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CANADIAN BIOSAFETY HANDBOOK

Second Edition

Canada

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Public Health Agency of Canada
100 Colonnade Road
Ottawa, ON K1A 0K9
Tel.: 613-957-1779
Fax.: 613-941-0596
PHAC email: PHAC.standards-normes.ASPC@canada.ca
CFIA email: standardsnormes@inspection.gc.ca

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TABLE OF CONTENTS

PREFACE ix

ABBREVIATIONS AND ACRONYMS. xi

CHAPTER 1 – INTRODUCTION 1

1.1 Scope. 2
1.2 Overview 2
1.3 The *Canadian Biosafety Standard* 3
1.4 How to Use the *Canadian Biosafety Handbook*. 4

CHAPTER 2 – BIOLOGICAL MATERIAL 5

2.1 Bacteria 6
2.2 Viruses 7
2.3 Fungi 7
2.4 Parasites. 7
2.5 Prions. 8
2.6 Zoonotic Pathogens. 8
2.7 Toxins. 9
2.8 Biotechnology. 10
2.9 Cell Lines and Cell Cultures 11

CHAPTER 3 – CONTAINMENT LEVELS AND CONTAINMENT ZONES 15

3.1 Containment Levels 16
3.2 Containment Zones 21
3.3 Identifying and Accessing a Containment Zone 22

CHAPTER 4 – RISK FACTORS, RISK GROUPS, AND RISK ASSESSMENTS 35

4.1 Pathogen and Toxin Risk Assessments and Risk Groups. . . 36
4.2 Containment Assessments. 39
4.3 Special Considerations 40
4.4 Risk Management 50

CHAPTER 5 – BIOSAFETY PROGRAM MANAGEMENT . . . 57

5.1 Administrative Controls 58
5.2 Risk Assessments and Planning 62
5.3 Implementation of a Biosafety Program 63
5.4 Measuring Program Effectiveness 66
5.5 Continuous Improvement of the Program 68

CHAPTER 6 – BIOSECURITY 71

6.1 Biosecurity Risk Assessment 72
6.2 Biosecurity Plans 75
6.3 *Human Pathogen and Toxin Act* Security Clearances . . . 78

CHAPTER 7 – MEDICAL SURVEILLANCE PROGRAM 83

7.1 Laboratory Exposures and Laboratory Acquired Infections/Intoxications 84
7.2 Pre-Placement Medical Evaluation 86
7.3 Vaccinations 87
7.4 Ongoing Medical Surveillance 87
7.5 Post-Exposure Response Plan 88
7.6 Additional Considerations for High Containment 88
7.7 Emergency Medical Contact Card 89

CHAPTER 8 – TRAINING PROGRAM 93

8.1 Training Needs and Objectives 94
8.2 Training Program Content 94
8.3 Identification of Trainees 97
8.4 Training Evaluation 98
8.5 Training Records 99
8.6 Training Program Review 99

CHAPTER 9 – PERSONAL PROTECTIVE EQUIPMENT 101

9.1 Types and Selection of Personal Protective Equipment . . . 102
9.2 Key Considerations for the Selection of Personal Protective Equipment 108
9.3 Use of Personal Protective Equipment 109

CHAPTER 10 – AIR HANDLING 117

10.1 Inward Directional Airflow 118
10.2 High Efficiency Particulate Air Filters 121

CHAPTER 11 – BIOLOGICAL SAFETY CABINETS 125

11.1 Classes and Descriptions 126
11.2 Installation of BSCs 139
11.3 Testing and Certification. 139
11.4 Proper Use 140

CHAPTER 12 – SAFETY CONSIDERATIONS FOR EQUIPMENT USED FOR BIOLOGICAL WORK 145

12.1 Centrifuges 146
12.2 Microtomes. 147
12.3 Blenders, Sonicators, Homogenizers, Shaking Incubators, and Mixers 147
12.4 Bunsen Burners 147
12.5 Microincinerators 148
12.6 Disposable Loops 148
12.7 Pipetting Aids 148
12.8 Vacuum Pumps and Systems. 149
12.9 Chemical Fume Hoods 151
12.10 Pass-Through Chambers 151
12.11 Cell Sorters. 152
12.12 Compressed Gas Cylinders 152
12.13 Additional Equipment Considerations for Prions. 152
12.14 Additional Equipment Considerations for Toxins 153

CHAPTER 13 – ANIMAL WORK CONSIDERATIONS . . . 155

13.1 Animal Characteristics 157
13.2 Animal Containment Zone Designs 158
13.3 Equipment. 168
13.4 Personnel Training 169
13.5 Handling and Restraint 169
13.6 Decontamination and Waste Management 170
13.7 Confinement 171
13.8 Special Considerations for Work with Prion-Infected Animals 172
13.9 Working with Non-Human Primates 173

CHAPTER 14 – LARGE SCALE WORK 177

14.1 Scope 178
14.2 Considerations for Large Scale Work 178
14.3 Fermenters 179
14.4 Regulatory Considerations 179

CHAPTER 15 – DECONTAMINATION 181

15.1 Principles of Sterilization, Disinfection, and Decontamination 182
15.2 Validation and Verification of Decontamination Technologies and Processes 183
15.3 Chemical Disinfectants 185
15.4 Autoclaves 193
15.5 Gaseous Decontamination 196
15.6 Effluent Decontamination Systems 198
15.7 Irradiation 199
15.8 Incineration 200
15.9 Dunk Tanks 200
15.10 Animal Carcasses and Anatomical Waste 201
15.11 Thermal and Chemical Decontamination of Biological Toxins 201
15.12 Additional Considerations for Prion Decontamination . . . 203

CHAPTER 16 – WASTE MANAGEMENT 207

16.1 Biomedical Waste 209
16.2 Storage and Disposal of Biomedical Waste 210

CHAPTER 17 – EMERGENCY RESPONSE PLAN 213

17.1 Emergency Response Plan Development 214
17.2 Emergency Response Plan Implementation 215
17.3 Spill Response 216

CHAPTER 18 – INCIDENT REPORTING AND INVESTIGATION 221

18.1 Incident Reporting 224
18.2 Incident Investigation 229

CHAPTER 19 – PATHOGEN AND TOXIN ACCOUNTABILITY AND INVENTORY CONTROL 233

19.1 Pathogen and Toxin Accountability 234
19.2 Inventories and Inventory Control Systems 237
19.3 Storage and Labelling 239

CHAPTER 20 – MOVEMENT AND TRANSPORTATION OF INFECTIOUS MATERIAL OR TOXINS . . . 241

20.1 Movement of Infectious Material or Toxins 242
20.2 Transportation of Infectious Material or Toxins 243

CHAPTER 21 – WORKING WITH RISK GROUP 1 BIOLOGICAL MATERIAL 247

21.1 Physical Design Considerations. 248
21.2 Operational Practice Considerations. 249
21.3 Routine Practices and Universal Precautions 251

CHAPTER 22 – DESIGN CONSIDERATIONS FOR NEW CONTAINMENT ZONES 253

22.1 Planning 254
22.2 General Building Layout 255
22.3 Building Mechanical Systems 257
22.4 Decontamination Technologies 260
22.5 Building Physical Components 262

CHAPTER 23 – REGULATORY OVERSIGHT OF HUMAN AND ANIMAL PATHOGENS AND TOXINS IN CANADA 268

23.1 Regulatory Authorities 268
23.2 Controlled Activities with Human Pathogens and Toxins 269
23.3 Importation of Animal Pathogens into Canada 273
23.4 Activities with Zoonotic Pathogens 276
23.5 Additional Regulatory Considerations for Pathogens and Toxins 276

CHAPTER 24 – GLOSSARY 281

CHAPTER 25 – RESOURCES 305

25.1 General Resources 306
25.2 Technical Standards and Codes 326
25.3 Website Addresses 330
25.4 Government of Canada Legislation 331
25.5 Other Applicable International Regulations 332

APPENDIX A – PLAN FOR ADMINISTRATIVE OVERSIGHT FOR PATHOGENS AND TOXINS IN A RESEARCH SETTING 334

APPENDIX B – PROPER HANDWASHING TECHNIQUE . . 337

INDEX 342

LIST OF FIGURES

Figure 3-1: Representative Diagram of a Mixed-use Facility Containing Multiple Containment Level 2 (CL2) and Containment Level 3 (CL3) Zones. 18

Figure 3-2: Representative Diagram of a Containment Level 4 (CL4) Zone Where Positive-Pressure Suits Are Worn.. 20

Figure 3-3: Representative Diagrams of Different Containment Level 2 (CL2) Zones in the Same Physical Space . . . 25

Figure 3-4: Representative Biohazard Warning Signage. 26

Figure 3-5: Representative Diagrams of Placement of Offices with Respect to Adjoining Containment Level 2 (CL2) Zone. 27

Figure 3-6: Representative Diagram of a Containment Level 3 (CL3) Small Animal Containment Zone (SA zone) Indicating the Containment Barrier and the Containment Zone Perimeter. 29

Figure 3-7: Representative Diagram of a Containment Level 3 (CL3) Large Animal Containment Zone (LA zone) that Includes Multiple Containment Barriers. 30

Figure 3-8: Representative Diagram of Anteroom Spaces in a Containment Level 3 (CL3) Zone Identifying the Critical Door. 33

Figure 4-1: Risk Assessment Matrix 53

Figure 7-1: Example of an Emergency Medical Contact Card. . . 90

Figure 10-1: Representative Diagram of a High Efficiency Particulate Air (HEPA) Filter Housing with Cut Away Showing HEPA Filters within the Housing. 122

Figure 11-1a: Illustration of a Class I Biological Safety Cabinet (BSC). 127

Figure 11-1b: Illustration of a Class I Biological Safety Cabinet (BSC 128

Figure 11-2: Illustration of a Class II Type A1 Biological Safety Cabinet (BSC) 130

Figure 11-3: Illustration of a Class II Type A2 Biological Safety Cabinet (BSC). 131

Figure 11-4: Illustration of a Class II Type B1 Biological Safety Cabinet (BSC). 132

Figure 11-5: Illustration of a Class II Type B2 Biological Safety Cabinet (BSC). 134

Figure 11-6: Illustration of a Class III Biological Safety Cabinet (BSC). 137
Figure 11-7: Representative Diagram Illustrating Location Considerations for Biological Safety Cabinets (BSCs). 138
Figure 11-8: Representative Diagram of a Recommended Layout of Materials and Workflow inside a Biological Safety Cabinet (BSC). 142
Figure 12-1: Representative Diagram of a Vacuum System Set-up for the Aspiration of Infectious Liquids 150
Figure 13-1: Representative Diagram of a Basic Animal Room . . 159
Figure 13-2: Representative Diagrams of Primary Containment Caging. 162
Figure 13-3: Representative Diagram of an Open Caging System. 163
Figure 13-4: Representative Diagrams of an Animal Cubicle . . . 164
Figure 13-5: Representative Diagram of Single Corridor and Dual Corridor Designs for Animal Containment Zones. 165
Figure 18-1: Visual Representation of Incidents Involving Pathogens and Toxins, including Exposures and Laboratory Acquired Infections/ Intoxications (LAIs). 223
Figure 18-2: Decision Chart to Assist in the Assessment of an Incident to Determine if an Exposure has Occurred and if Notification of the Public Health Agency of Canada (PHAC) is Required. 225

LIST OF TABLES

Table 4-1: Summary of Prescribed Toxins and Associated Trigger Quantities. 41
Table 9-1: Compatibility of Natural Rubber, Synthetic Rubber, and Plastic Polymer Gloves with Common Chemical Disinfectants 103
Table 11-1: Summary Table of Key Characteristics of Class II Biological Safety Cabinets (BSCs). 135
Table 15-1: Pathogens Ranked According to Relative Susceptibility to Chemical Disinfectants. . . 187
Table 15-2: Susceptibility of Microorganisms to Chemical Disinfectants 190
Table 15-3: Disadvantages of Chemical Disinfectants. 192

PREFACE

PREFACE

The Government of Canada's *Canadian Biosafety Handbook* (CBH), 2nd Edition, 2016, is a national guidance document for the safe handling and storing of human and terrestrial animal pathogens and toxins in Canada. It is a companion document to the *Canadian Biosafety Standard* (CBS), 2nd Edition, 2015 in which the physical containment, operational practice, and performance and verification testing requirements are set out to ensure the safe handling and storing of human and terrestrial animal pathogens and toxins. Activities in Canada involving human and animal pathogens and toxins are regulated by the Public Health Agency of Canada (PHAC) and the Canadian Food Inspection Agency (CFIA) in accordance with the *Human Pathogens and Toxins Act, Human Pathogens and Toxins Regulations, Health of Animals Act*, and *Health of Animals Regulations*.

The second edition of the CBH updates the guidelines originally published as Part II of the *Canadian Biosafety Standards and Guidelines* (CBSG), 1st Edition, 2013. The CBSG was developed as the result of a joint initiative undertaken by the PHAC and the CFIA to update and harmonize the following three Canadian biosafety standards and guidelines for the design, construction, and operation of facilities in which pathogens or toxins are handled or stored:

1. *Human pathogens and toxins: Laboratory Biosafety Guidelines*, 3rd Edition, 2004 (PHAC)
2. *Terrestrial animal pathogens: Containment Standards for Veterinary Facilities*, 1st Edition, 1996 (CFIA)
3. *Prions: Containment Standards for Laboratories, Animal Facilities and Post Mortem Rooms Handling Prion Disease Agents*, 1st Edition, 2005 (CFIA)

The CBH provides the core information and guidance on how to achieve the biosafety and biosecurity requirements specified in the CBS. The CBH systematically addresses the concepts required for the development and maintenance of a comprehensive risk-based biosafety management program.

The PHAC and the CFIA welcome comments, clarifications, and suggestions for incorporation into future editions of the CBS and the CBH. To this end, please send information with references (where applicable) for the continual improvement of the CBH to:

- PHAC email: PHAC.standards-normes.ASPC@canada.ca
- CFIA email: standardsnormes@inspection.gc.ca

ABBREVIATIONS AND ACRONYMS

ABBREVIATIONS AND ACRONYMS

Ag	Agriculture (i.e., CL2-Ag, CL3-Ag)
AIRS	Automated Import Reference System
ANSI	American National Standards Institute
ASHRAE	American Society of Heating, Refrigerating and Air-Conditioning Engineers
ASME	American Society of Mechanical Engineers
ASTM	American Society for Testing and Materials
BSC	Biological safety cabinet
BSE	Bovine spongiform encephalopathy
BSO	Biological safety officer
CAN	National Standard of Canada
CBH	*Canadian Biosafety Handbook*
CBS	*Canadian Biosafety Standard*
CBSA	Canada Border Service Agency
CBSG	*Canadian Biosafety Standards and Guidelines*
CCAC	Canadian Council on Animal Care
CCME	Canadian Council of Ministers of the Environment
CCOHS	Canadian Centre for Occupational Health and Safety
CDC	United States Centers for Disease Control and Prevention
CFIA	Canadian Food Inspection Agency

ClO_2	Chlorine dioxide
CJD	Creutzfeldt-Jakob disease
CL	Containment level (i.e., CL1, CL2, CL3, CL4)
CSA	Canadian Standards Association
CPC	Canadian Product Category
CSIS	Canadian Security Intelligence Service
CWC	Chemical Weapons Convention
CWD	Chronic wasting disease
DGR	Dangerous Goods Regulations
DNA	Deoxyribonucleic Acid
ED_{50}	Median effective dose
ELISA	Enzyme-linked immunosorbent assay
ERP	Emergency response plan
GAC	Global Affairs Canada
GMO	Genetically modified organism
HAA	*Health of Animals Act*
HAR	*Health of Animals Regulations*
HEPA	High efficiency particulate air
HIV	Human immunodeficiency virus

HPTA	*Human Pathogens and Toxins Act*
HPTA Security Clearance	*Human Pathogens and Toxins Act* Security Clearance
HPTR	*Human Pathogens and Toxins Regulations*
HVAC	Heating, ventilation, and air conditioning
H_2O_2	Hydrogen peroxide
IATA	International Air Transport Association
IBC	Institutional biosafety committee
ICAO	International Civil Aviation Organization
ID	Identification
IDA	Inward directional airflow
IEST	Institute of Environmental Sciences and Technology
in. w.g.	Inches water gauge
ISO	International Organization for Standardization
IUC	Intended Use Code
LA zone	Large animal containment zone
LAI	Laboratory acquired infection/ intoxication
LD_{50}	Median lethal dose (50% mortality rate of test group)
LRA	Local risk assessment

NACI	National Advisory Committee on Immunization
NaOCl	Sodium hypochlorite
NaOH	Sodium hydroxide
NHP	Non-human primate
NIH	United States National Institutes of Health
NSF	National Sanitation Foundation
OECD	Organisation for Economic Co-operation and Development
OIE	World Organisation for Animal Health
PAPR	Powered air purifying respirator (also known as Positive air-purifying respirator)
PHAC	Public Health Agency of Canada
PM room	Post mortem room
PPE	Personal protective equipment
PSDS	Pathogen safety data sheet
PVC	Polyvinyl chloride
QAC	Quaternary ammonium compound
RCMP	Royal Canadian Mounted Police
rDNA	Recombinant deoxyribonucleic acid
RG	Risk group (i.e., RG1, RG2, RG3, RG4)
RNA	Ribonucleic acid

SA zone	Small animal containment zone
sDNA	Synthetic deoxyribonucleic acid
SMACNA	Sheet Metal and Air Conditioning Contractors' National Association
SOP	Standard operating procedure
spp.	Species (plural)
SSBA	Security sensitive biological agent
TDGA	Transportation of Dangerous Goods Act
TDGR	Transportation of Dangerous Goods Regulations
TSE	Transmissible spongiform encephalopathy
UN	United Nations
UPS	Uninterruptible power supply
UV	Ultraviolet
vCJD	Variant Creutzfeldt-Jakob disease
VHP	Vaporized hydrogen peroxide
WHMIS	Workplace Hazardous Materials Information System

CHAPTER 1

INTRODUCTION

CHAPTER 1 – INTRODUCTION

The words in **bold type** are defined in the comprehensive glossary found in Chapter 24.

1.1 Scope

In Canada, all **facilities** that are **handling and storing** human **pathogens** or **toxins**, such as public health laboratories, teaching and research laboratories, diagnostic laboratories in hospitals, and vaccine production plants, are regulated under the *Human Pathogens and Toxins Act* (HPTA) and *Human Pathogens and Toxins Regulations* (HPTR).[1,2] Canadian facilities that import **animal pathogens**, infected animals, animal products or by-products (e.g., tissue, serum), or other substances that may carry an animal pathogen or toxin or parts thereof are regulated under the *Health of Animals Act* (HAA) and *Health of Animals Regulations* (HAR).[3,4] The Public Health Agency of Canada (PHAC) is responsible for the regulation of human pathogens and toxins under the authority of the HPTA and the HPTR. The PHAC and the Canadian Food Inspection Agency (CFIA) are jointly responsible for the importation and **transfer** of animal pathogens and toxins under the HAA and HAR.

The *Canadian Biosafety Handbook* (CBH), 2nd Edition, 2016 is intended as a companion document to the *Canadian Biosafety Standard* (CBS), 2nd Edition, 2015.[5] It is a guidance document for Canadian facilities where human and **terrestrial animal pathogens** are handled, that updates the guidelines originally published as Part II of the *Canadian Biosafety Standards and Guidelines* (CBSG), 1st Edition, 2013.[6] The CBH provides overall guidance on how to achieve the **physical containment requirements, operational practice requirements,** and **performance and verification testing requirements** for regulated facilities specified in the CBS. The CBH includes concepts that are fundamental to the development and maintenance of a comprehensive, **risk**-based **biosafety** management program; however, it does not provide specific guidance or **standard operating procedures (SOPs)** for individual pathogens.

1.2 Overview

Biosafety involves the consistent application of safety measures to minimize or prevent harm to laboratory personnel, building occupants, the public at large, the animal population, and the environment resulting from **exposure** to the **infectious material**, infected animals, or toxins handled in a **containment zone**. A biosafety program includes institutional plans and policies that facilitate the safe handling and storing of infectious material and toxins, and prevent the **release** of infectious material or toxins from the containment zone. Core elements of a biosafety program include a comprehensive training program, **medical surveillance program, emergency response plan (ERP)**, SOPs that follow safe work practices, and a **biosecurity** plan. A functional and practical biosafety program encompasses all the components that are relevant to the individual **laboratory work area, large scale production area**, or **animal work area**. Some facilities may include a single laboratory that performs

limited activities involving infectious material or toxins, while other programs may encompass multiple facilities on a campus performing diverse activities involving infectious material and toxins. Common safety measures in a biosafety program encompass **good microbiological laboratory practices**, appropriate **primary containment** equipment, and proper physical design of the containment zone. Increased public awareness has focused additional attention on the prevention of the misuse of pathogens and toxins, which has resulted in the fast-paced development of biosecurity, in its own right, and emphasized the value and integration of biosecurity into every biosafety program.

Risk assessments are the basis of all components of a biosafety program; they are critical for identifying the hazards associated with specific tasks or activities involving infectious material and toxins and for implementing the appropriate mitigation strategies. The development of a functional biosafety program requires an **overarching risk assessment** of all the work to be done with infectious material and toxins. In addition, **local risk assessments (LRAs)** specific to the work area are conducted to identify hazards based on the infectious material or toxin in use and the activities being performed. Risk assessments describing the hazardous properties of well-characterized human pathogens and toxins and recommendations for their safe handling have been developed into technical documents known as **Pathogen Safety Data Sheets (PSDSs)** that are readily available on the PHAC website (www.publichealth.gc.ca/pathogens). Fact Sheets for federally reportable **diseases** affecting terrestrial animals in Canada have also been developed and are readily available on the CFIA website (www.inspection.gc.ca/english/sci/bio/bioe.shtml).

A biosafety program can be integrated into an existing safety program or a national or international quality assurance program to improve and streamline overall safety, and promote understanding of, and compliance with, the **facility's** biosafety program. There are many resources and useful documents to facilitate the development and implementation of a biosafety program. In addition to the PSDSs and Fact Sheets described above, the PHAC and the CFIA provide numerous resources, such as biosafety training materials, templates, toolkits, posters, instructional videos, and more, available through an e-learning portal (www.publichealth.gc.ca/training). Throughout the CBH, there are references to additional complementary documents and resources which can be drawn on to develop the best possible programs to prevent the release of pathogens and toxins, and the exposure of personnel.

1.3 The *Canadian Biosafety Standard*

The CBS 2nd Edition, 2015 is a harmonized national standard for the handling and storing of human and terrestrial animal pathogens and toxins in Canada. The CBS specifies the physical containment, operational practice, and performance and verification testing requirements for the safe handling and storing of human and terrestrial animal pathogens and toxins. The CBS is used by the PHAC and the CFIA to verify the ongoing compliance of facilities regulated under the HPTA, HPTR, HAA, and HAR.

1.4 How to Use the *Canadian Biosafety Handbook*

The information provided in the CBH is intended as guidance on how to achieve the biosafety requirements specified in the CBS, and should not be interpreted as requirements. Where the guidance relates to a requirement from the CBS, the requirement matrix or matrices are referenced (e.g., CBS Matrix 4.1), and the wording used implies an obligation (e.g., must be done, to be done). Likewise, where the guidance relates to a requirement from the legislation (i.e., HPTA, HPTR, HAA, HAR), the specific section and subsection(s), where applicable, will be referenced (e.g., HPTA 33). In some cases, measures that are required only in **high containment zones** are indicated as "best practices" in lower containment zones and the wording implies a recommendation (e.g., should be done).

The CBH includes a detailed list of all abbreviations and acronyms used throughout; this list is located at the beginning of the document. Each abbreviation or acronym is spelled out upon first use in each chapter, with the abbreviation immediately following in brackets; the abbreviation is used exclusively throughout the remainder of the chapter.

The CBH contains a comprehensive glossary of definitions for technical terms, located in Chapter 24 of the document; words defined in the glossary appear in **bold type** upon first use in each chapter. Chapter 25 provides a list of the resources that were used to develop the CBH. In-text citations are listed in the references at the end of each chapter.

A full list of the external standards and other documents that are referenced in the CBH chapters is provided in Chapter 25.

