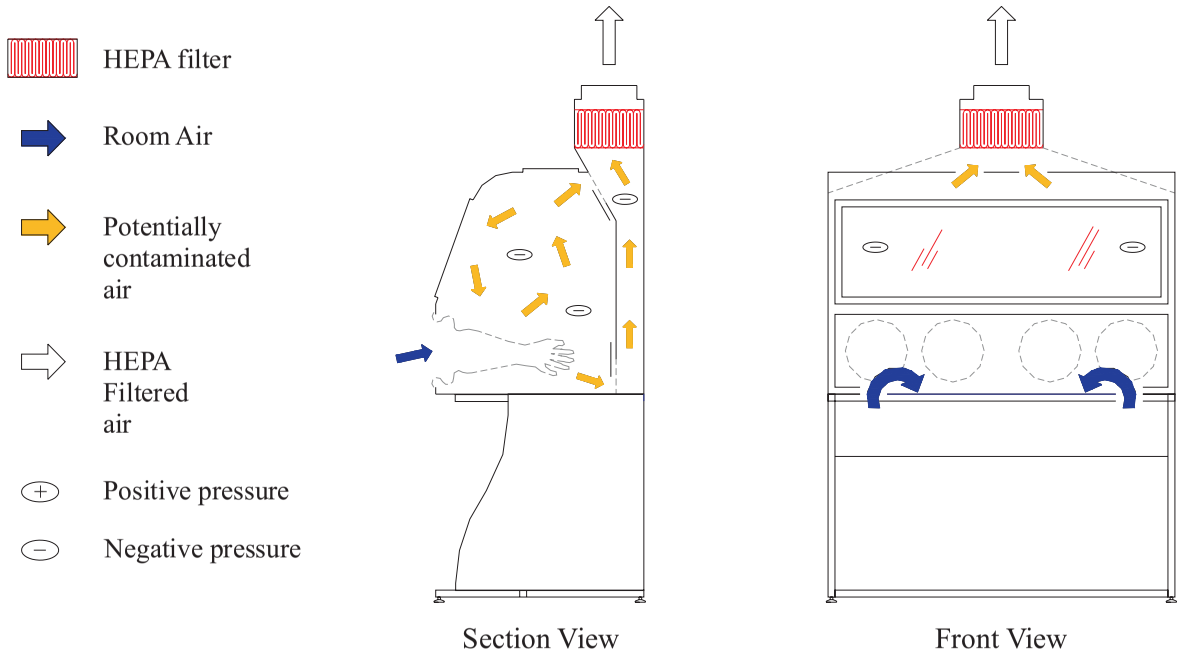

Fig. 1a. CLASS I BIOLOGICAL SAFETY CABINET

(Used in conjunction with the building system. Glove ports are optional.)