REFERENCES

[1] *Human Pathogens and Toxins Act (S.C. 2009, c. 24)*. (2015).

[2] *Human Pathogens and Toxins Regulations (SOR/2015-44)*. (2015).

[3] *Health of Animals Act (S.C. 1990, c. 21)*. (2015).

[4] *Health of Animals Regulations (C.R.C., c. 296)*. (2015).

[5] Government of Canada. (2015). *Canadian Biosafety Standard* (2nd ed.). Ottawa, ON, Canada: Government of Canada.

[6] Government of Canada. (2013). *Canadian Biosafety Standards and Guidelines* (1st ed.). Ottawa, ON, Canada: Government of Canada.

CHAPTER 2

BIOLOGICAL MATERIAL

CHAPTER 2 – BIOLOGICAL MATERIAL

Biological material refers to **microorganisms**, proteins, and nucleic acids, or anything that contains them (e.g., tissue) whether or not they are infectious or toxic. **Pathogens** are a subset of biological material that is capable of causing **disease** in humans or animals. Examples of human pathogens can be found in Schedules 2, 3, 4, and 5 of the *Human Pathogens and Toxins Act* (HPTA).[1] Examples of **animal pathogens** can be found on the Canadian Food Inspection Agency's (CFIA's) website. In the context of the *Canadian Biosafety Handbook* (CBH), the term "**infectious material**" is used throughout to collectively refer to pure **cultures** or isolates of pathogens as well as any material that may contain a pathogen (e.g., infected tissue sample) or part of one that retains its **pathogenicity**. A microbial **toxin** that is isolated from its parental organism or synthetically produced is not infectious by nature; therefore, toxins are not included in the term "infectious material". This chapter provides a brief overview of the basic characteristics of the various types of biological material that are important in the context of the CBH.[2,3,4,5]

2.1 Bacteria

Bacteria are single-celled prokaryotic organisms lacking a nucleus and other membrane-enclosed organelles.[2,3] Morphologically 0.5–5.0 µm in size, bacteria are spherical (cocci) or appear as rods (bacilli) that may be straight, curved, spiralled, or tightly coiled. Based on Gram-stain and morphology, thousands of bacterial species have been classified into one of the following three phenotypes: Gram-negative, Gram-positive, or mycoplasma (bacteria lacking a cell wall). The cell walls in Gram-negative and Gram-positive bacteria differ considerably. In Gram-negative organisms, the cell wall is composed of a plasma membrane, a peptidoglycan layer that comprises approximately 10% of the cell wall, and an outer membrane made of lipopolysaccharides and lipoproteins. In contrast, the cell wall of Gram-positive organisms is composed of a plasma membrane and a peptidoglycan layer that comprises up to 90% of the cell wall, but no lipid outer membrane. Bacteria also vary in their requirements for oxygen, being described broadly as either aerobic, microaerophilic, or anaerobic.

Some bacteria can induce an immune response (e.g., inflammation) in a host organism, secrete exotoxins, produce surface-associated endotoxins, or form spores that enhance survival and transmission outside of the host for extended periods of time. Bacteria that can infect and cause disease in humans or animals are referred to as pathogenic bacteria. Some bacteria are **opportunistic pathogens** that can colonize the body of a human or animal host and may not cause disease unless a disruption occurs in the host's immune system or natural barriers to infection (i.e., immunocompromised or immunosuppressed), or if the host is exposed to a high dose of the pathogen. In comparison, obligate pathogenic bacteria cannot survive outside of a host and must cause disease in order to survive and be transmitted from one host to another.[6] Examples of pathogenic bacteria include *Bacillus anthracis*, certain strains of *Escherichia coli*, *Mycobacterium tuberculosis*, and *Salmonella* species (spp.).

2.2 Viruses

Viruses are the smallest of replicating organisms.[2,3,5] Their small size (20-300 nm) allows them to pass through filters that typically capture the smallest bacteria. Viruses have no metabolism of their own and redirect existing host machinery and metabolic functions to replicate. Structurally, the simplest viruses consist of nucleic acids enclosed in a protein capsid (nucleocapsid). Enveloped viruses have a more complex structure in which the nucleocapsid is enclosed inside a lipid bilayer membrane; this membrane facilitates the virus's interaction with the host cell.

Viruses are classified by their replication strategy and by the organization of their genome (i.e., double-stranded deoxyribonucleic acid [DNA], single-stranded DNA, reverse transcribing, double-stranded ribonucleic acid [RNA], negative-sense single-stranded RNA, positive-sense single-stranded RNA, and subviral agents). There are many virus families that are able to infect human or animal hosts. Some are species-specific while others infect a wide range of host species. Some viruses are able to produce a persistent infection (i.e., host cell remains alive and continues to produce virus particles over a long period of time) or a latent infection (i.e., there is a delay of months or years between infection and the appearance of disease symptoms), or they may alter the host genome by integrating (e.g., integration of a retrovirus into the host genome). Examples of pathogenic viruses include influenza virus, human immunodeficiency virus (HIV), herpes virus, rabies virus, and Ebola virus.

2.3 Fungi

Fungi are eukaryotic microorganisms that can be easily distinguished from bacteria and other prokaryotes by their greater size and the presence of organelles; including a nucleus, vacuoles, and mitochondria.[2,3] Yeast normally grow as single cells, whereas moulds grow in branching chains. Of the 1.5 million estimated fungal species, over 500 are known to cause disease in human or animal hosts, including several species of yeast and moulds.[7]

Exposure to fungal spores can occur via the airborne route, inoculation, or close contact, depending on the species. In addition, some fungal species may produce and disperse mycotoxins (toxins are further described in Section 2.7). In general, human and animal tissues, including blood, are not considered a **risk** for the airborne dispersal of fungal spores. Most species of fungi are opportunistic pathogens and will generally only cause disease in immunocompromised individuals. Examples of pathogenic fungi include *Aspergillus fumigatus, Candida albicans, Blastomyces dermatitidis*, and *Histoplasma capsulatum*.

2.4 Parasites

Protozoa and helminths are parasites that live on or within a larger host organism at the host's expense.[8] Protozoa are single-celled eukaryotic microorganisms that lack a cell wall and are generally motile. Helminths are eukaryotic worms that may grow large enough to be

visible to the naked eye. Parasites that live within the tissues or cells of their host are known as endoparasites and cause infections that are generally treatable. Some endoparasites can persist for many years in the human body, even following treatment, and will re-surface to cause disease if the host becomes immunocompromised. Ectoparasites live on the external surface, or within the skin of their host, causing an infestation. The type and degree of injury inflicted on the host will vary based on the number of parasites present.

While most helminths in the adult stage of their life-cycle may be quite large and easily visible to the naked eye, they are generally only infectious during life-cycle phases in which they are very small (e.g., egg, larval stage). Since helminths in their infectious stage can be transmitted by ingestion, direct contact, injection, and inhalation, they present a risk similar to that of other microorganisms for accidental or unintentional exposure. Examples of pathogenic protozoa include *Plasmodium falciparum, Leishmania donovani, Cryptosporidium parvum, Giardia lamblia*, and *Trypanosoma cruzi*. Examples of pathogenic helminths include *Trichinella spiralis* (nematode), *Enterobius vermicularis* (pinworm), and *Hymenolepis nana* (tapeworm).

2.5 Prions

Prions are small, proteinaceous infectious particles that are generally accepted to be the cause of a group of progressive neurodegenerative diseases in humans and animals known as **transmissible spongiform encephalopathies (TSEs)**.[2,3,4] When an infectious prion enters a host, it induces the normally folded prion protein to convert to the disease-associated, misfolded prion isoform. The pathogenic isoform acts as a template that guides the misfolding of more prion proteins, eventually leading to the accumulation of large amounts of the extremely stable, misfolded proteins in infected tissue, causing tissue damage and cell death.

Prion proteins are extremely heat stable, able to bind with high affinity to metal surfaces, and can persist for long periods in the natural environment.[9] The most likely route of transmission to personnel handling infectious prions is through accidental inoculation or ingestion of infected tissues. TSEs are unique due to the long incubation times (up to 30 years) before disease symptoms appear.[10] Examples of TSEs in animals include bovine spongiform encephalopathy (BSE), scrapie, and chronic wasting disease (CWD). Examples of TSEs in humans include Creutzfeldt-Jakob disease (CJD), variant Creutzfeldt-Jakob disease (vCJD), Gerstmann–Straussler–Scheinker syndrome, fatal familial insomnia, and kuru. Some prions are **zoonotic pathogens**, such as the BSE prions.

2.6 Zoonotic Pathogens

The term "**zoonoses**" describes diseases that are transmissible between animals and humans; it encompasses both anthropozoonoses (i.e., diseases transmitted from animals to humans), and zooanthroponoses or "reverse zoonoses" (i.e., diseases transmitted from humans to

animals).[8,11,12,13] There have been several documented **laboratory acquired infections (LAIs)** involving zoonotic pathogens transmitted to humans by an animal infected with or carrying a zoonotic pathogen. The risk of zoonosis exists in activities involving animals experimentally infected with a zoonotic pathogen, as well as in activities involving first generation wild-caught animals that may be infected with or carrying a pathogen indigenous to the animal's natural environment. For example, Macacine herpesvirus 1 (formerly known as herpes B virus or cercopithecine herpes virus 1) is an **enzootic** virus present in up to 70% of captive macaques, including rhesus macaques and cynomolgus monkeys, and has been associated with at least 50 documented LAIs.[14,15] Documented zoonoses in humans have been caused by bacteria (e.g., *Salmonella* spp. can cause salmonellosis; *Yersinia pestis* can cause plague), viruses (e.g., rabies virus can cause rabies), parasites (e.g., *Toxoplasma gondii* can cause toxoplasmosis), and prions (e.g., BSE agent can cause vCJD).[16]

2.7 Toxins

Microbial toxins are poisonous substances that are a natural product of the metabolic activities of certain microorganisms (e.g., bacteria, fungi).[2,3] Toxins can cause adverse health effects, known as **intoxication**, which can include asymptomatic or symptomatic physiological changes, severe incapacitation, or death in a human or animal resulting from an exposure (i.e., ingestion, inhalation, inoculation, or absorption) to a toxin. Severe health effects may even occur in response to relatively low dose exposures of toxins. Toxins do not replicate and are not transmitted from person to person. The most likely route of transmission to personnel handling toxins is through accidental inoculation or by the exposure of mucous membranes to aerosolized toxins. Some toxins can be artificially produced by chemical synthesis or by recombinant DNA (rDNA) technology (rDNA technology is further described in Section 2.8.1). Microbial toxins are classified according to the organism from which the toxin is derived (e.g., bacterial, fungal). Microbial intoxication is typically associated with bacteria.

Two types of microbial toxins exist: exotoxins and endotoxins. Exotoxins are often heat-labile proteins and polypeptides that are produced and secreted by a variety of species, including both Gram-negative and Gram-positive bacteria. Bacterial exotoxins can exert their toxic effects on the host through the following five mechanisms: damage to cell membranes, inhibition of protein synthesis, inhibition of neurotransmitter release, activation of secondary messenger pathways, or activation of host immune responses. Examples of exotoxins include tetanus toxin, produced by the Gram-positive bacterium *Clostridium tetani*, and cholera toxin, produced by the Gram-negative bacterium *Vibrio cholerae*. Additionally, a family of heat-stable exotoxins exists, called enterotoxins, which exert their primary effects on the digestive tract. Some examples include Staphylococcus Enterotoxin Type B produced by *Staphylococcus aureus*, heat-stable enterotoxins produced by enterotoxigenic *Escherichia coli* (ETEC), and cereulide produced by *Bacillus cereus*. Endotoxins are structural molecules (i.e., lipopolysaccharides or lipooligosaccharides) that are embedded in the outer membrane of the cell wall of certain Gram-negative bacteria, such as *Escherichia coli* and *Shigella dysenteriae*. Endotoxins are relatively heat-stable and generally less toxic than exotoxins.

A subset of microbial toxins is regulated by the Public Health Agency of Canada (PHAC) and the CFIA under the HPTA, *Human Pathogens and Toxins Regulations* (HPTR), *Health of Animals Act* (HAA), and *Health of Animals Regulations* (HAR); an exhaustive list of regulated toxins affecting humans can be found in Schedules 1 and 5 of the HPTA.[17,18,19]

2.8 Biotechnology

Biotechnology describes the application of science and engineering to the direct or indirect use of living organisms or parts or products of living organisms in their natural or modified forms. Different methods can be used to alter the genetic material of an organism in order to create a different or novel organism. In nature, spontaneous mutation, crossbreeding, and natural selection lead to organisms and hybrids with new or advantageous traits or characteristics. Molecular methods, such as bacterial conjugation, transformation, and transduction, have been conventionally applied to introduce new genetic information into host organisms or cells for a variety of scientific or industrial purposes. Advancements in biotechnology have resulted in newer, more efficient techniques to create genetically modified organisms (GMOs) by the insertion, deletion, replacement, or alteration of genes or gene segments. There are many applications that employ biotechnology, including the production of antibiotics, hormones, enzymes, and antibodies. Modern biotechnology methods that are commonly used to create new or altered organisms are described below.

2.8.1 Recombinant DNA

Genetic material, either natural or synthetic, can be combined to construct novel rDNA. rDNA technologies, are widely used in modern-day research and industry and have many applications, including the production of transgenic animals, the cloning of microbial toxin genes, drug resistance genes, or other genes in expression vectors, as well as the production of full-length infectious viral clones.

While there are numerous beneficial uses for rDNA technology, there is also the risk that this technology can be used to create new pathogenic organisms or to increase the pathogenicity of existing organisms, whether intentionally or not.

2.8.2 Genetically Modified Organisms

GMOs are organisms (i.e., plants, animals, or microorganisms) that are created through the alteration of genetic materials in a way that does not occur naturally through mating or natural recombination. The best known method for creating GMOs is through the application of rDNA technologies. A GMO can be as simple as a point-mutated bacteria strain (e.g., *E. coli* DH5-Alpha) or rDNA cloned into a viral host (e.g., vaccinia virus vaccines) to overexpress a specific gene for further study.[20,21] More complex GMOs include transgenic and knock-out animals (e.g., severe combined immunodeficiency mice) whose genome has been altered by the insertion, removal, or alteration of DNA segments.[22]

2.8.3 Viral Vectors

Viral vectors are vehicles used to deliver genetic material into host cells for subsequent gene expression. These systems have been used for both research and gene therapy applications. Viral vector systems used for recombinant gene transfer are usually based on viruses present in the human population such as adenoviruses, herpesviruses, and retroviruses. Genetic modifications are typically made to these vectors to improve gene delivery efficiency and to enhance their safety.

Retroviral vector systems, including lentiviral vectors derived from HIV-1, are competent gene transfer vehicles that are widely used for their stable integration into the chromosomes of non-dividing and dividing cells, and for their long-term transgene expression.

2.8.4 Synthetic Organisms

Synthetic biology is a rapidly evolving interdisciplinary field of research that combines biology and engineering for the design, redesign, or fabrication of novel or existing natural biological components and systems.[23] Synthetic biology includes, but is not limited to, the use of synthetic DNA (sDNA) to design and construct new biological parts, devices, or systems. Synthetic biology demonstrates how rapid advances in life sciences are opening up potentially dramatic applications in fields such as health care, agriculture, industrial chemistry, and energy production.[24] It is expected to offer many significant and transforming products such as new and improved vaccines, medicines, diagnostic and infection surveillance tools, feed stocks, cleaner biofuels, and industrial processes.

Similar to rDNA technologies, synthetic biology also has the risk that this type of research may lead to the creation of new pathogenic organisms or increase the pathogenicity of existing organisms, whether intentionally or not.

2.9 Cell Lines and Cell Cultures

Cell lines and cell cultures are commonly used in diagnostic, research, and industrial laboratories. The PHAC and the CFIA do not regulate cell lines, but do regulate the pathogens or parts thereof that might be contained within them. Many cell lines do not inherently pose a risk to the individuals manipulating them in the **laboratory**; however, they have the potential to contain pathogenic organisms such as bacteria (e.g., mycoplasmas), fungi, viruses, or prions. This can occur either naturally through **contamination** by adventitious organisms (e.g., mycoplasma and moulds), or experimentally (e.g., transduction, transfection, or infection). Cell lines available from commercial sources are generally very well-characterized and the presence of infectious contaminants is documented. Some commercially available and established cell lines and cell cultures may contain parts of human or animal pathogens as a result of a previous infection (e.g., a latent virus or presence of a helper virus) or as the result of genetic engineering to include a pathogen's genetic information that retains pathogenicity. Such cell lines or cultures may be subject to regulation by the PHAC or the

CFIA, or both. Freshly prepared cell lines from a primary culture may have a higher risk of contamination, especially if the cell line was obtained from a source known to be or suspected of being infected with a pathogen. There have been documented LAIs associated with the manipulation of primary cell cultures.[25,26]

Bacterial and fungal contamination in cell lines can be readily identified; however, viruses are not as easily identified and can pose a significant hazard. Growth conditions (e.g., pH, temperature, medium supplements) may cause altered expression of oncogenes, expression of latent viruses, interactions between recombinant genomic segments, or altered expression of cell surface proteins. The presence of biologically active mycoplasma products, the stability of mycoplasma antigens, and the fact that a number of mycoplasmas are zoonotic pathogens may make them an additional hazard to consider when working with cell lines.

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CHAPTER 3

CONTAINMENT LEVELS AND CONTAINMENT ZONES

CHAPTER 3 – CONTAINMENT LEVELS AND CONTAINMENT ZONES

Containment (or "**biocontainment**") refers to the combination of physical design parameters and operational practices that protects personnel, the immediate work environment, the **community**, and the external environment from **exposure** to potentially hazardous **biological material**. The *Canadian Biosafety Standard* (CBS), 2nd Edition, describes the different **containment levels** for **facilities handling or storing** human or **terrestrial animal pathogens** and **toxins** regulated by the Public Health Agency of Canada (PHAC), the Canadian Food Inspection Agency (CFIA), or both agencies.[1] The specific **physical containment requirements** and **operational practice requirements** for each containment level are specified in the CBS Chapters 3 and 4, respectively. This chapter describes the different containment levels, the different types of work areas that may be found in a **containment zone**, and guidance to assist regulated parties in identifying and accessing containment zones within their own facilities. Note that all diagrams presented in this chapter are for illustrative purposes only and are not to scale; the optimal configuration of containment zones and sizes of rooms and doorways will vary according to **facility** type and program.

3.1 Containment Levels

Containment level describes the minimum physical containment and operational practices that a containment zone (i.e., an identified physical area that meets the requirements for a specified containment level) requires for the safe handling of **infectious material** or toxins. There are four containment levels ranging from a basic **laboratory** for work with biological material (containment level 1 [CL1]) to the highly sophisticated facilities for work with the highest **risk pathogens** (containment level 4 [CL4]). The CBS specifies the minimum specific physical containment requirements and operational practice requirements for containment level 2 (CL2), containment level 3 (CL3), and CL4 facilities that are regulated by the PHAC or the CFIA and that are authorized to handle or store human and **animal pathogens** or toxins. Due to the low risk to public health and the animal population from **Risk Group** 1 (RG1) biological material, there are no physical containment requirements or operational practice requirements for CL1 facilities.

3.1.1 Containment Level Categories

The following definitions provide a basic description of the different containment levels for activities with human or animal pathogens or toxins. The requirements and details specific to CL2, CL3, and CL4 can be found in the CBS.

3.1.1.1 Containment Level 1

Work with RG1 biological material can be safely performed in a basic **laboratory work area**, **large scale production area**, or **animal work area**, often described as CL1. CL1 incorporates features that provide the foundation for **biosafety** upon which the requirements for all higher level containment zones are built. Biosafety is primarily achieved through the use of **good microbiological laboratory practices** in addition to basic physical containment design elements, such as handwashing sinks, that serve to protect personnel and the environment from the biological material being handled.

Due to the low risk to public health and the animal population associated with RG1 biological material, there are no specific physical and operational requirements for CL1. The general recommendations for the safe handling of RG1 biological material are described in Chapter 21.

3.1.1.2 Containment Level 2

CL2 builds upon the basic laboratory foundation established for CL1. Biosafety and **biosecurity** at CL2 are achieved through operational practices and a core subset of physical containment requirements that are proportional to the risks associated with the pathogens and toxins handled therein. Operational practices for CL2 include administrative controls (e.g., biosafety program management, training) and procedures (e.g., work practices, **personal protective equipment [PPE]** use, and **decontamination**) that mitigate the risks associated with the activities conducted within the zone. Physical containment features include facility design (e.g., location of laboratory, surface finishes, access control) and provision of biosafety equipment, such as **primary containment devices** (e.g., **biological safety cabinets [BSCs]**) for certain activities.

A representative diagram of two CL2 zones is provided in Figure 3-1. A CL2 laboratory work area and a separate CL2 **small animal containment zone (SA zone)** are identified in this diagram; the solid red lines around the CL2 zones illustrate the **containment zone perimeter** (discussed in Section 3.3.1). This diagram depicts some basic physical features for CL2 zones, such as doors to separate public areas from the containment zones, primary containment devices (e.g., BSCs) located away from high traffic areas/doors, and sinks provided to facilitate handwashing upon exit from the containment zone.

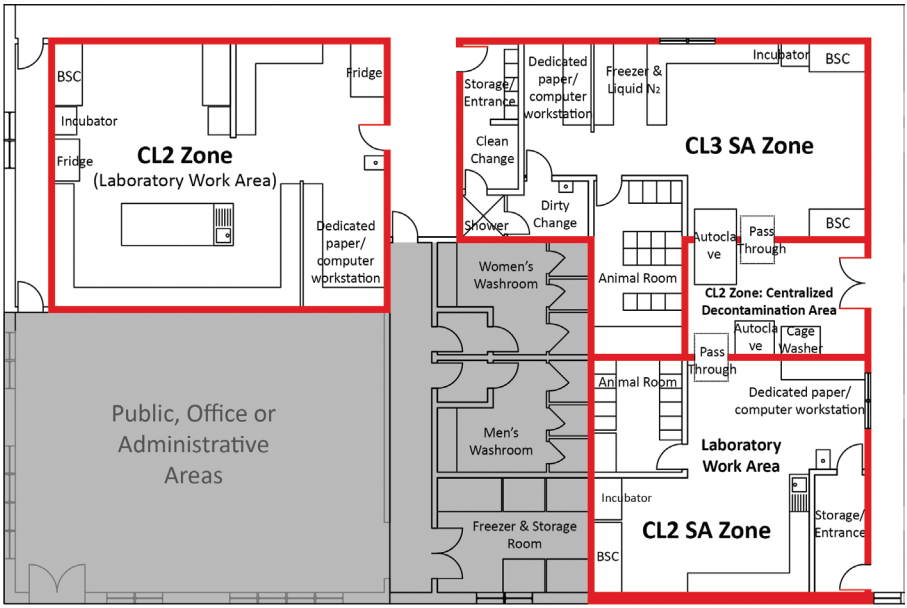


Figure 3–1:
Representative Diagram of a Mixed-use Facility Containing Multiple Containment Level 2 (CL2) and Containment Level 3 (CL3) Zones.

The solid red lines around the CL2 and CL3 zones illustrate the individual containment zone perimeters. Grey shading indicates rooms and areas that are outside of containment (e.g., public areas, offices, administrative areas, washrooms) that do not have any physical containment requirements.

3.1.1.3 Containment Level 3

Biosafety and biosecurity at CL3 are achieved through comprehensive operational practices and physical containment requirements. CL3 requires stringent facility design and engineering controls (e.g., **inward directional airflow [IDA], high efficiency particulate air [HEPA] filtration** of exhaust air), as well as specialized biosafety equipment (e.g., BSCs, centrifuges with sealed rotors) to minimize the **release** of infectious material into the surrounding rooms inside or outside the containment zone, or the environment outside. Additional engineering controls, such as **effluent decontamination systems**, may be needed in some cases (e.g., Risk Group 3 [RG3] **non-indigenous animal pathogens**) to control the risks associated with pathogen release into the environment. Operational practices at CL3 build upon those required for CL2, taking into consideration the increased risks associated with the pathogen(s) and laboratory activities being carried out with RG3 pathogens.

A representative diagram of a CL3 SA zone is provided in Figure 3-1. The solid red line surrounding the CL3 zone illustrates the containment zone perimeter of the CL3 zone in this example. This diagram depicts some basic physical features such as a door to separate public areas from the containment zone, primary containment devices (e.g., BSCs) located away from high traffic areas/doors, a handwashing sink provided (located in the **"dirty" change area** in this example), as well as **anterooms**/clothing change areas equipped with a walk-through body shower for personnel, **primary containment caging**, and **pass-through chambers** (optional).

3.1.1.4 Containment Level 4

CL4 is the highest level of containment available. CL4 requires a highly complex facility design that is a self-contained area within a building or, when necessary, a separate building. It includes enhanced engineering controls (e.g., HEPA filtration of exhaust and supply air), specialized biosafety equipment (e.g., BSC, effluent decontamination systems), and redundant biosafety features (e.g., two stages of HEPA filtration of exhaust air). CL4 requires the maximum level of operational practices (e.g., PPE use, work practices, medical surveillance) that build upon those required at CL3. CL4 zones necessitate the use of positive-pressure suits for personnel or, as an alternative, the use of a Class III BSC line in a laboratory work area that meets the necessary CL4 requirements.

A representative diagram of a CL4 zone where positive-pressure suits are worn (including a laboratory work area, an **animal room**, an **animal cubicle,** and a **post mortem room [PM room]**) is provided in Figure 3-2. Features include anterooms with showers separating "clean" and "dirty" change areas, BSCs, and double-door autoclaves.

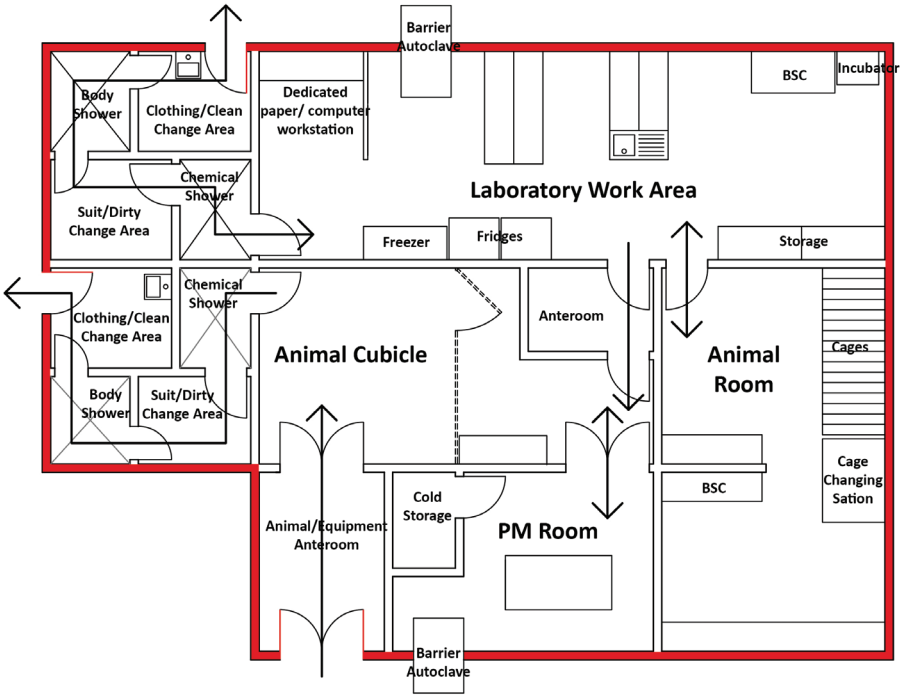


Figure 3–2:
Representative Diagram of a Containment Level 4 (CL4) Zone Where Positive-pressure Suits Are Worn.

The solid red line around the CL4 zone illustrates the containment zone perimeter. The thin black arrows across each doorway and through the anterooms indicate the direction of traffic flow for entry and exit.

3.2 Containment Zones

A containment zone refers to a physical area that meets the requirements for a specified containment level. This can be a single room (e.g., a laboratory) or a series of co-located rooms (e.g., several non-adjoining but lockable CL2 laboratory work areas), or it can be comprised of several adjoining rooms of the same containment level (e.g., CL3 suite comprised of dedicated laboratory work areas and separate animal rooms or cubicles). Dedicated **support areas**, such as anterooms (including showers and "clean" and "dirty" change areas, where required) are considered to be part of the containment zone, even though the **"clean" change area** is outside the **containment barrier** (see Section 3.3.2). A containment zone may include one or more work areas of different types (i.e., laboratory work area, large scale production area, animal work areas), as long as they are of the same containment level. The requirements for containment zones in facilities regulated by the PHAC and the CFIA are specified in the CBS.

3.2.1 Containment Zone Work Areas

The following different types of work areas describe, in general terms, where infectious material or toxins may be handled inside a containment zone. Each area is a designated area within the containment zone.

3.2.1.1 Laboratory Work Area

A laboratory work area is designed and equipped for performing ***in vitro*** activities, such as **scientific research, diagnostic activities**, commercial activities, or teaching, with infectious material or toxins. Samples of infectious material or toxins are handled for *in vitro* purposes only at volumes considered to be "laboratory scale" (i.e., generally less than 10 litres). Virus **propagation** in eggs can be conducted in a laboratory work area.

3.2.1.2 Large Scale Production Area

A large scale production area is designed specifically for the production (i.e., manufacture) of **large scale** volumes of infectious material or toxins for commercial activities, scientific research, or teaching purposes. Activities involving volumes of toxins or the *in vitro* **culture** of pathogens on a scale of 10 litres or greater are generally considered as large scale. A vaccine production facility is an example of a large scale production area. Large scale work is further described in Chapter 14.

3.2.1.3 Animal Work Area

An animal work area is designed specifically for ***in vivo*** activities with infectious material or toxins (i.e., activities involving whole living animals) for scientific research, diagnostic activities, teaching, or commercial purposes. Animal work areas include spaces specifically designed to house and handle living animals, and may also have designated areas to handle and store animal carcasses, such as a PM room. Animal containment zones describe containment zones that include multiple animal work areas (i.e., a containment zone that includes one or more animal rooms or animal cubicles). Animal containment zones are further described in Section 3.2.2.

3.2.2 Animal Containment Zones

A containment zone designed specifically for pathogen and toxin work performed *in vivo* (i.e., involving living animals) is referred to as an animal containment zone. A room designed to house animals in primary containment caging (i.e., filtered containment caging designed to prevent the release of infectious material and toxins) is referred to as an "animal room". A room or space designed to house an animal (or animals) where the room itself serves as **primary containment** is referred to as an "animal cubicle". In general, animal containment zones include a series of co-located animal rooms or animal cubicles, as well as anterooms, associated corridors and support rooms (e.g., storage and preparation areas) of equal containment level.

A zone where the animals are housed in primary containment caging inside animal rooms is termed a "small animal containment zone" (or SA zone). Alternatively, a zone where animals are housed in animal cubicles (i.e., the room itself provides the primary containment) is termed a "**large animal containment zone**" (or **LA zone**). LA zones may also include specific rooms within the containment zone where animal necropsies and dissections are conducted which are termed PM rooms. Since there are numerous additional physical containment and operational practice requirements necessary for animal containment zones where the room itself provides the primary containment (i.e., LA zones), the CBS distinguishes LA zones at CL2 and CL3 from other work areas and containment zones by designating them as CL2-Ag and CL3-Ag (i.e., CL2- and CL3-"Agriculture", respectively) in the requirements specified in Chapters 3, 4, and 5 of the CBS.

The designation as an SA zone or LA zone is dependent on the way in which the animal is housed (primary containment caging versus the room providing primary containment) rather than the actual physical size of the animal. In general, **large-sized animals** and **small-sized animals** are housed in LA zones and SA zones, respectively. In some cases, however, small-sized animals can be housed in an LA zone. For example, a room where small-sized animals, such as chickens, are housed in an open space inside a room or where small-sized animals, such as rodents, are housed in **open caging** only intended to restrict animals to an area (i.e., it does not include filtration to prevent the release of infectious material and toxins), the room itself provides primary containment, and it is therefore considered an animal cubicle (i.e., an LA zone), despite the actual size of the animals. Considerations for animal work are described in more detail in Chapter 13.

3.3 Identifying and Accessing a Containment Zone

It is essential to clearly identify the containment zone in order to determine compliance with respect to the corresponding requirements specified in the CBS. For example, without first identifying the containment barrier and the containment zone perimeter, it is impossible to determine the point(s) of entry and exit, to identify **critical doors**, or designate appropriate areas to don and doff PPE. Generally, in **high containment zones** (i.e., CL3 and CL4), the containment zone perimeter is determined during the design phase of the facility in order to adequately achieve all of the necessary physical containment requirements and to ensure the

appropriate physical containment and appropriate level of security to the facility. In contrast, for CL2 zones, in particular in older buildings that have not been recently renovated or updated, the determination of the containment zone perimeter can be more flexible. In CL2 and CL2 LA zones (i.e., CL2-Ag) where inward directional airflow (IDA) is not provided, there is no additional containment barrier created through the flow of air, and as such, the containment zone perimeter (doors and walls) acts as the physical containment barrier as well. Ultimately, it is the decision of the highest levels of the organization's structure (e.g., **senior management**, institutional biosafety committee) to determine how the containment zones within a facility are identified in consideration of security and access needs and challenges, as well as the **movement** and flow of materials and personnel, provided that compliance with the applicable requirements specified in the CBS are met. The concepts and considerations for containment zone perimeters and containment barriers are discussed below.

3.3.1 Containment Zone Perimeter

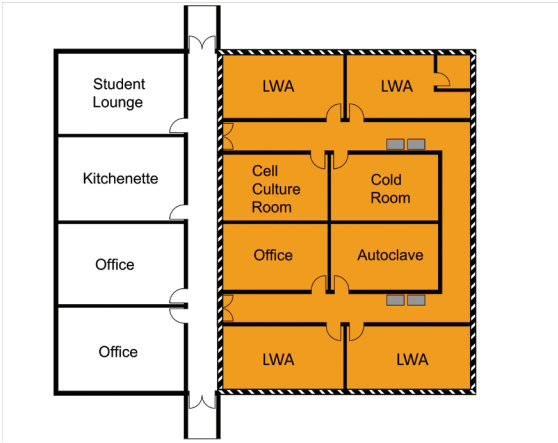
The containment zone perimeter refers to the outermost physical boundary of a containment zone (i.e., the walls, doors, windows, floors, and ceilings that enclose a single containment zone). There can be flexibility in identifying the containment zone perimeter, in particular for individual containment zones at CL2, as illustrated in Figure 3-3. In Figure 3-3(a), several co-located laboratories are grouped into a single CL2 zone, where all the rooms as well as the adjoining corridor connecting all of the rooms would be considered equal containment level (i.e., CL2). In this scenario, the containment zone perimeter follows the outer wall of this wing of laboratories. There are only two points of entry into/exit from the containment zone, which would require appropriate biohazard warning signage and closed, lockable doors, as per the physical requirements for access (CBS Matrix 3.3). In this example, the co-located office space (adjacent to the autoclave room) is located inside the defined containment zone and, consequently, all CL2 physical containment and operational practice requirements apply in order to demonstrate compliance within the zone. Figure 3-3(b), on the other hand, illustrates the same physical space of laboratories configured so that each room is a containment zone unto itself. In this scenario, there are seven separate CL2 zones identified, each with a single point of entry/exit (requiring lockable doors, biohazard signage, etc.). In Figure 3-3(b), the office space adjacent to the autoclave room is not identified as a containment zone and, therefore, is not required to meet any of the physical or operational requirements as specified in the CBS. Another point of interest is that the freezers located in the corridor in Figure 3-3(b) are located outside of the containment zone and would be required to be kept locked if they contain pathogens or toxins, and in some cases secured to the wall (e.g., if **security sensitive biological agents [SSBAs]** are present), to meet the requirements specified in the CBS. In contrast, the freezers in Figure 3-3(a), while in the same physical locations, are located inside the CL2 zone and do not require locks.

Figure 3-4 illustrates an example of biohazard warning signage that includes all of the elements required at the point of entry to a containment zone (i.e., the international biohazard symbol, containment level, name and telephone of contact person, and entry requirements [CBS Matrix 3.3]).

Figure 3-5 further illustrates the fluidity of the containment zone perimeter and the necessity to formally define the containment zone at CL2. In Figure 3-5(a) and (b), an office space is located next to a laboratory work area, accessible only through the laboratory itself. In Figure 3-5(a), the containment zone perimeter encompasses the laboratory work area as well as the office space, including both of these rooms in the CL2 zone. In this scenario, all of the physical containment and operational practice requirements for CL2 as specified in the CBS would apply equally to the laboratory work area and the office space (e.g., PPE requirements would apply in the office as well as in the CL2 laboratory work area). Figure 3-5(b), illustrates the same physical space; however, in this case, the containment zone perimeter is defined at the door to the office space. In this scenario, the office space is physically outside of the defined containment zone, and as such, the CBS requirements for CL2 would not be required for the office space. Lockable doors and other physical access requirements specified by the CBS for CL2 zones would need to be included at the entrance to the office and the appropriate operational practice requirements (e.g., exit procedures, doffing of PPE, and handwashing) would have to be followed when entering the office to be compliant with the CBS requirements. In a similar manner, entry procedures and PPE requirements would have to be followed when entering the CL2 zone from the office. It is also recommended in such a scenario, that the containment zone perimeter be visually indicated on the floor by demarcating it with a coloured line (e.g., coloured tape), to reinforce where the containment zone begins and ends. This configuration may not be achievable in CL2 work areas where inward directional airflow (IDA) is provided.

It is generally recommended to have floor plans of the containment zone and adjoining spaces, with the containment zone barrier and perimeter clearly marked, available to personnel. These can serve to assist in educating personnel and other individuals on the physical layout and the operational requirements of the containment zone; for ease of reference, these could be included in the Biosafety Manual.

(a) CL2 suite or wing.



(b) Individual CL2 work areas.

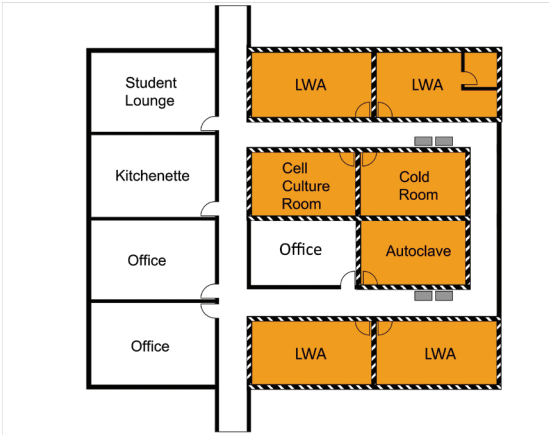


Figure 3–3:
Representative Diagrams of Different Containment Level 2 (CL2) Zones in the Same Physical Space.

Containment zones containing laboratory work areas (LWA) and support rooms are shown in orange; containment barriers are illustrated by a black hatched line. Freezers for storage of pathogens and toxins are indicated by grey rectangles. Configuration (a) illustrates a single CL2 suite or wing. Configuration (b) illustrates the same physical location where each work area is identified as a distinct CL2 zone. Note that a common cell culture room is considered a LWA.

AUTHORIZED PERSONNEL ONLY

THIS CONTAINMENT ZONE CONTAINS INFECTIOUS MATERIALS OR TOXINS

BIOHAZARD

CONTAINMENT LEVEL:_____

PRIMARY CONTACT PERSON: _____

PHONE NUMBER: _____

ALTERNATE EMERGENCY CONTACT: _____

PHONE NUMBER: _____

ENTRY REQUIREMENTS: _____

_____

_____

_____

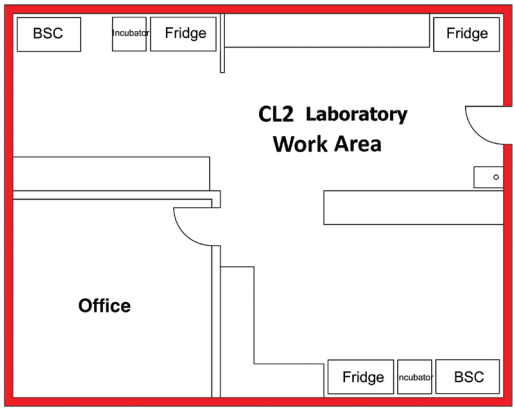
OPTIONAL INFORMATION TO INCLUDE IN BIOHAZARD WARNING SIGN:

- **ADDITIONAL SPECIAL PROVISIONS FOR ENTRY**
- **LIST OF RELEVANT PROCESS (ES) AND PRIMARY CONTAINMENT EQUIPMENT USED IN LARGE SCALE PRODUCTION AREAS**

Figure 3–4: Representative Biohazard Warning Signage.

Example of biohazard warning signage found at the points of entry to a containment zone. Biohazard warning signage must include the international biohazard warning symbol, containment level, name and telephone numbers of a contact person, and entry requirements (CBS Matrix 3.3). The sign may be further supplemented with additional requirements for entry, a list of relevant processes and primary containment equipment used in large scale production areas, or information on other hazards (e.g., chemical, radioactive) present in the containment zone.

(a) Office inside the CL2 zone.



(b) Office outside the CL2 zone.

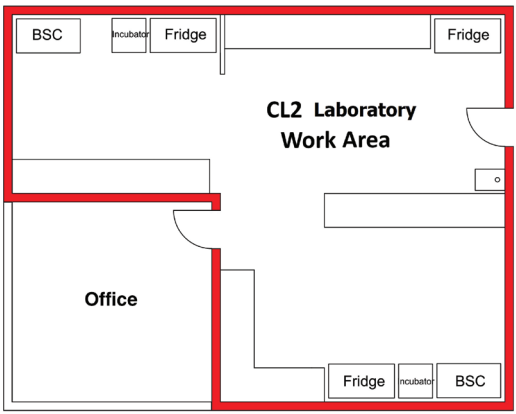


Figure 3–5:
Representative Diagrams of Placement of Offices with Respect to Adjoining Containment Level 2 (CL2) Zone.

Configuration (a) illustrates a CL2 zone that includes both the laboratory work area and office space. Configuration (b) illustrates the same physical location where the office is excluded from the containment zone. This configuration requires additional elements such keeping the office door closed and following appropriate PPE protocols for entry to and exit from the office, in order to be compliant with the CBS requirements.

3.3.2 Containment Barrier

The containment barrier, which refers to the boundary between "clean" and "dirty" areas inside a containment zone, is sometimes distinct from the containment zone perimeter. In some containment zones, most notably in high containment zones, a physical containment barrier of air is achieved through inward directional airflow (IDA) created by the presence of differential air pressures. This effect creates a physical barrier of air against the release of airborne or aerosolized infectious material and toxins through the door.

The containment barrier is always encountered at or inside the containment zone perimeter. In CL2 zones where inward directional airflow (IDA) or anterooms are not present, the containment barrier often overlaps with and corresponds to (i.e., is indistinguishable from) the containment zone perimeter; however, in high containment zones, the containment barrier is encountered inside the containment zone at the point where the "clean" change area of an anteroom in a CL3 or CL4 zone meets the walk-through body shower. This is illustrated in Figure 3-6 in the context of a CL3 zone.

In some containment zones, it is possible to have multiple containment barriers inside the same containment zone. For example, in a large CL3 LA zone (i.e., CL3-Ag), that encompasses several animal cubicles to accommodate activities involving several different pathogens, it is likely that containment barriers would be established at each animal cubicle and PM room, as well as establishing a containment barrier at the entry/exit to the entire CL3 LA zone (Figure 3-7). This configuration helps prevent the spread of **contamination** from animal cubicles and PM rooms, protects against cross-contamination of experiments, and protects personnel. In this case, personnel entering an animal cubicle from outside the containment zone would cross, at minimum, two containment barriers.

In contrast, the containment barrier of CL2 zones that do not require inward directional airflow (IDA) (e.g., laboratory work areas and SA zones) may be identified by a line on the floor demarcating the "clean" area from the "dirty" area. Unless otherwise identified, the containment barrier of most CL2 laboratory work areas would correspond to the containment zone perimeter.

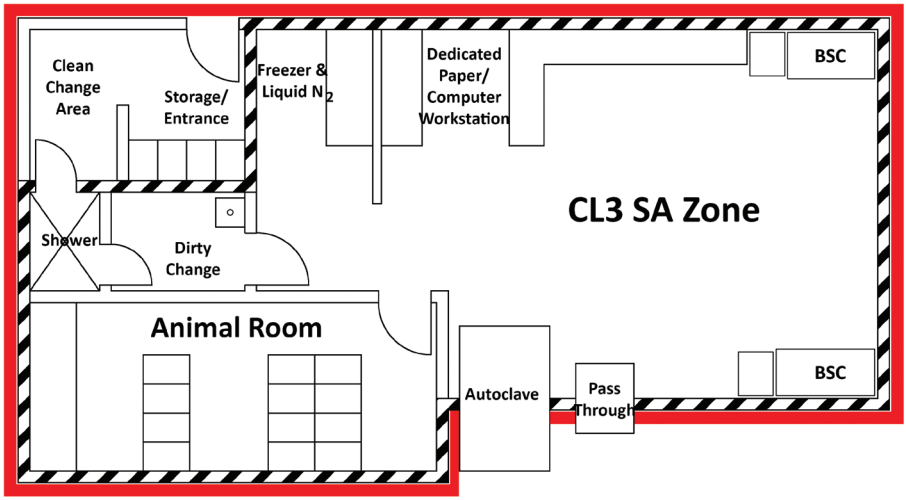


Figure 3–6:
Representative Diagram of a Containment Level 3 (CL3) Small Animal Containment Zone (SA zone) Indicating the Containment Barrier and the Containment Zone Perimeter.

The containment barrier is indicated with a black hatched line and the containment zone perimeter is shown with a solid red line.

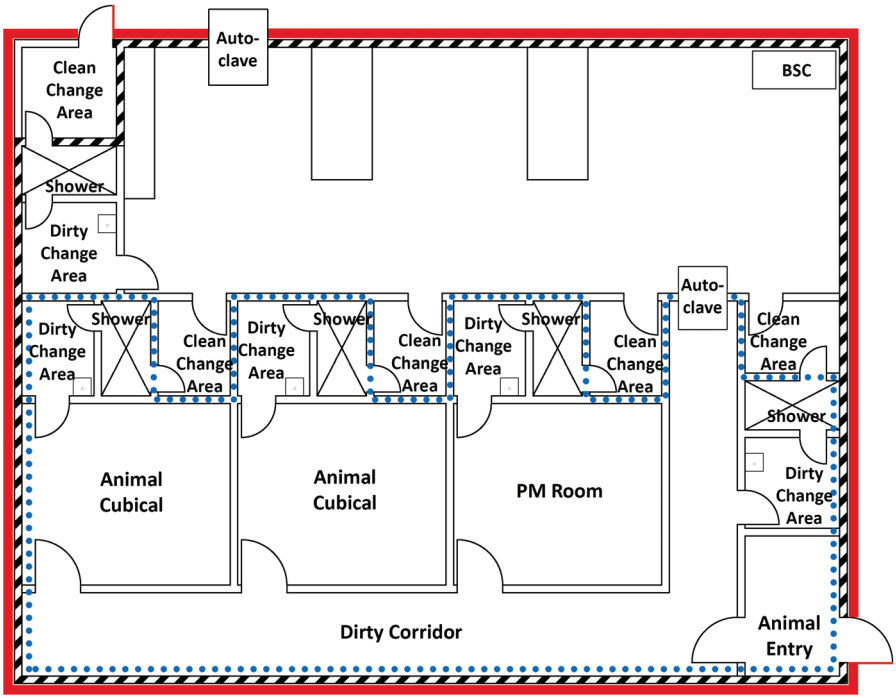


Figure 3–7:
Representative Diagram of a Containment Level 3 (CL3) Large Animal Containment Zone (LA zone) that Includes Multiple Containment Barriers.

The containment barrier of the containment zone is indicated with a black hatched line. The inner containment barrier (animal cubicles) is indicated with a blue dotted line and the containment zone perimeter by a solid red line.