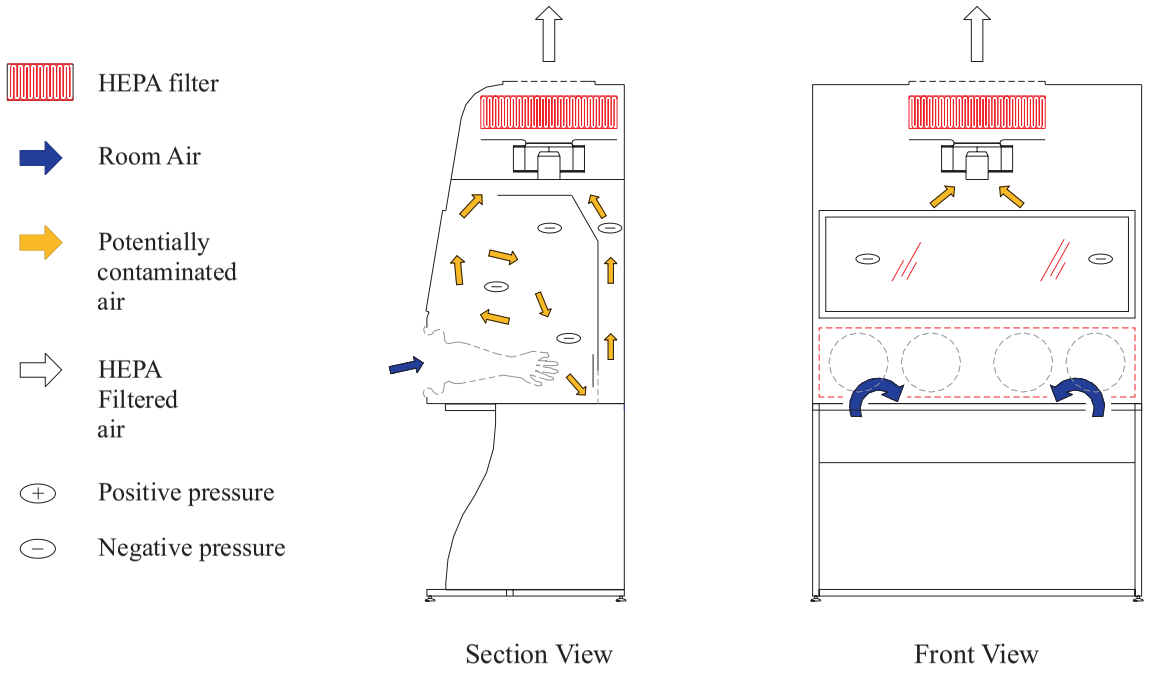

Fig. 1b. CLASS I BIOLOGICAL SAFETY CABINET

(Complete with internal motor/blower assembly. HEPA filtered exhaust air vented to the atmosphere. Glove ports are optional.)