3.3.3 Accessing the Containment Zone: Anterooms

An anteroom is a room or series of rooms inside the containment zone that separates "clean" areas from "dirty" areas (i.e., areas with a lower risk of contamination from those with a higher risk of contamination), for personnel and animal entry/exit across the containment barrier, and for entry to and exit from animal rooms, animal cubicles, and PM rooms. The presence of an anteroom at the point of entry or exit through a containment barrier creates an added buffer space to protect the outer environment from the infectious material and toxins handled within. An anteroom may also provide appropriate space at the entry/exit point(s) to don, doff, and store dedicated containment zone protective clothing and additional PPE, as required.[2,3]

3.3.3.1 Anteroom Configurations

The size and complexity of the anteroom(s) is dependent on the design and activities of the containment zone itself. In the most basic configuration, it can consist of a single room situated between an area free of contamination, which is not designated for activities involving infectious material or toxins (e.g., a public access corridor), and a "dirty" or contaminated area where infectious material or toxins are handled or stored. The anteroom(s) can serve as an access point into the work area for personnel, animals, material, and equipment. This single anteroom configuration may be incorporated into some CL2 zones, such as large scale production areas, SA zones, and LA zones. It should be noted, however, that not all containment zones at CL2 require an anteroom at the point of access. The CBS can be consulted to determine which work areas and containment zones require an anteroom.

Configurations for anterooms in CL3, CL3 LA zones (i.e., CL3-Ag), and CL4 zones are more complex and include a series of areas or rooms to prevent the spread of contamination from dedicated PPE and to prevent the migration of potentially contaminated air from the work area across the containment barrier by maintenance of inward directional airflow (IDA). Separating the "clean" and "dirty" change areas in anterooms with a walk-through body shower allows personnel to shower upon exit across the containment barrier to reduce the risk of release of infectious material contaminating the skin or hair. In CL3 zones where non-indigenous animal pathogens are not handled or stored, a **local risk assessment (LRA)** may be conducted to determine when a shower is or is not needed upon exit, based on the daily activities. For example, showers (upon exit) may be needed in cases where there is a risk of exposure to aerosolized or **airborne pathogens** (e.g., biological spill), or substantial contact with infected animals. Showers are always required upon exit from a containment zone actively handling non-indigenous animal pathogens.

Likewise, anterooms for CL4 zones where positive-pressure suits are worn can be very complex and may include not only "clean" and "dirty" change areas with a walk-through body shower, but also a suit change room (which may or may not be distinct from the "dirty" change area) and a chemical decontamination shower.

The location of the individual spaces within the anterooms is very important and dependent on the exit sequence required for personnel exiting the containment zone. Proper design of anterooms prevents the spread of contamination beyond the containment barrier, safeguards the individual's safety, and protects against a potential exposure to the chemical decontaminants in use.

3.3.3.2 Door Interlocks and Inward Directional Airflow

Inward directional airflow (IDA) is an essential component to maintain biocontainment in containment zones where it is required (CBS Matrix 3.5). The careful design and use of an anteroom on the containment barrier is the best way to protect the negative differential air pressures creating inward directional airflow (IDA), thereby protecting the integrity of the containment barrier. A critical door describes any door located directly on the containment barrier of a containment zone, animal cubicle, or PM room where inward directional airflow (IDA) is required. In order to maintain biocontainment and to prevent the reversal of inward directional airflow (IDA) (i.e., the migration of air from inside the "dirty" work area to the "clean" areas or outside the containment zone), the critical door must not be opened simultaneously with other doors, specifically the door leading into the anteroom from outside of the containment zone and the door(s) leading from the anteroom into the work area (i.e., laboratory work area, animal room, animal cubicle, PM room, or large scale production area). In CL3 LA zones (i.e., CL3-Ag) and CL4 zones, mechanical or electronic door **interlocks** are required to prevent the simultaneous opening of the critical doors with other anteroom doors that could result in a breach of containment (CBS Matrix 3.3). In CL2 LA zones (i.e., CL2-Ag) and CL3 zones, operational and administrative controls (e.g., **standard operating procedures [SOPs]** and appropriate signage) may be used in place of mechanical or electronic door interlocks to effectively prevent the simultaneous opening of critical doors with other key doors that could result in the release of contamination. There may be multiple critical doors identified in a containment zone, depending on containment zone design.

Figure 3-8 illustrates anteroom spaces providing entry from an access corridor into a CL3 work area through a series of 3 doors, and describes the combinations of doors that, in its design, must be prevented from opening simultaneously in order to maintain the containment barrier.

Figure 3–8:
Representative Diagram of Anteroom Spaces in a Containment Level 3 (CL3) Zone Identifying the Critical Door.

The containment barrier is indicated by the black hatched line and the containment zone perimeter is shown with a solid red line. Door "A" leads to the "clean" change area of the anteroom from outside the containment zone. Door "B", identified in yellow, is the critical door that separates the "clean" and "dirty" change areas. The "X" indicates the location of a walk-through body shower in the "dirty" change area. Door "C" leads to the CL3 work area (i.e., laboratory work area, animal room, animal cubicle, PM room, or large scale production area). In order to mitigate the migration of air from the "dirty" change area to the "clean" change area or outside the containment zone into the access corridor, critical door "B" would have to be interlocked or otherwise protected against simultaneous opening with door "A" (i.e., "A+B/B+A"). In order to mitigate the migration of air from the CL3 work area through the "dirty" change room and into the "clean" change area, critical door "B" would have to be interlocked with or otherwise protected against simultaneous opening with door "C" (i.e., "B+C/C+B").

REFERENCES

[1] Government of Canada. (2015). *Canadian Biosafety Standard* (2nd ed.). Ottawa, ON, Canada: Government of Canada.

[2] Canadian Council on Animal Care. (2003). *CCAC Guidelines on: Laboratory Animal Facilities - Characteristics, Design and Development*. Ottawa, ON, Canada: Canadian Council on Animal Care.

[3] Subhash, S. S., Baracco, G., Fennelly, K. P., Hodgson, M., & Radonovich, L. J. Jr. (2013). Isolation anterooms: Important components of airborne infection control. *American Journal of Infection Control*. 41:452-455.

CHAPTER 4

RISK FACTORS, RISK GROUPS, AND RISK ASSESSMENTS

CHAPTER 4 – RISK FACTORS, RISK GROUPS, AND RISK ASSESSMENTS

"Risk" is a function of the probability of an undesirable event occurring and the consequences of that event. For the safety of the community, it is essential to mitigate risks through various mechanisms, such as administrative and engineering controls, practices, and procedures. Risk assessments are conducted for many components of a biosafety program, including the evaluation of individual, community and environmental safety, biosecurity requirements, training needs, and regulatory compliance. Local risk assessments (LRAs), pathogen risk assessments, and toxin risk assessments are discussed in this chapter; overarching risk assessments and biosecurity risk assessments are discussed in Chapters 5 and 6, respectively. The requirements for risk assessments in facilities regulated by the Public Health Agency of Canada (PHAC) and the Canadian Food Inspection Agency (CFIA) are specified in Matrix 4.1 of the *Canadian Biosafety Standard* (CBS), 2nd Edition.[1]

4.1 Pathogen and Toxin Risk Assessments and Risk Groups

The PHAC and the CFIA conduct pathogen and toxin risk assessments to determine the risk group (RG) of a pathogen or toxin, which is in turn used to determine the appropriate containment level to safely conduct laboratory work and other activities with the pathogen or toxin. Risk assessments on well-characterized human pathogens are developed into technical documents by the PHAC known as Pathogen Safety Data Sheets (PSDSs) that are readily available to regulated and interested parties; fact sheets for federally reportable diseases affecting terrestrial animals in Canada have also been developed by the CFIA and are readily available on the CFIA website. Regulated parties are also encouraged to conduct pathogen and toxin risk assessments, especially on uncharacterized or modified pathogens and toxins. The PHAC and the CFIA can assist individuals in their pathogen and toxin risk assessments as necessary.

4.1.1 Conducting Pathogen and Toxin Risk Assessments

Pathogen and toxin risk assessments are based on three key elements: science, policy, and expert judgement. There is a qualitative component to a pathogen and toxin risk assessment, so a consistent approach should be used when determining risk groups, and uncertainties and assumptions should be clearly documented. In order to appropriately address all of the elements, individuals with varying expertise and responsibilities (e.g., facility director, principal investigator, senior microbiologist, biological safety officer [BSO], and institutional biosafety committee [IBC] members) should be included in the pathogen and toxin risk assessment process. Pathogen and toxin risk assessments should be reviewed routinely and revised when necessary to take into consideration relevant new information.

The pathogen risk assessment characterizes the risks associated with a pathogen based on the close examination of the following risk factors, which are the inherent characteristics of a pathogen that contribute to the risk it poses to humans and different animal species (risk factors for toxin risk assessments are discussed in Section 4.3.1):

- ***Pathogenicity*** *and* ***Virulence***: Is the pathogen able to infect and cause disease in humans or animals (i.e., pathogenicity)? What is the severity of disease in individuals or in different animal species (i.e., virulence; the degree of disease)?
- *Route of Infection:* How does the pathogen gain entry into hosts (i.e., ingestion, inhalation, inoculation, contact with skin or mucous membranes, or genitourinary)?
- *Mode of Transmission:* How does the pathogen travel to hosts? Is the pathogen transmissible by direct contact (e.g., close intimate contact or casual contact) or indirect contact (e.g., fomites, aerosolized droplets or airborne transmission)? Can the pathogen be transmitted by vectors or **zoonosis**?
- *Survival in the Environment:* How stable is the pathogen outside the host? Under which environmental conditions can it survive and for how long?
- ***Infectious Dose:*** What amount of pathogen is required to cause an infection in the host (measured in number of organisms)?
- *Availability of Effective Preventive and Therapeutic Treatments:* Are effective preventive measures available (e.g., vaccines)? Are effective treatments available (e.g., antibiotics, antivirals)?
- *Host Range:* What are the primary, intermediate, and dead-end hosts? Does the pathogen cause infection in a wide range of species, or is the host range more restricted?
- *Natural Distribution:* Is the pathogen present in Canada or is it exotic to Canada (i.e., non-indigenous)? Is it prevalent in a particular location, region, or human or animal population?
- *Impact of Introduction and/or* ***Release*** *into the Environment or the Canadian Public:* If the pathogen were introduced into the human or animal population or released into the environment (within Canada), what would be the economic, clinical, and biosecurity impact?

While most **infectious material** will clearly fall into one of the four risk groups outlined below, in some cases, the level of risk associated with the different risk factors can vary dramatically within a risk assessment. As a result, certain risk factors may be considered more important when determining the final risk group category. For example, if a pathogen is unlikely to cause disease in humans or animals, it may be irrelevant that it can survive in the environment for a long period of time or that there is no available treatment.

4.1.2 Risk Groups

It is very difficult to develop a comprehensive list of human and **animal pathogens** due to the emergence of new pathogens and the ongoing research into the characteristics of existing pathogens. Examples of human pathogens are included in Schedules 2-4 of the *Human Pathogens and Toxins Act* (HPTA), according to risk group; some of these pathogens are **zoonotic pathogens**.[2] Schedule 5 includes an exhaustive list of human pathogens prohibited in Canada. Examples of **terrestrial animal pathogens** can be found on the CFIA website. The following definitions provide the risk group categorization for both human and animal pathogens based on the risk to an individual human or animal, and the risk to health of the community.

4.1.2.1 Risk Group 1 (RG1; low individual and community risk)

A **microorganism**, nucleic acid, or protein that is either a) not capable of causing human or animal disease; or b) capable of causing human or animal disease, but unlikely to do so. Those capable of causing disease are considered pathogens that pose a low risk to the health of individuals or animals, and a low risk to public health and the animal population. RG1 pathogens can be opportunistic and may pose a threat to immunocompromised individuals. Due to the low risk to public health and animal population associated with RG1 material, there are no physical or operational requirements for handling them. Nonetheless, due care should be exercised and safe work practices (e.g., **good microbiological laboratory practices**) should be followed when handling these materials.

4.1.2.2 Risk Group 2 (RG2; moderate individual risk, low community risk)

A pathogen or toxin that poses a moderate risk to the health of individuals or animals, and a low risk to public health and the animal population. These pathogens are able to cause serious disease in a human or animal but are unlikely to do so. Effective treatment and preventive measures are available and the risk of spread of diseases caused by these pathogens is low. Examples of RG2 human pathogens are included in Schedule 2 of the HPTA.

4.1.2.3 Risk Group 3 (RG3; high individual risk, low community risk)

A pathogen that poses a high risk to the health of individuals or animals, and a low risk to public health. These pathogens are likely to cause serious disease in a human or animal. Effective treatment and preventive measures are usually available and the risk of spread of disease caused by these pathogens is low for the public. The risk of spread to the animal population, however, can range from low to high depending on the pathogen. Examples of RG3 human pathogens are included in Schedule 3 of the HPTA.

4.1.2.4 Risk Group 4 (RG4; high individual risk, high community risk)

A pathogen that poses a high risk to the health of individuals or animals and a high risk to public health. These pathogens are likely to cause serious disease in a human or animal, which can often lead to death. Effective treatment and preventive measures are not usually

available and the risk of spread of disease caused by these pathogens is high for the public. The risk of spread of disease to the animal population, however, ranges from low to high, depending on the pathogen. Examples of RG4 human pathogens are included in Schedule 4 of the HPTA.

4.2 Containment Assessments

Once the risk group has been determined by a pathogen and toxin risk assessment, there are several key factors to determine the appropriate level of **containment** at which the identified pathogen or toxin can be safely handled. Well-characterized pathogens that have had a pathogen risk assessment completed by the PHAC or the CFIA have already been assigned an appropriate risk group and containment level. The different containment levels are described in Chapter 3 and the requirements for containment level 2 (CL2) to containment level 4 (CL4) facilities that are regulated by the PHAC and the CFIA are specified in the CBS. In general, the containment level and risk group of the pathogen are the same (e.g., RG2 pathogens are handled at CL2); however, there are some exceptions. If the pathogen has been modified the containment requirements may need to be revised accordingly. These containment level changes reflect the risk mitigation strategies to address the specific modification of the pathogen.

Any change or addition to the **controlled activities** specified on a **licence** under the *Human Pathogens and Toxins Regulations* (HPTR) requires an amendment to be submitted to the PHAC prior to making the change.[3] Also, any change to the conditions of use specified on the **animal pathogen import permit** requires authorization from the appropriate issuing agency (i.e., the CFIA or the PHAC) prior to making the change. Further information on licences and animal pathogen import permits can be found in Chapter 23.

The following factors are considered when conducting a containment assessment (i.e., determining the specific **physical containment requirements**, **operational practice requirements**, and **performance and verification testing requirements**) for a pathogen:

- *Aerosol Generation:* Are equipment or procedures that may generate aerosols (e.g., pipetting, centrifugation, homogenization) being used? Personnel can be exposed to infectious aerosols or aerosolized toxin by direct inhalation of aerosolized droplets or by ingestion of droplets that settle on surfaces or hands.
- *Quantity:* What quantity of pathogen is being manipulated, and in what format (e.g., one large vessel, multiple small vessels)? **Large scale** processes (e.g., industrial fermentation, vaccine production) may have different containment requirements than laboratory scale work using the same pathogen.
- *Concentration of the Pathogen:* The concentration of the pathogen may vary depending on the work being performed (e.g., diagnostic specimens may contain a lower concentration of pathogen than pure **cultures**).

- *Type of Proposed Work:* What is the nature of the work (e.g., diagnostic activities, scientific research, *in vitro*, ***in vivo***, large scale)? For example, for *in vivo* work, the type of animal (e.g., host versus non-host species) and the inherent risks associated with that animal need to be considered when determining the appropriate containment level.
- *Shedding (specific to animals):* The shedding of pathogens should be considered when working with infected animals. Pathogens may be present in the saliva, urine or feces, and may also be exhaled by the animal. Due to the nature of zoonotic pathogens, additional precautions may need to be implemented whenever known or potentially infected animals are handled.

Some factors considered when determining the risk group may also be evaluated in the context of the containment assessment. For example, the concentration of the pathogen being handled may have less importance if the infectious dose is very high. On the other hand, aerosol generation becomes more important for pathogens transmitted via the inhalation route.

4.3 Special Considerations

Not all **biological material** will fall perfectly into a given risk group or containment level following a risk assessment. This could be the case for biological material (e.g., tissues, primary specimens) that may harbour pathogens, toxins, **prions**, or pathogens that have been modified or constructed. It is important to reiterate that an LRA be performed to determine the appropriate level of precautions to be taken for infectious material that is manipulated in a **containment zone**. Factors that should be considered when assessing the risks associated with activities involving these types of material are described below.

4.3.1 Toxins

Microbial toxins are not considered to be infectious material, nor can they be classified as standard toxic chemicals; therefore, special considerations are needed when performing a risk assessment on this type of material. When compared to microbial pathogens, it is fairly easy to control the spread of toxins. Toxins do not replicate and are not transmitted from host to host. The most likely route of transmission to personnel handling toxins is through accidental inoculation or by the **exposure** of mucous membranes to aerosols. Additional risks associated with toxins include static electricity when handling dried and lyophilized toxins and the minute lethal doses of some toxins.

Only certain microbial toxins, whether naturally derived from a microorganism or synthetically produced, are regulated by the PHAC and the CFIA under the HPTA, HPTR, *Health of Animals Act* (HAA) and *Health of Animals Regulations* (HAR).[4,5] An exhaustive list of the toxins regulated by the PHAC under the HPTA is included in Schedules 1 and 5 of the HPTA. The importation of microbial toxins derived from animal pathogens is regulated under the HAR; the importation of toxins derived from **non-indigenous animal pathogens** is regulated solely by the CFIA.

Certain regulated toxins are identified in the HPTR as "prescribed toxins" when present in a quantity greater than the identified **trigger quantity**, as described by Section 10(2) of the HPTR due to their **dual-use potential** (summarized in Table 4-1). "Prescribed toxins" identified by the HPTR are also referred to as **security sensitive biological agents (SSBAs)**, as they have additional biosecurity considerations. A toxin present in a part of a facility in a quantity greater than the trigger quantity is considered an SSBA and requires enhanced security measures (e.g., ***Human Pathogens and Toxins Act*** **Security Clearances [HPTA Security Clearances]**). A toxin in a total quantity at or below the trigger quantity is not considered an SSBA; however, it remains a toxin, and is subject to the CBS (i.e., the minimum containment level for handling a regulated toxin is CL2). SSBAs are discussed in further detail in Section 4.3.3 and Chapter 6.

Table 4–1: Summary of Prescribed Toxins and Associated Trigger Quantities. Reproduced from the HPTR 10(2).[3]

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

The principles of chemical safety and biosafety are both applicable when handling biological toxins capable of causing disease in humans or animals. In general, toxins capable of producing human and animal disease are safely handled in CL2; therefore, CL2 is the minimum containment level for a facility, such as a biochemistry laboratory, where activities with purified or chemically synthesized regulated toxins are performed in the absence of the parental microorganism or other pathogen.

4.3.1.1 Risk Factors for Toxin Risk Assessments

When handling toxins derived from microorganisms, a detailed risk assessment should include the following:

- exposure assessment to identify risks inherent to the procedure being performed (e.g., inoculation risk, aerosol generation, buildup of static electricity when handling powdered toxins);
- routes of exposure (i.e., ingestion, inhalation, absorption – dermal and ocular, and inoculation);
- concentration and amount of toxin being handled, and units of activity;
- indicators of toxicity;
  - LD_{50} (**median lethal dose**; amount of toxin that is lethal to 50% of the test population);
  - ED_{50} (**median effective dose**; amount of toxin that will cause a particular effect in 50% of the test population);
- rate of action (how long after exposure before effects are observed);
  - the effects of most neurotoxins are typically observed within minutes to hours after exposure;[6]
  - the effects of most cytotoxins are typically observed within hours to days after exposure;[6]
- severity and duration of illness (acute versus chronic effects);
- availability of vaccines or antitoxins;
- use of chemical safety practices appropriate to the techniques used (i.e., solvents, acids); and
- identification as a "prescribed toxin" as described by Section 10(2) of the HPTR and the associated trigger quantity (summarized in Table 4-1).

4.3.2 Prions

The primary routes of infection for prions are through ingestion and inoculation (characteristics similar to RG2 pathogens). Conducting risk assessments for prions is challenging due to the long incubation times (up to 30 years) before disease symptoms appear in human or animal hosts.[7,8,9,10,11,12] While the primary routes of prion transmission have been documented through

ingestion or inoculation, there is limited evidence to suggest that alternate routes may also be possible.[7,13,14,15] There have been no **laboratory acquired infections (LAIs)** documented to date from activities involving prions.[16] There are currently no treatments or vaccines available for transmissible spongiform encephalopathy (TSE) diseases. The protein structures of prions are very stable under extreme environmental conditions and they have been shown to remain infectious following standard treatment to inactivate other pathogens (e.g., autoclaving).[17,18] Activities involving prions can generally be conducted safely at CL2 with additional physical containment requirements and operational practice requirements, which are specified in the CBS. Additional recommendations and considerations for the **decontamination** of prions are described in Chapter 15.

4.3.3 Security Sensitive Biological Agents

SSBAs are human pathogens and toxins that have been determined to pose an increased biosecurity risk due to their dual-use potential. That is, they can be used for legitimate scientific applications, but they pose an increased biosecurity risk due to an inherent potential for development and use as a biological weapon. SSBAs are the human pathogens and toxins that are identified as "prescribed human pathogens" and "prescribed toxins" in the HPTA and HPTR. Prescribed human pathogens are all RG3 and RG4 human pathogens that are on the *List of Human and Animal Pathogens and Toxins for Export Control*, published by the Australia Group (as amended from time to time), except for Duvenhage virus, Rabies virus and all other members of the Lyssavirus genus, Vesicular stomatitis virus, and Lymphocytic choriomeningitis virus.[19] Prescribed toxins are all toxins listed in Schedule 1 of the HPTA that also appear on the *List of Human and Animal Pathogens and Toxins for Export Control*, published by the Australia Group (as amended from time to time), when in a quantity greater than the identified trigger quantity in a part of the facility where controlled activities with SSBAs are authorized, as described by Section 10(2) of the HPTR (prescribed toxins and trigger quantities are summarized in Table 4-1). Consequently, there are increased biosecurity requirements specified in the CBS for SSBAs. For ease of reference, the PHAC maintains an exhaustive list (as amended from time to time) of all SSBAs, including toxin trigger quantities, on the PHAC website (http://phac-aspc.gc.ca/lab-bio/regul/ssba-abcse-eng.php). Additional biosecurity considerations for SSBAs are discussed in Chapter 6.

4.3.4 Non-indigenous Animal Pathogens

Non-indigenous animal pathogens are pathogens that are listed in the World Organisation for Animal Health's *OIE-Listed diseases, infections and infestations* (as amended from time to time) and that are also exotic to Canada (i.e., foreign animal disease agents that are not present in Canada).[20] **Emerging animal disease** pathogens cause new infectious diseases resulting from the evolution or change of an existing pathogen, a known infectious disease spreading to a new geographic area or population, or a previously unrecognized pathogenic agent or disease diagnosed for the first time and which has a significant impact on animal health. Non-indigenous animal pathogens and emerging animal disease pathogens may only be imported into Canada under an animal pathogen import permit issued by the CFIA.

The appropriate containment level and any additional requirements to work with certain animal pathogens (e.g., non-indigenous animal pathogens and emerging animal disease pathogens) are determined by the CFIA through a pathogen risk assessment and containment level assessment. Factors that are assessed by the CFIA when evaluating a request to work with a non-indigenous pathogen include the following:

- disease control and potential impacts to the health of the Canadian animal population (including livestock and poultry) in the event of a breach of containment; and
- consideration of international practices.

The release of a non-indigenous animal pathogen or emerging animal disease pathogen into the environment could have a serious negative impact on the Canadian animal population; therefore, additional physical containment and operational practices beyond CL3 (or CL3 LA zones [CL3-Ag]) are required when handling these pathogens. As an example, Matrix 3.8 of the CBS specifies an **effluent decontamination system** is required in containment zones where non-indigenous animal pathogens are handled, so that all liquid **waste** is appropriately decontaminated prior to release from the containment zone.