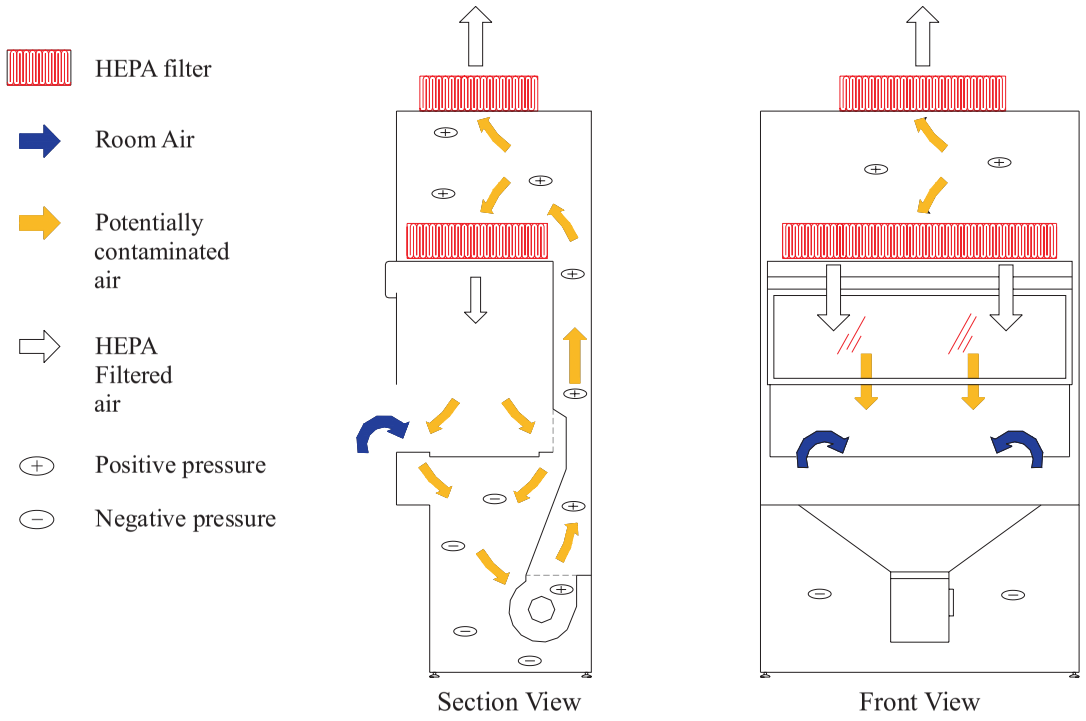

Fig. 2. CLASS II TYPE A1 BIOLOGICAL SAFETY CABINET

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

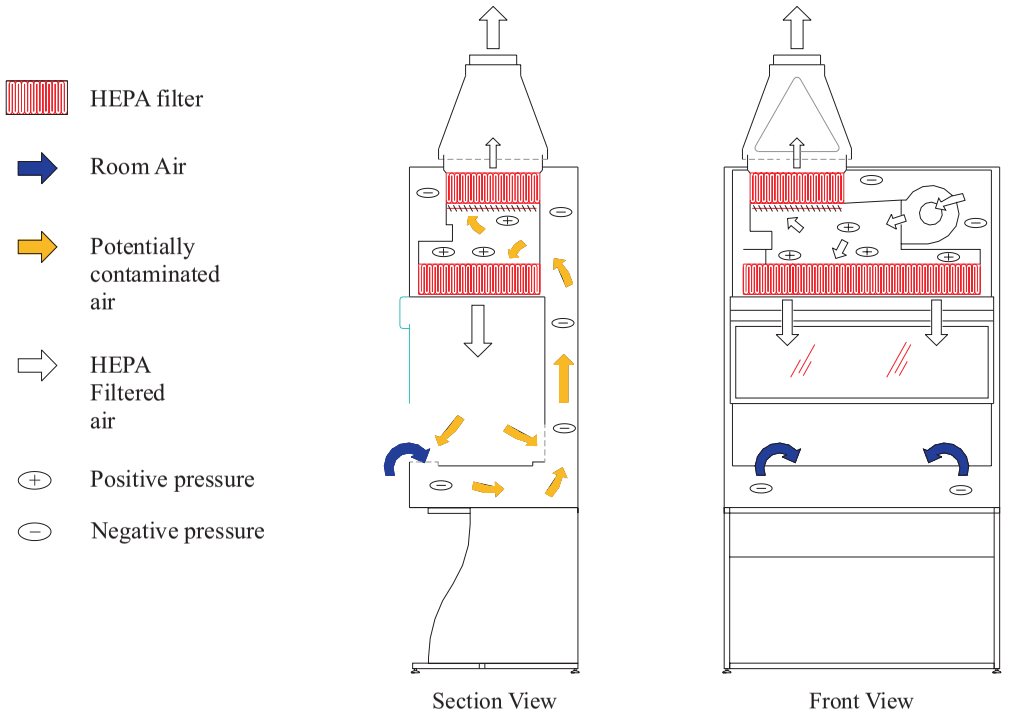

Fig. 3. CLASS II TYPE A2 BIOLOGICAL SAFETY CABINET

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

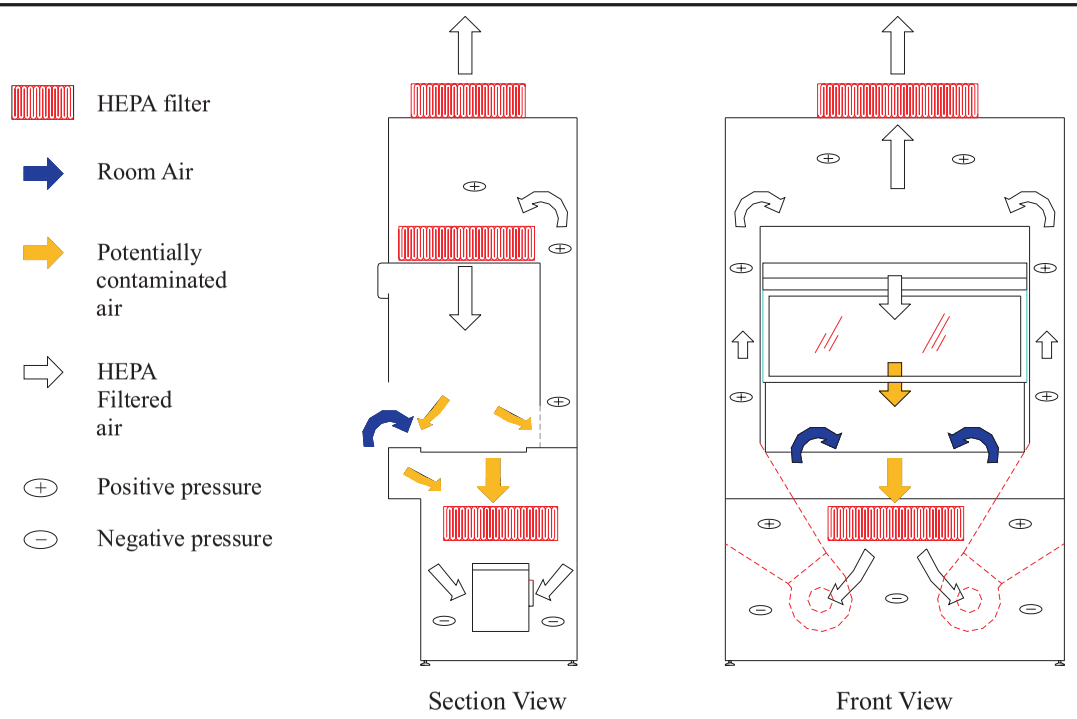

Fig. 4. CLASS II TYPE B1 BIOLOGICAL SAFETY CABINET

(Hard-ducted.)

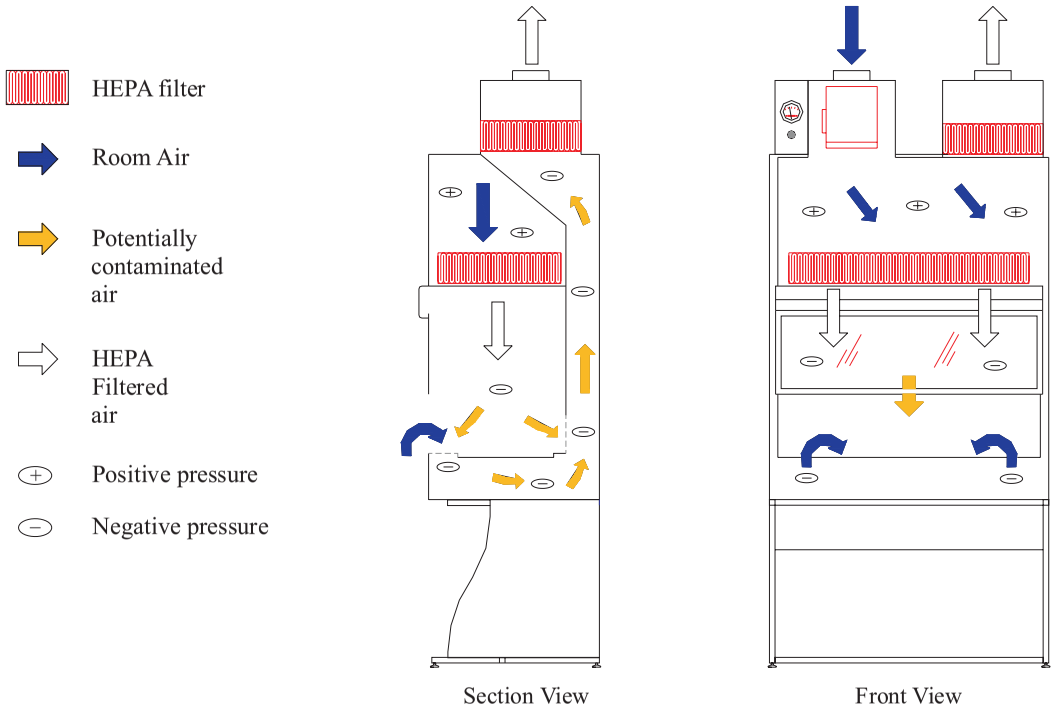

Fig. 5. CLASS II TYPE B2 BIOLOGICAL SAFETY CABINET

(Hard-ducted.)