Applicants of an animal pathogen import permit for non-indigenous animal pathogens or emerging animal disease pathogens are subject to **facility certification** by the CFIA before an animal pathogen import permit is issued. Activities involving non-indigenous animal pathogens and emerging animal disease pathogens that are also zoonotic pathogens (i.e., capable of causing disease in humans and animals) also require a licence under the HPTA issued by the PHAC. Facility certification by the CFIA and regulatory oversight for the importation of animal pathogens are further described in Chapter 23.

4.3.5 Parasites

Transmission of a parasite can occur via a wide range of mechanisms that closely mirror those of other human and animal pathogens. When performing an LRA to determine the appropriate containment level for parasites, the mode of transmission as well as the parasite life cycle stages should be considered, since not all stages are infective or pathogenic.

4.3.6 Large Scale Work

The PHAC and the CFIA generally consider activities involving volumes of toxins or the in vitro culture of infectious material on a scale of 10 litres or greater to be large scale; this could be a single vessel with a volume of 10 litres or greater, or in some cases, multiple vessels with a total volume of 10 litres or greater. Large scale production facilities such as industrial fermentation and vaccine production plants pose an increased risk to personnel and the environment due to the large quantities of infectious material or toxins being handled. As such, there are sometimes more stringent requirements specified in the CBS and additional considerations for large scale activities when compared to **laboratory work areas** handling laboratory scale volumes of the same pathogen at the same containment level. Consultation with the PHAC and the CFIA on a case-by-case basis will determine whether or not particular activities conducted in a containment zone are considered large

scale and, therefore, required to meet all of the additional requirements specified in the CBS specific for **large scale production areas**. Additional considerations for large scale work are described in Chapter 14.

4.3.7 Animal Work

Because of the unpredictable behaviour of animals and the potential for shedding of pathogens, working with pathogens in live animals may significantly increase the risk associated with any given procedure. Specific considerations for work involving pathogens and toxins with animals are provided in Chapter 13.

4.3.8 Biotechnology

4.3.8.1 Modifications that May Increase the Risk of a Pathogen

It is important for researchers to recognize the risks inherent to their activities and to take appropriate steps to mitigate those risks. Although the risk group and containment level may have been determined for a particular pathogen, modifications to a pathogen that increase risks posed by the pathogen may result in changes to specific physical containment or operational practice requirements from what is specified in the CBS. Modifications may be intentional (e.g., through the use of recombinant DNA [rDNA] technology) or incidental (e.g., resulting from pathogen evolution following passage through an *in vivo* model).

Experiments that decrease the risk posed by a pathogen (i.e., it has been attenuated) may be acceptable to be conducted with reduced physical containment and operational practice requirements. In these circumstances, a pathogen risk assessment is conducted to assign the attenuated pathogen to an appropriate risk group and containment level. For example, an attenuated strain of an RG3 pathogen may be determined to meet the risk profile for an RG2 pathogen and may be determined to be safe to handle at CL2, based on a pathogen risk assessment. In licensed facilities authorized to conduct controlled activities with human pathogens and toxins, the licence holder or the BSO is required to notify the PHAC in cases where the risk group of a human pathogen or toxin is modified, to initiate a discussion about the new risk classification of the modified strain and, if necessary, apply for a new licence or amend an existing licence (HPTA 12[2], HPTR [9][1][c][ii]).

Experiments that increase the risks posed by a pathogen can impact both the researcher and the community. For example, modifying a pathogen to become transmissible by the airborne route would increase the risks inherent in certain laboratory procedures that generate aerosols, as well as the impact on public or animal health if released from the laboratory. The HPTR Section 5 requires that the BSO and the licence holder be notified whenever an individual working under a licence authorizing controlled activities with human pathogens and toxins under the HPTA/HPTR intends to increase the virulence or pathogenicity of a human pathogen or the toxicity of a toxin. If such a modification changes the risk group of the pathogen or toxin, the PHAC is also to be notified (HPTR 26). Notifications can be submitted electronically to the PHAC through the Biosecurity Portal, accessible through the PHAC website (www.publichealth.gc.ca/pathogens), by telephone, email, or fax.

4.3.8.2 Genetically Modified Organisms

The use of rDNA technologies, to create genetically modified organisms (GMOs) may increase or decrease the risk group and containment level relative to the risk group and containment level of the parental organism, depending on factors such as the gene(s) being transferred, the modification to genes already present in the organism (e.g., point mutations, deletions), the expression of the gene(s) in the recombinant organism, the biological containment required for handling the host organism, the interactions between the gene(s) being transferred and the host vector systems, and the viability of the host vector systems.

An LRA is performed to appropriately assess the physical containment and operational practice requirements when the genetic manipulations that are performed:

- alter the pathogenicity or virulence of pathogens;
- affect the response of a pathogen to a pharmaceutical agent (e.g., changes in resistance to antibiotics);
- delete genetic material or introduce novel genetic material with potentially adverse effects (e.g., insertion of an oncogene);
- induce the production of toxins by recombinant microorganisms;
- broaden the host range or cell tropism of pathogens;
- create novel mechanisms or undesirable traits in transgenic animals;
- produce attenuated strains of recombinant pathogens that have lost virulence factors; or
- produce host bacterial or viral vector systems with limited ability to survive outside the containment zone.

Factors to consider when assessing GMOs include the following:

- containment level of the recipient organism;
- containment level of the donor organism;
- replication competency of the GMO;
- property of the donor segment incorporated into the recombinant particle;
- potential pathogenic factors associated with the donor segment; and
- novel hazards of the GMO that may not be well-characterized.

4.3.8.3 Viral Vectors

The risks associated with viral vector systems can be assessed by examining the considerations for GMOs outlined in Section 4.3.8.2, along with the choice of vector system, the safety features engineered into the system, and the nature of the transgene insert(s) encoded by the vector. The use of retroviral vector systems, including lentiviral vectors derived from human immunodeficiency virus type 1 (HIV-1), raises other possible risks that should be assessed.

More recent generations of lentiviral vector systems have been engineered with additional safety features to reduce the potential for production of replication competent retrovirus (i.e., a recombinant virus capable of self-replication), including the use of heterologous coat protein in place of native HIV-1 envelope protein, vector and packaging functions separated onto four or more plasmids, removal of genes essential for replication of wild type virus and the development of self-inactivating lentiviral vectors.[21]

The major risks involving viral vector systems include:

- potential for generation and **propagation** of replication competent retrovirus (RCR);
- potential for oncogenesis;
- potential for increased pathogenicity; and
- potential for **seroconversion**, even with non-replicating viruses (e.g., indication of HIV positive status resulting from exposure to a lentiviral vector).

4.3.8.4 Synthetic Biological Devices and Systems

The risks associated with devices and systems resulting from **synthetic biology** and synthetic DNA (sDNA) technologies are similar to the risks associated with GMOs and rDNA technologies. The principal difference is that synthetic biology seeks to redesign existing, or to design and construct novel biological functions and systems that are not found in nature, and, as such, assessing the potential risks associated with products of synthetic biology is somewhat more complex. As a first step, the risks associated with the parental organisms should be assessed. The properties and potential impacts of the individual parts, systems, or organisms with which researchers will be working (engineered or naturally occurring) also need to be assessed given that pathogenic and non-pathogenic organisms will each potentially contain both non-pathogenic and pathogenic components.

The risks of synthetic biology stem from: the organism manipulated (i.e., the chassis), the type and origin of the genetic material added, and the added risks of different components working in concert. Synthetic biology has the additional inherent risk that this type of research may have dual-use potential or the potential for misuse, whether intentional or not. Prior to performing experiments, consideration should be given not only to the source of the components, but to the potential of synergistic effects leading to a more pathogenic organism. This is often difficult to quantify when combining elements from multiple sources, combining elements that have never existed together in a natural organism, or when developing a biological function that does not exist in nature. Finally, an assessment of all the components working together as a new single system needs to be completed. Possibly the greatest challenge is identifying the risks associated with sDNA added to such organisms, and the possible unexpected interactions resulting from the expression of the engineered genome.

4.3.9 Infectious RNA

Purified positive-sense viral RNA is capable of causing infection and subsequent generation of complete, functional viruses in host cells.[22] Consequently, it is necessary to exercise additional care when manipulating genomic material of positive-sense RNA viruses. Examples of RNA viruses that produce infectious positive-sense RNA capable of causing disease in humans include poliovirus and hepatitis C virus.[23] Examples in animals include foot-and-mouth disease virus and classical swine fever virus.[24,25] West Nile virus is an example of a zoonotic virus with a positive-sense single stranded RNA genome.[23] An LRA should include the following considerations before handling infectious positive-sense viral RNA:[26]

- the efficiency of infection with positive-sense viral RNA is lower when compared to infection with whole virus particles;
- RNA can withstand significantly higher temperatures than proteins, which means that infectious positive-sense RNA can be extracted from heat-inactivated viruses;
- the DNA copy of certain RNA viruses is also infectious (e.g., poliovirus, retroviruses);
- the infectivity of positive-sense viral RNA is unaffected by virus-specific antibodies; and
- infectious single-stranded positive-sense viral RNA may have increased tropism (i.e., cell type and host range) when compared to whole virus particles.

4.3.10 Cell Lines

Cell lines are not considered infectious material, except when they harbour pathogens. Information on cell lines regulated by the PHAC and/or the CFIA can be obtained by contacting them directly or visiting their websites. The majority of cell lines are well-characterized and should be handled at the containment level of the pathogens they contain, if any. An LRA should be conducted prior to working with cell lines that are known to contain pathogens or potentially contaminated with pathogens, in order to determine the containment level appropriate for the contaminating organism of the highest risk group. One of the primary hazards of manipulating any cell line relates to the expression of latent viruses. Endogenous viral sequences have been found in a variety of cell lines derived from mammalian species, including humans. When handling non-recombinant cell lines, the risk assessment should include the following:

- *Source Organism:* cell lines derived from human or non-human primate tissue generally pose a greater potential risk for personnel than those derived from animals that are not closely related to humans;
- *Source Tissue:* provides an indication of possible contaminants and latent (e.g., oncogenic) viruses;
- *Type of Cell Line:* less will be known about primary cultures and lab-generated continuous cell lines than intensively characterized commercial cell lines; and
- *Source Population:* the particular breeding group or colony of the organism from which the cell line was derived may have a higher risk for some pathogens.

With respect to the handling of recombinant or genetically modified cell lines, the risk assessment should include the following considerations, 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 should be considered;
- vector used for transformation;
- transfer of viral sequences;
- transfer of virulence factors;
- activation of endogenous viruses;
- recombinant gene product; and
- presence of helper virus.

4.3.11 Primary Specimens

Primary specimens are those derived directly from a human or animal. The HPTA and HPTR do not apply to human pathogens or toxins that are in an environment in which they naturally occur (i.e., in primary specimens). It should be noted, however, that specimens obtained from an animal that has been intentionally exposed to a human pathogen or toxin (e.g., experimental infection or inoculation) are subject to regulation under the HPTA and HPTR. Additionally, the HAA and HAR apply to any imported primary specimen that contains an animal pathogen or part of one that retains its pathogenicity, regardless of whether its presence is naturally occurring or not.

Pathogens may be transmitted from symptomatic and asymptomatic individuals.[27] Accordingly, it is always prudent that primary specimens, such as blood, blood components (e.g., serum, plasma), other bodily fluids (e.g., urine, feces, saliva, milk), or tissues taken from human or animal subjects that have been exposed to a pathogen or toxin, be considered as potentially infectious.

Routine practices are infection control guidelines developed by the PHAC for health care settings to protect individuals from exposure to potential sources of pathogens.[27] Routine practices and universal precautions to prevent the transmission of pathogens through occupational contact with human tissue or bodily fluids are discussed in Chapter 21.[28,29]

Diagnostic activities involving primary specimens that do not involve propagating, concentrating or purifying the pathogen (e.g., enzyme-linked immunosorbent assay [ELISA], extraction of genetic material, fixation of tissue samples for histology) are regularly carried out in hospitals, public health laboratories, and veterinary diagnostic laboratories. In most cases, the risks associated with this type of work are considered lower than propagation and *in vivo* work. Based on the risks associated with the pathogen suspected of being within the primary specimen and the laboratory procedures, the physical containment and operational requirements for activities with primary specimens may sometimes be lower than the requirements for handling pure cultures (i.e., derogated). Although the PHAC and the CFIA assign containment levels for pathogens, the CBS is performance-based, allowing

facilities to use LRAs to determine the mitigation strategies for their activities dependent on the situation. In situations where a sample is suspected to contain a pathogen from a risk group higher than the containment level of the testing facility, additional operational practices or **transfer** to a facility with an appropriate containment level may be required, and can be determined in consultation with the PHAC and the CFIA.

4.3.12 Autologous Cells, Tissues, and Specimens

The experimental infection of cells, tissues, or other specimens derived from the person conducting the experiment may endanger that individual. Such practices are described as "self-to-self" experimentation and are prohibited (CBS Matrix 4.6). Any procedure being conducted by an individual that involves the *in vitro* transformation or some other genetic modification of cells derived from his or her own body (i.e., **autologous cells**) could result in the development of malignant disease (e.g., if the cells are modified to express an oncogene) or the expression of an unusual protein with pharmacological properties (e.g., if the cells are modified to express a toxin). Such experiments put the individual at risk since any innate immune protection that is normally available to destroy foreign cells would be bypassed. Personnel should not conduct these types of experiments in laboratory areas or containment zones in which they work and they should never donate or collect their own specimens or tissues, or those of any other personnel, within the containment zone.

4.3.13 Handling Risk Group 1 Biological Material

The regulations administered by the PHAC and the CFIA do not apply to RG1 organisms; therefore, the CBS does not specify requirements for these activities. Nonetheless, RG1 biological material poses a low risk to the health of individuals or animals and this material should be handled safely in a basic laboratory or **animal work area**. Due care should be exercised and safe work practices (e.g., good microbiological laboratory practices) are strongly encouraged when handling these materials. Work with RG1 material is further discussed in Chapter 21.

4.4 Risk Management

Risk management of human and animal pathogens and toxins involves understanding the legislative requirements related to conducting activities with such material (e.g., **importing**, handling, and possessing), as well as the abilities of the individuals concerned and the limitations of the facilities where the material is being handled and stored. Pursuant to the applicable legislation (i.e., HPTA, HPTR, HAA, and HAR), organizations that handle or store human or animal pathogens or toxins are required to comply with the CBS and may be subject to inspection by the PHAC or the CFIA. The risks associated with pathogens and toxins are managed through ensuring compliance with the applicable legislation and periodically conducting LRAs. It is the responsibility of facility personnel to complete an LRA specific to their containment zone and related processes (CBS Matrix 4.1), although the BSO or other designated individual may be consulted. The PHAC or the CFIA may also be consulted when determining the risk group and containment level for a particular pathogen. Administrative controls and roles and responsibilities are discussed further in Chapter 5.

The process to undertake risk assessments for infectious material and toxins follows the same principles as those found to address hazards or risks in most occupational health and safety programs.[30] The accepted mechanisms to control an identified occupational health and safety hazard apply to biosafety and biosecurity as well. These controls are:

- *Elimination (including substitution):* Is there a pathogen or process that poses less of a risk than the one selected that will provide the same result?
- *Engineering Controls:* This includes the selection and use of **primary containment devices** (e.g., **primary containment caging**, **biological safety cabinet [BSC]**, closed vessels, and heating, ventilation, and air conditioning [HVAC] systems).
- *Administrative Controls:* These are the controls that can alter the way in which the tasks are done and can include policies and **standard operating procedures (SOPs)**.
- ***Personal Protective Equipment (PPE):*** The PPE selected and worn by individuals to reduce or minimize the potential exposure to infectious material or toxins.

Safety legislation and other safety resources often refer to this list as the hierarchy of control, meaning that the controls should be considered in the order they are presented. PPE should be the last form of control considered when conducting the LRAs.

4.4.1 Local Risk Assessments

LRAs are site-specific risk assessments that are conducted to identify hazards based on the pathogen, infectious material, or toxin in use and the activities to be performed. They examine specific elements of the biosafety program and may support the broader overarching risk assessment. Personnel who work in the containment zone with the infectious material and toxins are in the best position to provide input for an LRA to identify the hazards associated with their day-to-day activities and potential measures to mitigate risks. Depending on the nature of the procedure or tasks to be performed, there may be other hazards (e.g., chemical, radiological, physical) involved that may necessitate a broader task analysis as part of a larger or general health and safety program. In this case, the LRA to assess the biosafety risks involved with infectious material and toxins may already be incorporated into or included in the broader hazard analysis. The BSO should be involved in the development of the LRA. If the organization has an IBC, as described in Chapter 5, it would be beneficial for the committee to also be involved. They could also liaise with the PHAC and the CFIA for more information or to confirm the outcome of their assessment.

4.4.1.1 Identification of Tasks and Procedures

The first step of an LRA is to identify the tasks and procedures where infectious material and toxins will be used within the containment zone. The potential for the infectious material or toxins to cause harm to the personnel, the community, and the environment should also be assessed. It is important to include all of the known and potential risks associated with the work and the infectious material or toxins. This stage of an LRA is critical since it is not possible to effectively determine the risk associated with any hazard unless the activity with which it will be used has been properly identified.

4.4.1.2 Break Down Tasks into Steps

All containment zone activities involving infectious material and toxins should be described. Examples of activities include large scale production, diagnostic work, and *in vivo* work with **small-sized animals** or **large-sized animals**. It is important to break down the tasks identified within the activities into steps, in order to minimize the amount of work needed for each LRA and assess the actual risk effectively. If only one step has been modified or altered in a certain task from the steps identified in a previous LRA, the impacts of the one step would need to be assessed. The quantity and concentration of infectious material or toxins used during the activity is also critical to understanding the potential risks in each step.

4.4.1.3 Identify Potential Exposure Risks for Each Step

Risk (i.e., the probability of an undesirable event occurring and the consequences of that event) can be characterized based on the infectious material or toxins in use and the activities being performed. Each task (or step) will entail a different potential of leading to an exposure (e.g., sonication has a high probability of generating aerosols). The potential for exposure will also depend on the pathogen's characteristics (e.g., pathogenicity, virulence, route of transmission), the form of the pathogen (e.g., liquid culture, solid matrix, lyophilized spores), and the quantity of pathogen (e.g., amount, volume, concentration). Assigning probabilities and consequences to all possible combinations of these factors will allow the related risk for each step to be determined. Figure 4-1 provides a matrix that can help visualize the concept by plotting probability and consequence to identify the risk.

4.4.1.4 Determine Appropriate Mitigation Strategies for Each Risk

In the context of LRAs, risk mitigation strategies are biosafety practices that are put into place to minimize the identified risk. The mitigation strategies selected should always be appropriate to the level of risk. The controls listed in Section 4.4 of this chapter provide the order in which the controls should be considered and assessed. Examples of these strategies include the use of primary containment devices, SOPs outlining the use of good microbiological laboratory practices and appropriate decontamination practices, and the use of appropriate PPE. These strategies should be developed, implemented, and regularly reviewed and updated. In some cases, the concept of acceptable risk may also come into play. Acceptable risk is based on the premise that zero risk is unachievable, and a tolerable or "acceptable" level of risk is determined by a risk assessment. If the risks associated with the infectious material, toxins, or activities concerned are deemed to be too high, the project may need to be modified or cancelled.

Risk Level

Likelihood of occurrence	Very Low	Low	Moderate	High	Very High
Very High	Moderate	High	High	High	Very High	Very High
High	Low	Moderate	High	High	High	Very High
Moderate	Low	Low	Moderate	High	High	High
Low	Low	Low	Low	Moderate	High	High
Very Low	Very Low	Low	Low	Low	Moderate	High
	Very Low	Very Low	Low	Low	Low	Moderate

Consequences of occurrence

Figure 4–1: Risk Assessment Matrix

Risk can be evaluated by plotting the likelihood of an event occurring and the impact of that event, should it occur.

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CHAPTER 5

BIOSAFETY PROGRAM MANAGEMENT

CHAPTER 5 – BIOSAFETY PROGRAM MANAGEMENT

A **biosafety** program is designed to prevent **infections** and illnesses among personnel and to protect the public, the environment, and animal population from harm by preventing the inadvertent **release** of **pathogens** or **toxins**. An effective biosafety program will promote and reinforce safe work practices, improve safety performance, and increase regulatory compliance through a combination of training, documentation, inspections, evaluation, review, and clear communication. A biosafety program may include a **biosecurity** component, or have a separate biosecurity program, to protect against the theft, loss, or intentional misuse of pathogens, toxins, or other **infectious material**. The management of a biosafety program involves ensuring all aspects of the biosafety program are in place. The requirements for biosafety program management in **facilities** regulated by the Public Health Agency of Canada (PHAC) and the Canadian Food Inspection Agency (CFIA) are specified in Matrix 4.1 of the *Canadian Biosafety Standard* (CBS), 2nd Edition.[1]

The level of detail and complexity of the biosafety program will depend on the nature (i.e., size, structure, complexity) of the organization and the activities performed by it. In organizations that carry out limited activities with pathogens, toxins, or infectious material, the task of developing a biosafety program may simply entail broadening the scope of an existing safety program to incorporate their **facility's** specific biosafety needs. In more complex organizations such as universities, it may be necessary to have biosafety personnel dedicated to ensure that the goals of the biosafety program are met. Although some more complex facilities may choose to maintain their biosafety and biosecurity components as two separate programs, biosecurity is included as a component of a biosafety program within the CBS and this document. This chapter outlines considerations on how a biosafety program can be effectively managed as well as the core elements of a biosafety program, which are further explored in the following chapters.

5.1 Administrative Controls

Integral to the success of any biosafety program is a strong commitment and involvement by everyone within the organization, including **senior management**, supervisors, the **biological safety officer (BSO)**, and individual personnel. Administrative controls implemented from the highest levels of the organizational structure (i.e., senior management) by means of policies and procedures help protect workers throughout the organization from **exposure** to human and **animal pathogens** and toxins. The roles and responsibilities for a successful biosafety program are outlined in this section. The specific administrative controls required at the operational level are specified in Matrix 4.1 of the CBS for facilities regulated by the PHAC or the CFIA. The *Plan for Administrative Oversight in a Research Setting* (see Appendix A), required for any applicant for a licence for controlled activities with human pathogens and toxins who intends to carry out **scientific research**, is a tool that can be used to document the administrative controls already in place.

5.1.1 Biosafety Policy

A high-level biosafety policy or code of practice, which is specific to the institution, is encouraged; it could be a standalone biosafety policy, code, or plan, or a biosafety policy integrated into a pre-existing health and safety policy or plan. The biosafety policy can outline senior management's commitment to biosafety, the guiding principles, the applicable biosafety and biosecurity standards (e.g., CBS, International Organisation for Standardization [ISO], Organisation for Economic Co-operation and Development [OECD] *Principles of Good Laboratory Practice*, Canadian Council on Animal Care [CCAC]) and legislation (i.e., *Human Pathogens and Toxins Act* [HPTA], *Human Pathogens and Toxins Regulations* [HPTR], *Health of Animals Act* [HAA], *Health of Animals Regulations* [HAR], and applicable federal/provincial/territorial legislation), the protection of personnel, the program objectives, accountabilities and responsibilities, and the consequences and disciplinary actions for deliberate or repeat non-compliance.[2,3,4,5,6,7,8] A policy can establish the **internal accountability system** with respect to pathogens, toxins, and other regulated infectious material (e.g., infected or intoxicated animals, animal products or by-products that contain a pathogen or toxin). Communicating the policy to all personnel is essential in ensuring that all workers are aware and informed of their responsibilities as well as the consequences for non-compliance. Pathogen and toxin accountability and the internal accountability system are further discussed in Chapter 19.

5.1.2 Program Intent

The **program intent** describes the planned work to be performed in a **containment zone** and serves to document the scope of a facility's activities. It includes the pathogens, toxins, and other regulated infectious material the facility plans to use. Documenting the types of activities conducted in the facility at the broadest level (e.g., what type of business is conducted, such as academic/instructional, hospital/health care, public health surveillance, environmental surveillance, veterinary/animal health, research and development, manufacturing or production) is a good starting point for outlining the program intent. From there, the scope of work for the activities planned inside the facility, including ***in vitro*** activities with pathogens and toxins (e.g., routine **diagnostic activities**, scientific research, or **large scale** production), and ***in vivo*** activities with pathogens and toxins (e.g., work with small-sized animals or large-sized animals). Where *in vivo* work with pathogens and toxins is to be performed, the program intent includes a list of all animal species that will be used. The program intent can be documented at an organizational level; although in some cases where diverse activities are conducted within the same organization or facility (e.g., universities), it may be appropriate to develop a program intent at the level of the containment zone.