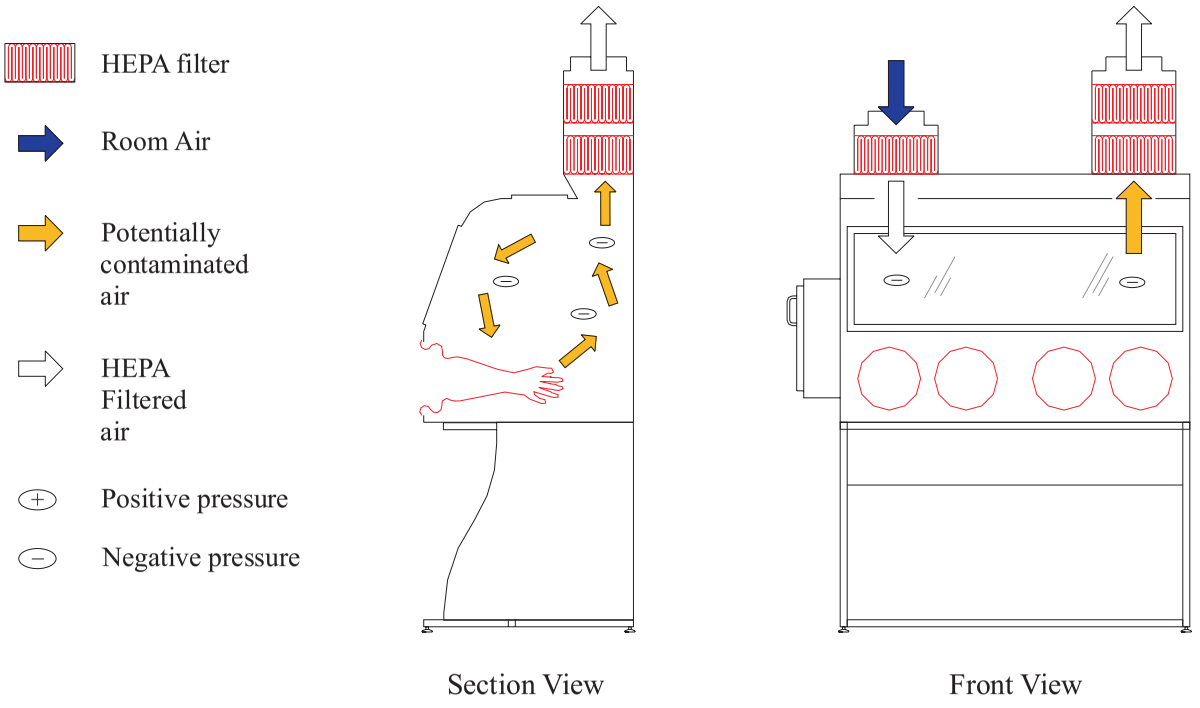

Fig. 6. CLASS III BIOLOGICAL SAFETY CABINET

(Hard-ducted.)

Chapter 10
Regulatory Aspects for Handling Infectious Substances

10.1 Importation and Transfer of Human Pathogens

The *Human Pathogens Importation Regulations* (SOR/94-558) (HPIR) are the regulatory authority for facilities wishing to import human pathogens into and transfer specimens within Canada. These regulations were developed to ensure that facilities have appropriate containment for the pathogens they wish to handle. Any facility wishing to import a human pathogen requiring containment levels 2, 3 or 4 must have a valid Health Canada permit before importation. Pathogens requiring containment level 1 facilities are not regulated by the HPIR, and therefore a permit is not required for their importation. Applications for permits to import human pathogens can be obtained either by calling the Office of Laboratory Security directly at (613) 957-1779 or by downloading the application form from the Office of Laboratory Security's Website at http://www.phac-aspc.gc.ca/ols-bsl/

Similarly, a copy of the HPIR and frequently asked questions about the importation process can also be accessed at the Office of Laboratory Security's Website.

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 these *Guidelines.* For facilities wishing to import pathogens requiring containment levels 3 and 4, Health Canada certification that the laboratory meets the requirements of the *Laboratory Biosafety Guidelines* is required before a permit is issued. The requirements for certification of a containment laboratory are detailed in Chapter 5 . Facilities wishing to import pathogens requiring containment level 2 are

to perform a self-inspection to ensure that the facility meets the *Laboratory Biosafety Guidelines'* requirements and the self-inspection is subject to verification by Health Canada inspectors at any time. Additionally, the Office of Laboratory Security's Website (given above) hosts a Risk Group listing of human pathogens, detailing the recommended Risk Group for each pathogen.