In containment zones where **non-indigenous animal pathogens or emerging animal disease pathogens** are handled, changes to program intent (e.g., introduction of a new pathogen or toxin or introduction of a new animal species) or to **standard operating procedure (SOPs)** that may impact **biocontainment** or biosafety are submitted to the CFIA prior to implementing the change to allow the CFIA to confirm the changes are acceptable in order to maintain **containment** (CBS Matrix 4.1).

5.1.3 Roles and Responsibilities

In most organizations, senior management is the ultimate authority and is responsible for delegating appropriate authority for biosafety. Senior management is also responsible for ensuring that adequate resources are available to support the biosafety program and compliance with legal requirements, and for ensuring that biosafety concerns are appropriately prioritized and addressed. Senior management establishes policies and practices that describe the reasonable precautions to be undertaken for the prevention of the release of pathogens and toxins. They also play a role in the continuous improvement and relevance of the biosafety program.

Managers and supervisors are responsible for ensuring that personnel comply with biosafety related legislation. Where **controlled activities** involving human pathogens and toxins are authorized by a **licence** under the HPTA, the individual identified as the licence holder is ultimately accountable for activities carried out with the pathogens and toxins in a licensed facility. All personnel conducting controlled activities with human pathogens and toxins in a licensed facility have specific responsibilities laid out in the HPTA and HPTR (summarized in Section 1.3 of the CBS).

Where animal pathogens or toxins have been imported to a facility in Canada under an **animal pathogen import permit** under the HAA and HAR, the individual identified as the importer on the permit is ultimately accountable for the imported pathogens, toxins, or other regulated infectious material. All personnel handling imported animal pathogens, toxins, and other regulated infectious material in a facility authorized to import or receive imported material via **transfer**, under an animal pathogen import permit under the HAA and HAR have specific responsibilities (summarized in Section 1.4 of the CBS). It remains the responsibility of the regulated party to understand their obligations under the HPTA, HPTR, HAA, and HAR, as applicable, and regulated parties are encouraged to refer to the specific sections of the legislation for a complete understanding of the requirements.

Whenever possible, a senior officer from within the organization (i.e., senior management) should be identified as the licence holder and/or importer so that the appropriate institutional oversight of the pathogens, toxins, and other infectious material may be allocated. Pathogen and toxin accountability is further discussed in Chapter 19.

5.1.4 Biological Safety Officer

The biosafety representative, or BSO, is an individual with the appropriate knowledge who has been delegated the responsibility for the oversight of biosafety and biosecurity practices, including the organization's biosafety program. In many facilities, the biosafety representative or BSO is a role that can be assigned to a qualified person who performs these duties on a part-time basis (e.g., senior microbiologist, **laboratory** technician) or that can be assigned to a qualified person on a full-time basis, as determined by the organization. In some large organizations, there may be several individuals carrying out the functions of the biosafety representative or BSO to manage the key elements of the

biosafety program. The HPTA and HPTR have been designed to reflect the key role played by BSOs in risk reduction in institutions **handling and storing** human pathogens and toxins and are designed to provide the designated BSO with the authority and controls necessary to facilitate their work, by specifying qualifications, functions, and power of a BSO.

The biosafety representative or BSO must have the knowledge appropriate to the **risks** associated with the activities being performed with pathogens and toxins in the facility, possess knowledge of the HPTA, HPTR, HAA, and HAR, and any other applicable federal or provincial/territorial legislation, possess knowledge of the **containment levels** in the facility, as well as knowledge of the applicable biosafety and biosecurity policies, standards, and practices appropriate to the risks associated with the activities being performed with pathogens and toxins in the facility. It is encouraged that the BSO updates or improves his or her knowledge of biosafety-related topics on a regular basis. This enables the BSO to remain well informed and current in areas related to managing the risks associated with the pathogens and toxins handled or stored within the organization. In accordance with Section 36 of the HPTA, a designated BSO must be identified in a facility that is applying for a licence to conduct controlled activities with human pathogens and toxins. Section 8 of HPTR further specifies the qualifications of the designated BSO in licensed facilities.

The BSO plays a key role in assisting scientific and technical staff in navigating the administrative and regulatory obligations associated with biosafety. The responsibilities of a BSO are distinct from those of the occupational health and safety committee. The biosafety representative, or designated BSO, is responsible for oversight of biosafety and biosecurity practices, including the overall management of the biosafety program, which may include policy development, implementing the program, monitoring compliance, conducting risk assessments and internal inspections/audits, overseeing and documenting biosafety-related training, assisting in investigations of **incidents**, compiling and providing any reports/documentation, as required by the regulatory agencies, and maintaining liaison with containment zone personnel, support staff, housekeeping personnel, and contractors on biosafety-related matters, as well as the continual improvement of the program (CBS Matrix 4.1; HPTR 9[1]). The biosafety representative or designated BSO is also responsible to verify the accuracy and completeness of licence applications or renewals, animal pathogen import permit applications, and transfer requests, as well as to communicate with the PHAC and the CFIA on behalf of the licence holder or animal pathogen import permit holder, as appropriate. Although the responsibilities of a BSO may be separate from that of the occupational health and safety committee, it is recommended that the BSO be a part of the committee in order to provide the appropriate safety linkages. Section 9 of the HPTR further specifies the functions and power of the designated BSO in licensed facilities.

A change in the designated BSO may occur for a number of reasons, such as a change in employment or a prolonged absence (e.g., sabbatical, parental leave). Licence holders are required to notify the PHAC without delay when the designated BSO has changed (HPTA 36[6]). Consequently, considerations should be made within the organization to determine circumstances when a new BSO needs to be designated and the PHAC notified. It may be beneficial to identify alternate biosafety contacts to handle the day-to-day BSO responsibilities during short-term absences of the designated BSO (e.g., vacation).

5.1.5 Institutional Biosafety Committee

An institutional biosafety committee (IBC) can also be involved in the management of a biosafety program. The BSO, or biosafety representative, should liaise with the IBC through regularly scheduled meetings and should present specific biosafety problems, concerns and policy/protocol improvements to be considered and addressed. The IBC can assist the BSO with risk assessments, biosafety protocol reviews and approvals, disputes about biosafety matters, or other biosafety or biosecurity concerns. Careful consideration should be given to the composition of the IBC, which should include several individuals with varying expertise. It is recommended that the IBC include at least one member from the research or technical staff, a representative from management, a medical advisor who can be consulted as required, and the BSO. Depending on the facility, other members may be included, such as facility technical staff or an animal care technician.

5.2 Risk Assessments and Planning

Risk assessments are conducted to identify hazards and appropriate mitigation strategies, and to evaluate whether or not existing mitigation measures are commensurate with the level of risk. There are many types of risk assessments related to handling pathogens and toxins, one of which is the **overarching risk assessment**, described below. A comparison should be made with requirements specified in the CBS Chapters 3, 4, and 5, as well as existing best practices, to clearly identify gaps that need to be addressed.

The first step in conducting a biosafety or **biosecurity risk assessment** is always to determine the pathogens, toxins, and other infectious material that are present, or planned to be present, so that the associated risks can be determined and addressed. It may be necessary to review existing **inventories** (including material in **long-term storage**), and research proposals in order to fully build an accurate perspective of the risks present in the facility. A survey of the facility in the context of planned program activities should also be conducted to identify the containment levels of existing laboratories and any gaps in facility design and engineering controls based on applicable regulations, standards, and guidelines. Determining the existence of shared laboratory workspace within the facility (e.g., multiple investigators, multiple agents, and various organizations) is a consideration for this survey as these spaces may have an impact on the way the biosafety program is managed. There are several additional international biosafety standards and guidelines available that may provide further assistance for best practices and performing biosafety and biosecurity risk assessments.[9,10,11,12] Other types of risk assessments that are intended to be more focused in scope are discussed in Chapter 4 (**pathogen risk assessments** and **local risk assessments [LRAs]**) and Chapter 6 (biosecurity risk assessments).

5.2.1 Overarching Risk Assessments

When developing a biosafety program, an overarching risk assessment is conducted to identify the hazards and appropriate mitigation management strategies (CBS Matrix 4.1). The overarching risk assessment process is a broad assessment that supports the biosafety program as a whole and may encompass multiple containment zones within an institution or organization.

The overarching risk assessment identifies hazards through a systematic review of the type of **biological material** that is present, including the identification of personnel who are handling it, the locations where the material is handled and stored, and the activities being conducted (e.g., routine diagnostic activities, scientific research, large scale, recombinant work, animal work). It helps identify the most important biosafety issues and provides an opportunity to assign resources where they can be the most effective. An overarching risk assessment informs the development of biosafety program risk mitigation strategies, which may include the use of engineering and administrative controls, practices and procedures, and training. This assessment includes a wide-ranging analysis of the hazards and possible exposure or release scenarios, which may involve examining factors such as the full spectrum of the different types of work to be performed, and the various equipment and procedures required. An overarching risk assessment provides a top-down view of the risks associated with the biosafety program, and may be supported by LRAs, which are more focused assessments to examine specific elements of the program.

The overarching risk assessment may also include a risk communication plan designed to effectively address public concerns about the risks associated with the facility and its operation. An effective risk communication plan is proactive and begins at the early planning stages of facility construction and continues after operation begins. This may include early engagement of, and open communication with, the public. Trust, transparency, and availability of information that will not compromise biosecurity are integral elements of a successful risk communication plan, and public engagement should be maintained throughout the lifetime of the facility.

5.3 Implementation of a Biosafety Program

While biosafety programs will differ from one organization to another, there are a number of common core program elements that must be present. These building blocks, when assembled upon a foundation of strong management commitment and planning, will provide a solid framework for an effective biosafety program. The complexity of any specific program element will depend on the outcome of the overarching risk assessment and the nature of the organization and its activities. An organization or institution may decide to incorporate their biosafety program into an existing management system to limit duplication and increase efficiencies.

5.3.1 Biosafety Manual

A **Biosafety Manual** containing institutional policies, programs, and plans is to be developed, implemented, and kept up to date (CBS Matrix 4.1). The Biosafety Manual is the most common and effective tool for documenting the biosafety program and describing how the organization/facility will achieve the goals and objectives of the program. It is also one of the most effective tools to make personnel aware of the hazards, risks, mitigation strategies, emergency response, and safe work practices, and which personnel can consult as the need arises to review updates or refresh their memory on these issues. Depending on the detail and complexity of the program, the Biosafety Manual may be a standalone document or be incorporated into one or more general health and safety manuals within the organization. A description of the biosafety program itself, as well as each of the core elements described below, are included in the Biosafety Manual so that all personnel are aware of how the program is structured and the responsibilities relevant to all personnel.

5.3.2 Biosecurity Plan

A biosecurity plan is to be developed and implemented by facilities where pathogens or toxins are handled or stored to describe and outline the security measures designed to prevent the loss, theft, misuse, diversion, or intentional release of pathogens and toxins from the facility (CBS Matrix 4.1). Further details on biosecurity are provided in Chapter 6.

5.3.3 Medical Surveillance and Evaluation Program

A **medical surveillance program** is to be developed, implemented, and kept up to date for facilities where pathogens or toxins are handled or stored (CBS Matrix 4.2). The basic purpose of this program is to help prevent illnesses related to exposure of laboratory personnel to infectious material or toxins, and to detect such illnesses when they occur, in order to protect the health of the **community**. Further details and considerations related to medical surveillance and evaluation programs are described in Chapter 7.

5.3.4 Training Program

A training program, based on a **training needs assessment**, is to be developed, implemented, evaluated, and improved and updated as necessary in order to identify current and future training needs of the facility, and gaps in the current training program (CBS Matrix 4.3). Training is a core element of the biosafety and biosecurity programs so that personnel are adequately informed on the risks associated with the pathogens and toxins with which they will work, as well as the approved safe work practices and mitigation strategies. Further details and considerations related to training programs are outlined in Chapter 8.

5.3.5 Safe Work Practices and Standard Operating Procedures

Good microbiological laboratory practices, described in Chapter 21, lay the foundation for all safe work practices involving infectious material (CBS Matrix 4.6). All procedures that will involve potentially infectious material or toxins are assessed to ensure that safe work practices have been established (CBS Matrix 4.1). Safe work practices can be documented in SOPs so that they can be easily understood and implemented by all personnel.

SOPs are detailed, step-by-step procedures that are introduced during training, and that are read prior to performing the procedure for the first time, for refamiliarization with procedures that are performed infrequently, and whenever the SOP is amended. They provide documentation that can be reviewed by internal or external auditors, and can facilitate evaluation of compliance with program requirements. Safe work practices and SOPs specific to the containment zone (e.g., **personal protective equipment [PPE]**, entry and exit procedures, and **waste** management) are developed to address specific biosafety issues for the containment zone and added to the Biosafety Manual so that they are documented and accessible for all containment zone personnel (CBS Matrix 4.1).

5.3.6 Emergency Response Planning

An **emergency response plan (ERP)** outlines the action(s) to be taken in the event of situations such as a spill, exposure, release of pathogens or toxins, infected animal escape, personnel injury or illness, power failure, fire, explosion, or other emergency situations (e.g., flood, earthquake, hurricane) (CBS Matrix 4.9). The ERP should take into account the physical structure of the building as well as the location (e.g., exposure to weather extremes, earthquakes, floods). This type of plan is for protecting human and animal health and safety as well as safeguarding property and the environment. Further details on ERPs are described in Chapter 17.

5.3.7 Regulatory Compliance

Regulatory compliance requires an understanding of the relevant legislation and regulations, including the HPTA and HPTR where human pathogens and toxins are handled and stored, as well as the HAA and HAR where imported animal pathogens and toxins are handled and stored. It is therefore important for any facility where pathogens or toxins are handled or stored to establish a liaison, through the BSO or other biosafety representative, with the applicable regulatory bodies, including the PHAC, the CFIA, and other Canadian governmental, non-governmental, provincial or territorial, and municipal authorities, as required. Further details on regulatory oversight are provided in Chapter 23.

5.4 Measuring Program Effectiveness

A management system outlines the framework of processes and procedures that can be applied by an organization to meet specific goals. In general, management systems follow a cycle of planning, implementing, measuring, and improving (or the "Plan-Do-Check-Act cycle", as described by the ISO), whereby the management system itself is continually improved.[2]

In order for any management system to be effective, its performance should be tracked and measured against the program's goals and objectives. For a biosafety program, that would include the following:

- how infections and illnesses among personnel are being prevented;
- how a release of pathogens or toxins is being prevented;
- how compliance with legislation is being achieved; and
- how safety is being promoted.

Internal mechanisms are put in place to determine how the biosafety program is functioning; performance measurements provide qualitative and quantitative information that can be collected and analyzed to evaluate the program's success. The tools described below are commonly used to evaluate a biosafety program.

5.4.1 Incident Reporting and Investigations

Incident reports, subsequent investigations, and corrective actions can provide an indication of biosafety program effectiveness by identifying deficiencies and gaps in procedures or in the program itself. Incident reports and investigations are required in specific situations such as upon discovery of a **laboratory acquired infection/intoxication (LAI)**, an exposure, or a failure of a **containment system** or device. Incidents can serve as an indicator of a program's success, even though they are generally under-reported and thus challenging as a quantifiable measure. Additional information on incident reporting, documentation, and investigation can be found in Chapter 18.

5.4.2 Records

Records are documents pertaining to the biosafety program or to biocontainment systems that provide evidence or information accounting for something that has occurred (e.g., accomplished, achieved, performed, or maintained). They serve to document most activities, including training, containment zone access, importation, maintenance and repair, equipment monitoring/calibration, **decontamination**, and shipping, receiving, and transfer. They can be used to assess whether essential biosafety elements are met (e.g., review of who has access to which containment zone, and if they have received training; which pathogens are handled and stored in the facility; whether autoclave cycles are always effective).

Records are kept on file to provide evidence that a specific activity was performed and to document the results achieved (CBS Matrix 4.10). It is important that records are legible and clearly identify the activity, product, or service involved. Historical records are to be retained for a specified period of time, should be easy to retrieve, and protected from damage or loss.

5.4.2.1 Authorized Persons

The CBS requires that facilities maintain a record of all individuals entering and exiting the containment zone where SSBAs are present, as well as containment level 3 (CL3, which include CL3 **large animal containment zones [LA zone]**), and containment level 4 (CL4) zones (CBS Matrix 4.10). This can be accomplished by maintaining an entry and exit log, or electronically by having all personnel use an electronic key card to scan in and out of the containment zone.

In addition, the HPTA requires licence holders to establish and maintain a list of all persons authorized by the licence holder to access any part of the facility (e.g., building and room numbers) to which the licence applies (HPTA 31). This list is to be provided to the PHAC if requested. This list is to include, but is not limited to, personnel, students, researchers, visitors, cleaning staff, maintenance staff, and contractors, whether or not they handle pathogens or toxins. It is left to the facility to decide how the list of authorized persons is managed. Each researcher or laboratory manager should be aware of who is authorized to enter their licensed areas. It may also be possible to obtain the information from student or human resources records.

5.4.3 Inventories

Pathogen and toxin accountability and **inventory** control processes allow pathogens, toxins, and other regulated infectious material to be readily located, when necessary, and permit missing items to be more easily identified. Any discrepancies noted can serve to identify potential areas for improvement, such as improving the existing inventory system, re-training of personnel, or implementation of a new system. Further details on pathogen and toxin accountability and inventory control are provided in Chapter 19.

5.4.4 Internal Inspections and Audits

Internal inspections and audits are an important component of any biosafety program and are designed to help prevent mishaps, incidents, and exposures, by proactively identifying hazards, deficiencies, or areas for improvement. These internal inspections and audits are conducted or coordinated by the BSO, with or without the participation of facility personnel, supervisors, directors, management, the IBC, or even occupational health and safety committees independently of PHAC and CFIA inspections. Many definitions exist for the terms "audit" and "inspection" and these terms are often used interchangeably. For the purposes of this document, internal inspections are conducted in person, scheduled regularly, thoroughly documented, and follow a documented procedure that outlines the items to be inspected in compliance with internal and external requirements. Internal audits are more periodic, focused, and can be carried out in person or be paper-based. Documentation from inspections and audits clearly specify the corrective actions to be implemented.

In general, internal inspections are conducted on an annual basis at a minimum (CBS Matrix 5.1), although it may be advisable to review critical elements of the program more frequently. The BSO, members of the IBC, senior management, or other similarly trained staff can conduct these internal inspections by walking through the **facility**. These walk-throughs provide an opportunity to observe the physical work environment, equipment, work practices, and correct use of PPE. It is also advantageous to interview personnel and supervisors, listen to any concerns, and review relevant documents and records.

Periodic audits between internal inspections can be useful to enforce and promote compliance. Audits can be random and unannounced and should be performed by individuals who are independent of the activity being audited.

Internal inspection and audit reports detail the findings of the inspection/audit and any corrective actions to be implemented to address deficiencies or non-compliance items. Internal inspection and audit procedures should include prompt follow-up on deficiencies, target dates for corrective action, and **verifications** to confirm implementation of corrective actions.

5.4.5 Regulatory Reporting Requirements

The HPTA and HPTR specify the reporting requirements for the licence holder in facilities that have been authorized to conduct controlled activities with human pathogens and toxins. In addition, regular reporting to the PHAC or the CFIA may be required based on conditions of licence or conditions of animal pathogen import permit, or upon request of either agency. For example, an annual report of performance and verification tests, as specified in Matrix 5.1 of the CBS, is required based on a condition of licence, as well as a condition of an animal pathogen import permit. Further details on regulatory reporting requirements are provided in Chapter 19.

5.5 Continuous Improvement of the Program

A successful biosafety program is regularly reviewed at the program management level and continually improved to remain relevant, applicable, and effective. Regular program reports (e.g., quarterly, semi-annually, annually) can be used as a tool to compare achievements against the program's objectives and goals. In addition, a third-party can be enlisted to conduct an objective review of the individual elements as well as the program management system as a whole to identify any gaps that may exist. The review will help address broad questions related to the program management system such as:

- Is the system in place and is it working?
- Are the appropriate procedures, processes, and plans in place to meet the program objectives and goals?
- Is the program adequately communicated to, and understood by, personnel?
- Does the program need to be updated?
- Is the system adapted in response to changes?
- Are adequate resources available to maintain the system?

Senior management should also review the biosafety program at regular intervals to ensure that the program remains effective. For example, the review may be used to determine the program's effectiveness at complying with legal requirements, based on existing legislation. Senior management can also ensure that the existing system continues to reflect the long-term goals and objectives of the institution or organization.

The biosafety program review can also identify non-compliance or other potential problems that may lead to non-compliance. Corrective action should be taken whenever non-compliance is identified, and preventive action should be taken to prevent the occurrence of non-compliance. These actions provide a framework that can help keep the program on track with its goals and objectives and an emphasis on personnel safety.

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CHAPTER 6

BIOSECURITY

CHAPTER 6 – BIOSECURITY

While the concepts of **biosafety** and **biosecurity** are closely related, the distinction between the two is important, particularly when considering **facilities** where **infectious material** or **toxins** are handled and stored. "Biosafety" describes the **containment** principles, technologies, and operational practices that are implemented to prevent unintentional **exposure** to **pathogens** or toxins, or their accidental **release**. In comparison, "biosecurity" refers to the security measures designed to prevent the loss, theft, misuse, diversion, or intentional release of infectious material or toxins. These concepts are not mutually exclusive and are inherently complementary, as the implementation of good biosafety practices serves to strengthen biosecurity programs and vice versa.

Just as the approach to biosafety is **risk-** and performance-based, so too is the approach to biosecurity, and facilities may be able to meet the intent of regulatory requirements through a combination of alternative physical measures and operational procedures. The minimum **physical containment requirements**, **operational practice requirements**, and **performance and verification testing requirements** related to biosecurity for regulated facilities in Canada are included in the requirements specified in the *Canadian Biosafety Standard* (CBS), 2nd Edition.[1] This chapter aims to outline considerations and approaches to establish a robust biosecurity program.

It is important to note that the term "biosecurity" used here is different from the concept of "agricultural biosecurity", which is outside the scope of the CBH. Agricultural biosecurity is intended to protect livestock and Canada's food supply from **disease**, and consists of preventive measures to minimize the possibility that a disease will enter an animal or plant population, and to minimize the spread of a pathogen within already infected premises.

6.1 Biosecurity Risk Assessment

The preliminary step in developing a biosecurity program is to conduct a **biosecurity risk assessment**. There are several resources available to assist with biosecurity risk assessments and developing biosecurity programs.[2,3,4,5] Similar to other risk assessments described in this volume (see Chapters 4 and 5), the complexity and detail of the biosecurity program is dependent on the level of risk posed by the pathogens, infectious material, or toxins in possession. It is recommended that the biosecurity risk assessment be reviewed on an annual basis and updated as necessary to address any change that affects the level of risk (i.e., introduction of a new pathogen, construction of a new facility). The following are the key elements that are included in a biosecurity risk assessment.

6.1.1 Identify and Prioritize Assets

The first step of the biosecurity risk assessment is to identify all of the relevant assets. In the context of biosecurity, "**assets**" would include all of the pathogens, infectious material, and toxins in the possession of the facility; however, other materials, equipment, non-infectious material, animals, knowledge and information, and even people may also be identified as assets. At minimum, it is required to maintain an **inventory** of pathogens, toxins, and other

regulated infectious material in **long-term storage** (i.e., greater than 30 days) in the facility, including their **risk group(s)** and location. For higher risk material (i.e., **security sensitive biological agents [SSBAs]**, Risk Group 3 [RG3], and Risk Group 4 [RG4]), it is required to have a means to allow for the timely detection of a missing or stolen sample. Good practice would dictate that other factors such as the concentration, quantity, and state of the material, also be included in the inventory. With this information at hand, the potential for the intentional misuse of the pathogens or toxins can be determined and the asset can be prioritized based on the consequences of this misuse. The impact that the loss would have on facility operations should also be considered.