Many human pathogens are pathogens of animals as well. Animal pathogens are regulated by the Canadian Food Inspection Agency (CFIA) (see section 10.4, Importation of Animal Pathogens, in this chapter). For importation of pathogens that are common to both animals and humans, an import permit is required from the CFIA as well as Health Canada. 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.

10.2 Export of Pathogens

Many pathogens and associated equipment that are destined for export from Canada require permits. Canada is a signatory to the 1972 Biological and Toxin Weapons Convention. This international Convention stresses the goal of non-proliferation of biological and toxin weapons through the prohibition of the development, production, stockpiling or acquisition of microbiological (biological) and toxin weapons and their destruction. The Department of Foreign Affairs and International Trade Canada currently controls certain toxicological and biological agents as well as their related equipment, components, materials and technology, under item 2007 of the Export Control List of this international Convention. 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/

10.3 Transportation

The transportation of infectious substances is an essential part of routine laboratory procedures in both research and diagnostic settings. Samples must be transported by road and/or air to assist researchers collaborating with other researchers at removed locations, or to carry out primary diagnostic tests on samples obtained from ill patients. Although there has never been a reported case of illness associated with a transportation accident involving an infectious substance[1], transportation accidents involving infectious substances do occur[2]. Therefore, it is important that infectious substances be packaged and transported according to tested and approved methods.

The transportation of infectious substances within Canada is regulated by the *Transportation of Dangerous Goods Regulations* (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). Additionally, shippers of risk group 4 materials are required to have an emergency response assistance plan to respond to any shipping emergency occurring anywhere in Canada. More information regarding the transportation of infectious substances within Canada can be obtained by calling Transport Canada, Dangerous Goods Standards, at (613) 990-1059, by writing to them at Place de Ville, Tower C, 330 Sparks St., 4th Floor, Ottawa ON K1A 0N8, or by visiting the Transport Canada Dangerous Goods Website at
http://www.tc.gc.ca/civilaviation/commerce/dangerousgoods/

The air transportation of infectious substances internationally is regulated by the International Civil Aviation Organization (ICAO)[3]. 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: Judith Code, Chief, Dangerous Goods Standards, Commercial and Business Aviation, Transport Canada, at (613) 990-1060 (mailing address as indicated for Transport Canada above).

Shipping infectious substances by air also falls under the *Dangerous Goods Regulations* (DGR) of the International Air Transport Association (IATA)[4]. 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/

10.4 Importation, Transfer and Containment of Animal Pathogens

The *Health of Animals Act* ,1990, and the *Health of Animal Regulations* give the 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. Please refer to the *Health of Animals Act* and the Regulations for complete information.

Permits are required for the importation of all animal pathogens into Canada. In the case of pathogens that affect both humans and animals, import permits are required from both Health Canada and the CFIA. If an agent is brought into Canada under an import permit that restricts its distribution,

further approval must be obtained from the CFIA before transferring the agent to another location.

The CFIA also establishes the conditions under which animal pathogens will be maintained and 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. The CFIA publication *Containment Standards for Veterinary Facilities*[5] 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 that are considered nonindigenous to Canada form a portion of this database and are severely restricted. 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

References

1. WHO. *Guidelines for the safe transport of infectious substances*. Geneva: Division of Emerging and Other Communicable Diseases Surveillance and Control, WHO/EMC/97.3, 1997.
2. Transport Canada. *1999 statistics*. Canadian Transport Emergency Centre, 2000.
3. International Civil Aviation Organization. *Technical instructions for the safe transport of dangerous goods by air. 2001-2002.* Doc 9284-AN/905, 2000.
4. International Air Transport Association. *Dangerous goods regulations.* 43rd edition. January 2003.
5. *Containment standards for veterinary facilities*. Ottawa: Agriculture and Agri-Food Canada, Minister of Supply ans Services Canada, No. 1921/E, 1996.

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