Pathogens and toxins with **dual-use potential** (i.e., where the inherent qualities of a pathogen or toxin allow for its use in legitimate scientific applications, as well as for intentional and malicious misuse as a biological weapon to cause disease in humans or animals) are of the greatest biosecurity concern. These are the human pathogens and toxins that have been determined to have a potential for misuse and are identified as "prescribed pathogens" and "prescribed toxins" in the *Human Pathogens and Toxins Act* (HPTA) and *Human Pathogens and Toxin Regulations* (HPTR), and collectively specified in the CBS and this volume as SSBAs.[6,7]

Assets are prioritized according to their biosecurity risk based on a number of key factors, including the consequences of malicious use, the ease of use of the material, and the impact of loss of material on the facility.

6.1.2 Identify and Define Threats and Vulnerabilities

Individuals, organizations, or groups that may pose a risk to the security of assets present within the facility (e.g., theft) should be identified and listed. Such individuals or groups are considered adversaries or threats. Adversaries can be categorized as "**outsider threats**" (i.e., the threat from someone without authorization or access to the assets, **containment zone**, or facility and who may not have a formal relationship with the facility) and "**insider threats**" (i.e., an authorized individual who has access to the assets as part of his or her job and has the potential to steal or deliberately misuse the assets). Potential insider threats can include disgruntled current employees, including individuals with malevolent intent who intend to steal, release, or divert pathogens or toxins; or employees with access to pathogens and toxins that are coerced or manipulated into providing access or expertise to unauthorized individuals. Former employees, terrorist groups, organized criminal groups, extremist protest groups, persons suffering from mental illness, and opportunistic criminals are examples of outsider threats.

The biosecurity risk assessment involves an assessment of threats to determine if and how each threat could gain access to, damage, or misuse assets such as pathogens or toxins.[4] Threat analysis considers the motive, means, and opportunity for each threat. The threat analysis also provides an indication of **vulnerabilities**. These are the weaknesses in existing security measures that may impact on the "means and opportunity" of the threat to access an asset, and therefore on the likelihood of an event occurring. Identified vulnerabilities can be addressed with mitigation strategies.

6.1.3 Determine Risk Levels and Mitigation Strategies

The biosecurity risk level is determined based on an analysis of the risk associated with each asset (or group of assets with similar characteristics) in combination with each threat. The highest biosecurity risks are those events with the greatest consequences, even if it is fairly unlikely they would occur, followed by events with moderate consequences that are more likely to occur.

Risks can be mitigated using physical security measures, enhanced screening for personnel, a clear accountability framework for pathogens and toxins, effective **incident** and emergency response protocols, and information security measures. Assets can be managed according to risk level as follows:

- assets that are deemed to be at low risk of unauthorized access need minimal management and control measures;
- assets that are at medium or high risk of unauthorized access will require moderate management and risk mitigation; and
- assets that are at very high risk require extensive management and controls.

Senior management within the organization is responsible for determining the acceptable levels of risk for identified scenarios (i.e., risk tolerance), as well as the resources available to mitigate the risks. Possible mitigation strategies for the identified vulnerabilities should be outlined and include preventive measures that may be implemented to counter the identified risk, and it may be found that identified risks are already controlled through existing biosafety and/or biosecurity measures. Any risks that have not been mitigated or that have been deemed acceptable should be documented along with an explanation of the decision.

Mitigation strategies developed to protect against unacceptable risks can be used to develop a biosecurity plan that will complement the biosafety program.

6.1.4 Develop Risk Statements and Risk Registers

A risk statement provides an accurate picture of a risk and is a key tool used in the risk management process.[8] Risk statements serve to identify and document biosecurity risks during the biosecurity risk assessment. A risk statement for a threat involves at least two elements: the *event* and the potential negative *impact* of such an event should it occur. A risk statement (i.e., the threat) can be structured to read: "If [*event*] occurs, the consequences could result in [*negative impact*]".

A risk register is a common project management tool to document the results of qualitative and quantitative risk analysis and risk response planning. It is essentially a list of all of the identified risk statements and the risk level in a format that can be easily reviewed, modified, and updated as necessary.[9]

6.2 Biosecurity Plans

A biosecurity plan is a key biosafety program element for any facility where infectious material or toxins are handled or stored. The biosecurity plan is based on the biosecurity risk assessment and can be simple or complex, depending on the pathogens, infectious material, and toxins possessed by the facility and the structure or complexity of the facility or organization.[10] The biosecurity plan should address the unacceptable risks posed by both outsider and insider threats. The biosecurity plan should be developed through a collaborative process that involves facility staff members, such as scientific directors, principal investigators, **laboratory** personnel, administrators, information technologists, occupational health and safety personnel, security personnel, and engineering staff. Involving personnel responsible for the facility's overall security in this process is crucial as certain biosecurity measures may already be in place as part of an existing security program. It may also be appropriate to involve local law enforcement in the development of the biosecurity plan. Regular reviews and updates of the biosecurity plan keep the biosecurity measures, policies, and procedures accurate and effective to maintain biosecurity. Matrix 4.1 of the CBS specifies the minimum requirements for biosecurity plans and biosecurity risk assessments.

6.2.1 Elements of a Biosecurity Plan

Once the biosecurity risk assessment is complete, a biosecurity plan tailored to the facility can be developed, implemented, evaluated, and continually improved as necessary. Integrating the elements of the biosecurity plan within the overall biosafety program will minimize duplication of information and allow for a more efficient biosafety management system. A biosecurity plan addresses the elements that follow. At minimum, a biosecurity plan must document that the risks associated with each element have been assessed and describe the strategies, if any, that are already in place or have been added to mitigate these risks. The PHAC and the CFIA have developed an additional guideline that elaborates on a number of the biosecurity topics introduced in the CBH and serves as a resource for stakeholders seeking additional information and to establish a comprehensive and robust biosecurity plan; please visit the PHAC or CFIA website for further information.

6.2.1.1 Physical Security

Physical security elements of a biosecurity plan aim to reduce the risk of unauthorized access to identified assets and other sensitive materials (i.e., protect against outsider threats). Adequate physical security measures should be in place to minimize opportunities for the unauthorized entry of individuals into containment zones and the unauthorized access to infectious material or toxins in the facility. An evaluation of the physical security measures should include a thorough review of the premises, building, containment zones, and storage areas. **Security barriers** (i.e., a physical structure designed to prevent entry by unauthorized personnel), such as locked doors and windows, **controlled access systems**, and secure containers or storage equipment, can be incorporated to increase the security of a containment zone and to restrict access to **authorized personnel** only. Security barriers can be considered at the property or building perimeter, facility, containment

zone, and pathogen or toxin-specific level. Security barriers at the points of access to the containment zone (e.g., lockable doors, manned security stations or checkpoints), access control measures (i.e., limited or restricted), mechanisms to detect unauthorized access and attempts (e.g., security cameras, access control system software records), additional security barriers (e.g., lock boxes or lockable freezers), and maintenance of security barriers are key considerations when determining the appropriate level of physical security. The minimum physical security requirements for regulated containment zones are specified in Matrices 3.1, 3.2, and 3.3 of the CBS; operational practice requirements relating to biosecurity practices are included in Matrices 4.5 and 4.6 of the CBS.

6.2.1.2 Personnel Suitability and Reliability

Hiring managers should screen candidates to establish they have the appropriate credentials, skills, and personal traits to undertake the work, and are the best fit for the position prior to being granted access to pathogens and toxins or other assets.[11] Academic credentials and prior experience may qualify an individual's scientific ability, but they do not always measure the individual's suitability to handle or access pathogens and toxins. Personnel suitability and reliability policies and procedures should be established to address the risk from a potential insider threat; the training, experience, competency, and other suitability requirements for personnel who handle or have access to pathogens or toxins should be clearly defined and documented. Employee pre-appointment screening is a crucial step in determining personnel suitability. Procedures may also be needed for approving and granting visitor access.

An ongoing reliability assessment program aims to verify that access to pathogens and toxins granted to an individual continues to be justified based on the established criteria for personnel suitability. Moreover, an ongoing assessment program also aims to identify insider threats from personnel who have previously been determined to be suitable for access. Circumstances that may affect an employee's ability to safely and securely perform their duties can also affect that individual's ***Human Pathogens and Toxins Act*** **Security Clearance (HPTA Security Clearance)** status. These may include, for example, participation in criminal activities, immigration or financial concerns, dramatic changes in behaviour, attitudes, demeanor, or actions (e.g., increasingly withdrawn, anger or aggression, unexplained absences, signs of alcohol or drug use), or willful non-compliance with policies and legislation. The availability of programs that identify and offer assistance to employees who are experiencing problems may be considered as a possible method to reduce these risks.

6.2.1.3 Accountability of Pathogens and Toxins and Inventory

Pathogen and toxin accountability procedures are established in order to track and document pathogens and toxins, which include all regulated infectious material in long-term storage (i.e., greater than 30 days) within the containment zone or organization, so that material can be located when necessary and missing items can be identified more readily. Effective inventory measures for pathogens and toxins can be a successful way to deter a variety of insider threats. The level of detail of the inventory system is determined based on the risk associated with the pathogens, toxins, and other infectious material being

handled and stored. For example, where SSBAs, RG3, or RG4 pathogens are in long-term storage, the inventory will require more detail than Risk Group 2 (RG2) pathogens so that the samples of specific pathogens, toxins, and other regulated infectious material can be easily identified, and consequently located or determined to be missing or stolen in a timely manner (CBS Matrix 4.10). Provisions for maintaining accountability during shipping, receiving, monitoring, and storage of packages that contain pathogens, toxins, and other regulated infectious material should also be incorporated into the biosecurity plan. Pathogen and toxin accountability and inventory systems are discussed in detail in Chapter 19.

6.2.1.4 Information Management and Security

Information management and security policies and procedures are created to protect sensitive information from unauthorized access or theft and to ensure the appropriate level of confidentiality. Examples of sensitive information may include facility biosecurity plans, employee information, access codes, passwords, infectious material and toxin inventories, and storage locations. In some cases, scientific information may be considered sensitive information (e.g., cloning procedure to reconstitute an extinct virus).[12] Information management and security policies should govern the classification and handling of sensitive information and address how the information is collected, documented, transmitted, accessed, and destroyed. Proper access control to sensitive information, containment zones and associated areas is often the first step to mitigating the risk of information misuse by outsider threats.

The protection of information should be consistent with the level of risk posed by the material in question. In certain environments, access to records and documentation pertaining to activities with pathogens and toxins is restricted to authorized personnel only (CBS Matrix 4.10).

Clear policies or protocols for basic information technology security, such as strong user passwords, discouraged or limited use of unsecured wireless connections, and the use of a virtual private network (VPN) to communicate between several offices, are general considerations for information security. The use and control of mobile electronic devices (e.g., tablets, personal data storage devices) and digital cameras should be considered as a vulnerability to information security, as they can be easily hidden from sight and are capable of storing or transferring information on media that can be removed and stored separately.

6.2.1.5 Incident and Emergency Response

The incident and emergency response elements of a biosecurity plan should be integrated into the overall biosafety program for greater efficiency (i.e., a component of the **emergency response plan [ERP]**). For example, it is recommended that a mechanism is included for the removal of unauthorized individuals. All incidents should be reported. Reporting of biosecurity-related incidents (e.g., missing pathogens or toxins, unauthorized entry or access to sensitive information, loss of keys or passwords) to the **biological safety officer (BSO)** should be encouraged so that incidents can be appropriately documented, investigated, and reported as necessary. Depending on the incident, the BSO may consider reporting the incident to local law enforcement and may be obligated to report the incident to the Public Health Agency of Canada (PHAC) under the conditions of **licence**. More details about ERPs and incident investigation are provided in Chapters 17 and 18, respectively.

6.3 *Human Pathogen and Toxin Act* Security Clearances

The PHAC has determined that an HPTA Security Clearance is an important employee screening procedure to complement suitability assessments in an effort to mitigate the risk from potential insider threats in Canadian facilities authorized to conduct **controlled activities** with SSBAs. In accordance with the HPTA, an individual must possess a valid HPTA Security Clearance issued by the PHAC to enter a part of a facility in which controlled activities with SSBAs have been authorized under a licence (HPTA 33). The HPTA Security Clearance issued by the PHAC is a comprehensive background check of law enforcement and intelligence databases that also includes a credit check. An individual without an HPTA Security Clearance may only enter the part of a facility in which controlled activities with SSBAs have been authorized if there are no SSBAs in that part of the facility, if any SSBAs that are present are locked up and inaccessible, or if accompanied and supervised by a person who holds a valid HPTA Security Clearance for that part of the facility (HPTA 33). Security screening is a key biosecurity element of the regulatory framework under the HPTA to assess the credibility and suitability of all individuals who will be authorized to access SSBAs. The HPTA Security Clearance cannot be processed without consent; the applicant is screened based on the information provided on the security screening application form. Throughout this section, the relevant sections of the HPTA or HPTR are indicated for ease of reference.

6.3.1 HPTA Security Clearance Process

The HPTA Security Clearance process involves an electronic records check by the Royal Canadian Mounted Police (RCMP), and the Canadian Security Intelligence Service (CSIS), as well as a credit history check. The RCMP determines if an individual has a criminal record in Canada and conducts law enforcement record checks to determine if the individual has engaged in or has been associated with criminal activities that would indicate unacceptable risk. The CSIS reviews domestic and foreign threats to Canadian security posed by both individuals and organized entities, and assesses the applicant's loyalty to Canada as it pertains to threats to national security. In accordance with Section 12 of the HPTR, applicants for an HPTA Security Clearance will be requested to submit additional information to support their application, including but not limited to: a copy of their birth certificate, copies of two valid pieces of government issued photo identification, fingerprints taken at an RCMP accredited facility, and a statement signed by the licence holder certifying that the applicant requires an HPTA Security Clearance for a particular part or parts of a facility. Foreign nationals will also be required to supply a copy of their *curriculum vitae*, a valid visa if applicable, and the results of a police record check from every jurisdiction they have. At the time of publication, the service standard to review and issue an HPTA Security Clearance had not been established, although it is estimated that the entire security screening process may take up to 80 working days or longer. Longer delays may be encountered due to incomplete applications or additional investigations by the RCMP or CSIS. The decision to issue, suspend, refuse, or revoke an HPTA Security Clearance is made by the Minister of Health, or his or her delegate, in accordance with the HPTR. If an applicant or HPTA Security Clearance holder has had their clearance denied, suspended, or revoked, they will have the option to appeal by requesting a reconsideration of this decision within 30 calendar days

after the day on which the notice of denial, suspension, or revocation was received. Further information on the HPTA Security Clearance Application Form and the appeal process can be obtained by visiting the PHAC website (www.publichealth.gc.ca/pathogens).

6.3.2 Exemptions

Personnel in licensed facilities that are authorized to handle and store toxins identified in Schedule 1 of the HPTA in quantities equal to or less than the **trigger quantity** specified in the HPTR 10(2) do not need an HPTA Security Clearance. Access to an RG3 or an RG4 pathogen that is also an SSBA and that has been modified to the extent that it no longer meets the risk profile described by the definition of the respective risk groups in the HPTA may not require an HPTA Security Clearance (e.g., a vaccine strain of an RG3 pathogen that has been attenuated to a point that it meets the risk profile of an RG2 pathogen).

6.3.3 Validity and Portability

An HPTA Security Clearance issued by the PHAC is valid for a period of up to 5 years and authorizes access to the part(s) of the facility (or facilities) identified on the initial application. The HPTA Security Clearance is issued to an individual and is transferable between licensed facilities. In order to access a facility not indicated on the initial HPTA Security Clearance application without being accompanied by an authorized individual, an HPTA Security Clearance holder will be asked to provide the PHAC with a signed statement by the licence holder of the new licensed facility certifying that they require an HPTA Security Clearance (HPTR 18). In this case, the HPTA Security Clearance holder may not enter the new licensed facility until the signed statement by the licence holder has been provided to the PHAC. A new supporting letter signed by the licence holder will be requested each time an HPTA Security Clearance holder moves to a different licensed location during the tenure of their clearance.

6.3.4 Suspension and Revocation

An HPTA Security Clearance may be suspended by the PHAC in the event that it receives any information that, should it have been obtained in support of the initial application, may have resulted in a refusal to issue an HPTA Security Clearance. The suspension will stand until such time as the analysis of this new information is complete and the suspension is lifted or the HPTA Security Clearance is revoked. An HPTA Security Clearance will be revoked if the PHAC determines that the holder of a clearance is no longer deemed to be suitable.

6.3.5 Notification of Criminal Offences

An individual who has been issued an HPTA Security Clearance is obliged to notify the PHAC if he or she is convicted of a criminal offence (HPTR 19). Depending on the nature of the offence, a review of the individual's HPTA Security Clearance may be initiated. The notification can be submitted electronically to the PHAC by email or through the Notification of Criminal Offence form accessible through the PHAC website (www.publichealth.gc.ca/pathogens). Failure of an individual to report conviction of a criminal offence may result in suspension or revocation of his or her HPTA Security Clearance.

6.3.6 Accompaniment and Supervision

An individual without an HPTA Security Clearance may only enter the part of a facility in which controlled activities with SSBAs have been authorized when accompanied and supervised by a person who holds a valid HPTA Security Clearance for that part of the facility (HPTA 33). In this case, the escort must have an HPTA Security Clearance issued by the PHAC and may only accompany and supervise one person who does not hold a clearance at any one time (i.e., a 1 to 1 ratio). The escort must be in the same room and monitor the activities of the person who does not hold a clearance at all times (HPTR 23); a direct line of sight of the individual without an HPTA Security Clearance should be maintained at all times. Individuals without an HPTA Security Clearance are not considered authorized personnel and are, therefore, not permitted to have access to SSBAs, except under direct supervision as described above. An individual who has been refused an HPTA Security Clearance or whose HPTA Security Clearance has been suspended or revoked may not enter the part of a facility where controlled activities with SSBAs have been authorized at any time, even under accompaniment and supervision (HPTR 24).

6.3.7 Shared Facilities

Shared spaces located inside the part of a facility where controlled activities with SSBAs have been authorized under a licence may present access challenges for all personnel. Facility management, directors, and the licence holder will have to determine how best to address these challenges. There are essentially two options available, each with benefits and challenges, dependent on scheduling and secure storage areas for the SSBAs:

1. each individual who works in the shared spaces holds a valid HPTA Security Clearance so that they can freely access the part of the facility when SSBAs are present, regardless of whether or not he or she actually needs access to the SSBAs; or
2. access by persons without an HPTA Security Clearance (i.e., those who do not actually need access to the SSBAs) to shared facilities is limited to times when:
    - there are no SSBAs present;
    - the SSBAs are locked away and inaccessible; or
    - they are accompanied and directly supervised by an individual who holds a valid HPTA Security Clearance for that part of the facility (i.e., 1 to 1 ratio).

Option #1 provides more flexibility in terms of what work can take place at what time in the shared space, but increases the reliance on operational controls to manage the number of personnel who would have access to the SSBAs. This option can also weaken a security posture by breeding complacency (i.e., if it is assumed everyone accessing the room possesses an HPTA Security Clearance, and these numbers are significant, staff may be less likely to challenge an unfamiliar individual). In contrast, Option #2 addresses the challenges with the former option, with less reliance on operational controls, but may depend on strict scheduling of activities.

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CHAPTER 7

MEDICAL SURVEILLANCE PROGRAM

CHAPTER 7 – MEDICAL SURVEILLANCE PROGRAM

The basic purpose of a **medical surveillance program** is to help prevent and detect illnesses related to the **exposure** of personnel to **pathogens** or **toxins**. The focus of this program is primarily preventive, although it also provides a response mechanism through which a potential infection can be identified and treated before serious injury, **disease**, or secondary transmissions occur. The medical surveillance of personnel handling pathogens and toxins can often be integrated into an existing workplace medical surveillance program (e.g., occupational health and safety programs for chemical or radiological hazards).

The requirements for a medical surveillance program are specified in Matrix 4.2 of the *Canadian Biosafety Standard* (CBS), 2nd Edition.[1] The medical surveillance program is developed and based on an **overarching risk assessment** and **local risk assessments (LRAs)** in order to identify the pathogens and toxins handled, stored, or encountered in the **containment zone** or throughout the organization, and to identify the associated **risks**. A description of the medical surveillance program is included in the containment zone's **Biosafety Manual** so that it is available for reference to all personnel. It is important to update the medical surveillance program accordingly whenever changes are made to a **laboratory** program (e.g., when different pathogens or toxins will be introduced or new procedures or activities will be carried out). It may be appropriate to involve an occupational health professional or a local health care provider (e.g., physician, nurse, local hospital), as well as emergency responders (e.g., local paramedic, fire, and police department personnel), in the process of developing the medical surveillance program, especially with programs involving higher risk pathogens.

This chapter presents a number of aspects to be considered in developing a medical surveillance program. The level of detail and the complexity of the program will depend on the nature (i.e., size, structure, complexity) of the organization, the activities carried out involving pathogens and toxins, and the safety-related provisions of applicable legislation. Some components that may be considered when developing a medical surveillance program include a pre-placement medical examination of personnel; serum screening, testing or storage; immunizations; and other tests, as determined by an LRA.

The medical surveillance program complements medical emergency procedures, which form part of a **facility's emergency response plan (ERP)**. ERPs and **incident** investigation and reporting are described further in Chapters 17 and 18, respectively.

7.1 Laboratory Exposures and Laboratory Acquired Infections/Intoxications

Individuals who work in areas where **infectious material** or toxins are handled or stored are at risk of exposure to these pathogens and toxins and the adverse consequences of an exposure event (i.e., infection or **intoxication**). The term that is commonly used to describe diseases associated with workplace exposures to infectious material or toxins in a laboratory setting is **laboratory acquired infections/intoxications (LAIs)**; however, the term exposure

more accurately includes both infections and intoxications (i.e., resulting from exposure to toxins), whether symptomatic or asymptomatic in nature, as well as those that can be linked to a containment zone but that occur outside of a laboratory environment (e.g., infection of an office worker in a licensed facility by a pathogen handled or stored in that facility).

In addition to the immediate risk to individuals handling infectious materials, exposed persons can pose a risk to the **community** via transmission of infections within or outside the laboratory setting. Although it may be difficult to determine the root cause(s) in all cases, exposures resulting in LAIs are not uncommon. The most recent comprehensive epidemiological review found 5,527 cases and 204 deaths reported worldwide from 1930 to 2004.[2] While LAIs do still occur and are documented, the incidence of LAIs appears to have declined over the years; this may be attributable to enhanced **biosafety** practices, improved design of **containment facilities** and equipment, or simply due to under-reporting of incidents.[3,4,5] Despite this apparent decline, exposures and LAIs continue to occur, and data on these incidents may be used by biosafety professionals to better understand and quantify the risk associated with a given pathogen or a specific laboratory activity. Likewise, the information can be used to improve biosafety and **biocontainment** standards, guidelines, training, equipment and systems, and best practices, as well as medical surveillance programs (e.g., immunization, post-exposure prophylaxis, or treatment recommendations). The Public Health Agency of Canada (PHAC) is currently collecting incident reports documenting LAIs or exposures to human pathogens and toxins to analyze this information and help shape current and future biocontainment and biosafety practices in Canada.

Exposure to an infectious material or toxin is not always immediately followed by symptoms or overt disease. In addition, LAIs themselves can be symptomatic or asymptomatic in nature. Some facilities may employ medical surveillance practices that could identify a **seroconversion**, which may provide an additional source of information for recognition or confirmation of recent or previous infection or disease. Seroconversion can occur following initial infection and clearance of the pathogen, and may indicate a post-infection latency period prior to onset of the disease associated with certain pathogens (e.g., human immunodeficiency virus [HIV], *Mycobacterium tuberculosis*, hepatitis C virus, and **prions**). Sound judgement is needed in evaluating historical LAI data, as the accuracy of statistics may be impacted due to the likelihood of under-reporting of incidents. Under-reporting of exposures and LAIs may be attributed to a variety of factors, including:

- a lack of mechanisms for the reporting and tracking of exposures and LAIs;
- recognition and reporting of only symptomatic or laboratory-confirmed cases of disease;
- limited publication of LAI cases in scientific or medical journals due to factors such as space limitations;
- uncertainty as to whether an illness is due to an exposure that occurred in the laboratory setting or the community;
- a lack of interest or motivation to report common incidents or incidents involving a commonly used pathogen; and
- fear of reproach or reprisal.

The *Human Pathogens and Toxins Act* (HPTA), Section 13, requires that any exposure to human pathogens or toxins that may cause disease or any disease that may have been caused by an exposure to a human pathogen or toxin in the facility be reported to the PHAC without delay.[6] This reporting allows the PHAC to assess the severity of the exposure incident and assist the facility in their response, if requested or necessary. The PHAC can also provide expertise and assistance to the facility in developing corrective actions to address the cause of the incident and prevent a recurrence. Information provided in an **exposure notification report** following an exposure incident will allow the PHAC to monitor developing trends, prompt the issuance of biosafety advisories, and amend or update best practices in biosafety practices and training, and at the same time analyze this data at the national level to inform current and future biocontainment and biosafety directions. Local investigation, documentation, and reporting for all types of incidents are intended to capture near misses and LAIs for which no clear exposure event can be identified. Incident reporting and investigation are further discussed in Chapter 18.

7.2 Pre-Placement Medical Evaluation

In some circumstances, it may be beneficial to conduct pre-placement medical evaluations for all personnel. This section describes options when considering the implementation of pre-placement medical evaluations. A pre-placement medical evaluation may be conducted for new personnel or when personnel are given new responsibilities, prior to commencing activities with human pathogens, toxins, or **zoonotic pathogens**. The primary purpose of such an evaluation is to assess the initial health status of the individual and identify if there are any underlying medical conditions that may increase the risk of harm associated with the anticipated job activities. This evaluation may include an interview with the institutional occupational health care provider or completion of a personal medical history questionnaire to document the individual's previous and current medical problems; current medications; known allergies to medications, animals, or environmental allergens; and prior immunizations. Personnel who are immunocompromised or immunosuppressed (e.g., through medical therapy, pregnancy, diabetes, or other conditions) may be particularly susceptible to infection or intoxication, unable to take post-exposure treatment, or experience more severe illness if they develop disease following exposure to a pathogen or toxin. A complete physical examination is rarely necessary as part of this process but may be appropriate.

Before commencing any **controlled activities**, the individual should be informed of the hazards associated with, and the signs and symptoms of disease(s) caused by, the pathogens and toxins to be manipulated, and of all preventive measures available against the pathogens or toxins, such as vaccinations or other treatments, along with the risks and benefits of these vaccinations and treatments. They should also be informed of the steps to follow in the event of potential exposure, including appropriate first aid measures, incident reporting, timely post-exposure prophylaxis and medical treatments. In addition, the early signs and symptoms of a possible infection or intoxication with the pathogen(s) or toxin(s) being handled should be described to personnel, and they should be told what immediate steps to take if they develop these symptoms. In a clinical diagnostic setting, it may not always be possible or practical

to advise personnel of all potential pathogens that they may encounter; rather, it may be more reasonable to inform personnel of symptoms of key concern in situations when illnesses caused by unusual pathogens have been diagnosed in the laboratory.

Personnel with a considerable risk of exposure to pathogens may be encouraged to provide a blood sample for serum testing and storage prior to the initiation of work with the pathogen(s). Such samples can be stored long-term and later used to determine pre-existing immunity from prior vaccination or infection, and to establish a baseline seroreactivity for comparison with supplementary blood samples collected following a potential exposure.

7.3 Vaccinations

Vaccines are highly regulated and complex biological products designed to induce a protective immune response both effectively and safely. The availability of vaccines or other prophylaxis should be evaluated, and these should be offered to personnel as required prior to commencing work with a pathogen. Periodic testing of antibody titres should be conducted post-vaccination to determine if the required level of protective immunity has been achieved and is being maintained, or if a booster vaccination is necessary. Should an individual decline or not respond immunologically to a vaccination that is deemed a prerequisite for working in a containment zone, a re-evaluation of placement, the implementation of additional environmental controls, or the use of additional **personal protective equipment (PPE)** should be considered.

Further recommendations on vaccines can be obtained from health care professionals specializing in this area or from the National Advisory Committee on Immunization (NACI). The NACI is a national advisory committee of medical and health sciences experts that makes recommendations to the PHAC on the use of vaccines in Canada, including the identification of groups at risk (e.g., occupational groups, age groups) for vaccine-preventable diseases. All NACI recommendations are published in the *Canadian Immunization Guide* with additional statements and updates published in the *Canada Communicable Disease Report* (CCDR).[7,8]

7.4 Ongoing Medical Surveillance

Ongoing medical surveillance for personnel who are at risk of exposure to pathogens or toxins may provide an indication of occupational exposure. Personnel should be encouraged by the supervisor, without fear of reprisal, to disclose any changes in their health status that could increase their risk of exposure or disease susceptibility. This could include developing an immunodeficiency or a temporary condition, such as the need to take prescribed antibiotics, impaired vision, or even stress. Routine or periodic medical evaluations are generally not necessary; however, such evaluations may be appropriate in the case of personnel with a substantial risk of exposure to pathogens or toxins since they may permit earlier recognition of an infection that may be due to a laboratory exposure. Any clinical tests (e.g., serum testing) requested by a medical advisor or practitioner should be limited to approved, commercially

available tests with adequate sensitivity to identify an infection or previous infection (i.e., seroconversion). Serum samples collected during the pre-placement evaluation can be used to establish a baseline or "pre-exposure" reference for any tests to be conducted as part of the medical surveillance program. While medical test results are only reported to the patient, individuals who discover a positive infection or seroconversion that may be associated with a laboratory-related exposure have an obligation to inform their supervisor or internal organizational authority (i.e., **biological safety officer [BSO], licence** holder), who, in turn, is legally required to notify the PHAC of the exposure (HPTA 13).

7.5 Post-Exposure Response Plan

Post-exposure response plans outline the specific procedures to follow and actions to be taken in the event of a known, suspected, or potential exposure to a pathogen or toxin (e.g., reporting, medical testing, and treatment) and could be a component of an overall ERP. For containment zones where pathogens or toxins are handled or stored, a post-exposure response plan may be created in consultation with the occupational health care provider or practitioner, the institutional biosafety committee, the BSO, and the occupational health and safety advisor. Incident reporting and investigation are discussed in further detail in Chapter 18.

7.6 Additional Considerations for High Containment

Any potential occupational exposure that occurs in a **high containment zone** (i.e., **containment level** 3 [CL3], which includes CL3 **large animal containment zones [LA zone**; CL3 Ag], or containment level 4 [CL4]) should be promptly evaluated, as infection with a higher risk pathogen may lead to severe illness or death. The pathogens manipulated in CL4 zones are typically exotic and an LAI would represent a serious health concern for the community. Ensuring adherence to all medical surveillance protocols and procedures by all containment zone personnel, including facilities and support personnel, is particularly important in **high containment zones**. It is strongly recommended that an infectious disease specialist be involved in the development of the medical surveillance program, including risk assessment, pre-placement evaluations, and development of a post-exposure response plan. Additionally, it is required (CBS Matrix 4.2) for CL4 zones and strongly recommended for CL3 and CL3 LA zones (CL3-Ag), that the post-exposure response plan be prepared in consultation with local health care facilities, to keep health care providers informed of the pathogens being handled and that the appropriate procedures and treatments are in place. Specific quarantine procedures for potentially infected personnel may need to be established prior to an exposure incident. In CL4 zones, it is also required that the supervisor contact any containment zone personnel with unexpected work absences to determine if the absence is due to an illness that could be related to activities with the pathogens in use (CBS Matrix 4.2).

7.7 Emergency Medical Contact Card

Emergency medical contact cards are issued by the employer to personnel working with non-human primates (NHPs), personnel working with pathogens or toxins that cause diseases unlikely to be recognized by a physician, and all personnel working in CL4 zones to provide a means to facilitate communication with health care providers and other individuals, particularly during emergency situations. The card should summarize important information regarding the pathogen(s) or toxin(s) that are handled by the individual, such as routes of infection or intoxication, transmission, symptoms, and preventive and therapeutic treatments. This measure is also recommended for personnel working in CL3 and CL3 LA zones (i.e., CL3-Ag). In the event of an unexplained illness, this card can be presented to hospital or heath care facility staff, or emergency responders. The containment zone supervisor should provide guidance as to when the card should be carried by the personnel (e.g., at all times on the premises except inside the containment zone, or at all times during a period that an active study involving the particular pathogen is being conducted). It is the responsibility of the facility to determine when the emergency medical contact card is to be carried by personnel. An example of an emergency medical contact card can be found in Figure 7-1.

EMERGENCY MEDICAL CONTACT CARD

NAME: _____

DATE ISSUED: _____

I WORK WITH:

- ☐ _____ RISK GROUP 4 PATHOGENS
- ☐ _____ RISK GROUP 3 PATHOGENS
- ☐ _____ NON-HUMAN PRIMATES
- ☐ _____ TOXINS
- ☐ _____ OTHER

This card is to be kept in the possession of the laboratory employee and presented to a physician if an illness occurs that may be associated with a pathogen used within the laboratory (see reverse).

FRONT

TO THE PHYSICIAN

This employee works in an environment where pathogenic microorganisms are present. Please contact the individuals listed below for information on the agents to which this individual may have been exposed.

FACILITY NAME: _____

ADDRESS: _____

_____

CONTACT 1: _____

NAME TEL. (Home / Work / Cell)

CONTACT 2: _____

NAME TEL. (Home / Work / Cell)

BACK

Figure 7–1: Example of an Emergency Medical Contact Card.

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CHAPTER 8

TRAINING PROGRAM

CHAPTER 8 – TRAINING PROGRAM

A training program is essential to the success of a **biosafety** program. It is critical that personnel be knowledgeable about the hazards present in the work environment and the practices and tools that can protect them from these hazards. The training program encompasses both education and training. Education relates to the provision of general information or theoretical knowledge. Personnel can be educated on work-related hazards through various means, including classroom courses, videos, e-learning, on-the-job training, and printed materials, such as manuals, **Pathogen Safety Data Sheets (PSDSs)**, and posters. Training refers to more practical and hands-on job-specific instruction, including the demonstration of practices and procedures. These two concepts are complementary and necessary to create a robust training program. A supervisor or facility director is generally responsible for ensuring that **containment zone** personnel receive proper training. The **biological safety officer (BSO)** or biosafety representative plays a role to promote and monitor that training related to biosafety and **biosecurity** policies, standards, and practices is arranged and documented in regulated **facilities** where human and **animal pathogens** and **toxins** are handled and stored in accordance with the *Human Pathogens and Toxins Regulations* (HPTR) (HPTR 9[1][c][i]) and the *Canadian Biosafety Standard* (CBS), 2nd Edition.[1,2] The required elements of a biosafety training program are specified in Matrix 4.3 of the CBS.

8.1 Training Needs and Objectives

The specific content of the training program will vary between organizations and even between containment zones within the same **facility**. A **training needs assessment** is the first step in developing an effective training program. This assessment is performed to identify the current and future training needs of the facility, and gaps in the existing training program. The results of a training needs assessment should be used as the foundation for determining instructional objectives, the selection and design of instructional programs, the implementation of the programs, the retraining cycle, and the evaluation of the training provided. The needs assessment should take into consideration the **risks** identified through the **pathogen** and **biosecurity risk assessments**, and the specific issues that can be mitigated through training.

The **Biosafety Manual** describes the core elements of the biosafety program, including training program objectives and goals. The training objectives should be measurable and clearly identify the desired behaviour or skill to be learned in the training.

8.2 Training Program Content

All training programs share certain components and requirements. Combining biosafety and biosecurity training with other workplace training requirements could be beneficial, as well as an efficient use of resources. Training on the Biosafety Manual and **standard operating procedures (SOPs)** requires personnel to be familiar with the contents of the Biosafety Manual, such as the biosecurity and **emergency response plans (ERP)**. Personnel demonstrating knowledge and proficiency in the SOPs on which they were trained will be able to safely handle the pathogens and toxins they will encounter in the workplace and respond accordingly in an emergency situation.

Training related to the potential hazards associated with the work carried out is particularly important and may include the following elements:

- information on the nature of **infectious material** and toxins used in the workplace and how to identify them;
- signs and symptoms of **disease** caused by **exposure** to the pathogens that will be handled. In facilities where a wide variety of pathogens could potentially be handled (e.g., diagnostic facilities), a broader approach may be considered (i.e., training on general signs and symptoms of concern rather than the symptoms for each pathogen);
- safe work practices and physical control measures, including handling and disposal of infectious material or toxins (i.e., **decontamination** and **waste** management), and the correct choice, use, and maintenance of **personal protective equipment (PPE)**;
- instruction on relevant safety information (e.g., PSDSs) and on how to find and use these materials; and
- information on the legislative and regulatory requirements related to activities involving the infectious material or toxins concerned.

There are many teaching and training resources available to assist in the development of a training program. The Public Health Agency of Canada (PHAC) and the Canadian Food Inspection Agency (CFIA) provide numerous resources, including biosafety training materials (such as the "Principles of **Laboratory** Biosafety" e-learning course), templates, toolkits, posters, instructional videos, and more, available through an e-learning portal (www.publichealth.gc.ca/training).

8.2.1 Biosecurity Training

Biosecurity training for all personnel is essential to establish a culture of responsibility and security awareness and may include the following elements:

- awareness of **insider threats** and **outsider threats**;
- behaviours of concern;
- identifying and removing a suspicious person;
- policies concerning access to **security sensitive biological agents (SSBAs)**;
- escort procedures;
- self and peer reporting procedures;
- corrective actions, procedures and policies;
- information security;

- security policies, including:
  - entry and access procedures and prevention of "tailgating";
  - preventing the sharing of unique means of access;
  - reporting the loss or compromise of passwords;
  - how to identify and report suspicious persons or activities; and
  - **inventory** documentation and records management;
- responding to an alarm; and
- responding to a security breach.

8.2.2 Training on Containment Systems and Equipment

Training programs are to include information on the physical design and operation of the containment zone and **containment systems** that are relevant to the individual being trained (CBS Matrix 4.3). This should include a review of the types of **primary containment devices** and containment systems associated with the containment zone, a basic overview of how each functions, their correct use and operation, and how to determine if the equipment or systems are functioning properly or not, to protect against a release or exposure to a pathogen or toxin. This training should also include a review of secondary containment systems (e.g., backup containment systems) where these have been incorporated into the containment zone.

Personnel are to demonstrate knowledge and proficiency in the SOPs on which they were trained, including SOPs for the correct operation of containment systems, devices, and other equipment. Examples of primary containment devices and containment systems that should be reviewed during biosafety training include, but not limited to:

- **biological safety cabinets (BSCs)**;
- heating, ventilation, and air conditioning (HVAC) and control systems;
- **decontamination technologies** (e.g., autoclaves, **effluent decontamination systems, dunk tanks**);
- **primary containment caging** systems used in **small animal containment zones (SA zones)**;
- centrifuges; and
- laboratory equipment and apparatus used for activities with pathogens and toxins.

8.3 Identification of Trainees

Identification of the intended audience is a key component of designing a training program, since it permits the identification of specific training needs and the type of training suitable for the learning styles of the target audience.

8.3.1 New Personnel

Implementation of an orientation program for new personnel gives them the requisite instruction prior to **exposure** to work-related hazards. Training of new personnel should include all training elements identified in Section 8.2 of this chapter, and any other relevant topics (e.g., a review of the organization's history, safety program, policies, personnel rights and responsibilities, general Workplace Hazardous Materials Information System (WHMIS) information). Hands-on training and extra guidance and supervision should be provided during the initial period of employment for a new employee. In addition to formal training, on-the-job experience is also important. Trainees may carry out activities with infectious material and toxins within the containment zone, provided that they are supervised by **authorized personnel**.

8.3.2 Existing Laboratory Personnel

Training is an ongoing process and is therefore not limited to new personnel. Existing personnel may require training or education with respect to new procedures, work in new environments, or work with new infectious material or toxins. Refresher training, provided at a frequency determined by a training needs assessment review or when warranted by a change in the biosafety program, helps keep personnel knowledgeable about the hazards, risks, resources, and control measures in their workplace. Annual refresher training on emergency response procedures is required (CBS Matrix 4.3). Refresher training also provides an opportunity to educate personnel with respect to any new information about the infectious material or toxins being used, changes in recommended practices, or changes in regulatory requirements.

8.3.3 Other Personnel

A training program needs to consider all personnel who will access the containment zone; not just those handling the infectious material and toxins. Visitors, contractors, janitorial staff, security staff, and maintenance staff require training on the hazards, risks, and control measures, in accordance with anticipated activities and supervision by authorized personnel while conducting activities in a containment zone (CBS Matrix 4.3).

8.3.4 Learning Conditions

Incorporation of adult learning principles into the design of biosafety education and training programs will promote program success. This may include focusing on motivation, reinforcement, retention, and transference of existing skills and knowledge. Since people differ in their learning styles, a variety of teaching methods and tools are recommended in order to reach a broader audience. Training is most effective when a variety of education tools are used, such as a presentation combined with visual aids, videos, self-directed tutorials, and problem solving activities. Acting out scenarios or rehearsing emergency drills will reinforce knowledge and skills acquired through other teaching methods. Trainers should also consider accessibility issues, such as language barriers and hearing impaired participants, and adjust their approach accordingly.

8.4 Training Evaluation

A variety of methods can be used to evaluate the uptake of knowledge and skills conveyed in a training program. The evaluation method that is selected (e.g., written test, hands-on evaluation) should effectively measure the trainee's skill development and knowledge acquisition. Pre- and post-training tests or quizzes are helpful tools for measuring whether the learning objectives were achieved. The evaluation of workplace practices and behaviour through facility audits, inspections, or regular monitoring by supervisors can also provide a useful indication of how well the training was understood, and whether retraining or review of the training program is warranted. Training evaluation forms may be handed out at the end of a training course or session to solicit feedback from trainees. This will provide valuable feedback on the effectiveness and efficiency of the course content, instructor(s), and teaching practices, and can assist in improving the training program.

An annual (or periodic) performance review of individuals with access to human or animal pathogens or toxins provides an opportunity to gauge their adherence to, and understanding of, biosafety and biosecurity procedures. The annual review also provides an opportunity for the supervisor to review and reinforce the importance of biosafety and biosecurity, to discuss the requirements of the *Human Pathogens and Toxins Act* (HPTA), HPTR, *Health of Animals Act* (HAA), *Health of Animals Regulations* (HAR), and the CBS, and address any potential problems that have affected work performance in the past or may impact future performance.[3,4,5]

8.5 Training Records

Training and retraining records document the participation and successful completion of training. These records may include attendance sheets, orientation checklists, examinations, certificates, or other types of records (e.g., a description of the training with signatures of the attendee, trainer, and supervisor), as deemed appropriate by the organization. Training and retraining records provide documentation of the date the course or training was provided, the names of individual participants/trainees, and the type or name of the course/training to facilitate tracking of retraining requirements. Biosafety training and retraining records may be combined with other occupational health and safety program training records, where applicable. All training and retraining records should be kept up to date and the most recent version should be kept on file (i.e., if training is repeated, updated, or a refresher course given, only the most recent record of training needs to be retained for a given individual). Minimum retention periods for training records can be found in Matrix 4.10 of the CBS. Such records will be used to determine refresher training needs.

8.6 Training Program Review

Regular evaluation of the content of the training program will help identify areas that need to be updated to ensure that the information is accurate and relevant. It is recommended that the program be reviewed and updated annually at a minimum, or whenever changes occur in working conditions, procedures, hazards, or hazard information. Training and retraining records should also be included in the review of the biosafety and biosecurity programs as a measure of training program performance (e.g., frequency of training sessions, number of attendees, variety of topics/programs). This will provide an opportunity to adjust resources so as to optimize the training program.

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CHAPTER 9

PERSONAL PROTECTIVE EQUIPMENT

CHAPTER 9 – PERSONAL PROTECTIVE EQUIPMENT

Personal protective equipment (PPE) refers to devices and clothing designed to minimize the **risk** of **exposure** to various hazards. PPE is the last line of defence to protect personnel and to minimize the risk of transmitting **pathogens** and **toxins** to the public and the animal population. PPE helps to prevent the **release** of pathogens and toxins on contaminated individuals (or their clothes) by providing a barrier between the individual and the **infectious material** and toxins being handled or stored. PPE should be the last form of control considered as it provides an additional barrier to protect against exposure to hazardous materials in the event of failure in the administrative or engineering controls. PPE requirements are described in Matrix 4.4 of the *Canadian Biosafety Standard* (CBS), 2nd Edition.[1]

In Canada, occupational health and safety is regulated provincially, territorially, and federally, and requirements pertaining to PPE have been incorporated into the relevant occupational health and safety legislation. In general, the employer is responsible for ensuring that appropriate PPE is available, properly maintained, used, and that personnel are appropriately trained on how to use it. The PPE requirements specified in the CBS are specific to **biosafety** and not intended to supersede any provincial or territorial legislation, nor the regulatory requirements of local occupational health and safety legislation. This chapter provides guidance on the types and general use of the PPE commonly used in **containment zones** where infectious material and toxins are handled or stored.

9.1 Types and Selection of Personal Protective Equipment

PPE can include respirators, hand and foot protection, head and eye protection, and full-body protection. Selection of PPE is based on a **local risk assessment (LRA)** of the containment zone and is specific to the work to be performed.

9.1.1 Hand Protection

Gloves protect hands from **contamination** and reduce the risks associated with ingestion (e.g., hand-to-mouth transfer) or absorption through the skin. Gloves provide a protective barrier when handling infectious material, toxins, infected animals, or material potentially contaminated with a pathogen or toxin (e.g., tissues, **cultures**, blood, and body fluids). Gloves can be made from many different materials, and the type of glove selected will depend on the specific activity and hazard concerned; they should be clean, disposable, and fluid-resistant for handling infectious material or toxins.

Fluid resistance of gloves is affected by conditions of use including type and concentration of chemicals used, duration of use, temperature, physical stress, and material thickness.[2] Table 9-1 provides general recommendations for glove materials commonly used for work involving pathogens, infectious materials, toxins, and chemical disinfectants based only on chemical compatibility data. Glove material compatibility with a chemical can be assessed by measuring the amount of time elapsed between exposing the outer surface of a glove to a chemical and detecting the chemical on the inside of the glove (i.e., breakthrough time); measuring the flow of the chemical through the glove material (i.e., permeation rate); and assessing the degradation in the physical properties (e.g., brittleness, softening, swelling) of the glove material as a result of exposure to the chemical.[3]

Table 9–1: Compatibility of Natural Rubber, Synthetic Rubber, and Plastic Polymer Gloves with Common Chemical Disinfectants.[4,5,6,7]

Disinfectant Type	Chemical Disinfectant	Natural Rubber Latex	Synthetic Rubber		Plastic Polymer
			Neoprene	Nitrile	Polyvinyl Chloride (PVC)
Oxidizing Agents	Sodium Hypochlorite (<15%)	G	G	F	VG
	Iodine	G	G	G	G
	Hydrogen peroxide (30%)	VG	VG	VG	VG
	Sodium Hydroxide (50%)	VG	VG	VG	VG
	Ammonium hydroxide	G	G	G	VG
Phenolics	Hexachlorophene	G	G	G	–
Quaternary ammonium compounds	N, N Didecyl Dimethyl Ammonium Chloride	G	G	VG	–
Aldehydes	Glutaraldehyde (25%)	VG	VG	VG	VG
	Formaldehyde (37% in 1/3 methanol/water)	F	G	VG	VG
Alcohols	Ethanol (92%)	F	VG	VG	G
	Isopropyl alcohol	G	VG	VG	VG
Bisbiguanides	Chlorhexidine digluconate (4%)	F	F	VG	–

F Fair; these gloves show moderate degradation effects, a moderate permeation rate, and have breakthrough times less than 30 minutes.
G Good; these gloves show very little degradation upon exposure to the chemical, have breakthrough times of greater than 30 to 60 minutes, and slow permeation rates.
VG Very good; these gloves show very little degradation upon exposure to the chemical, have breakthrough times of greater than 60 minutes, and slow permeation rates OR were recommended as the preferred glove type by the tester.
– No data available.

In addition to chemical compatibility, the degree that the glove material impedes dexterity or punctures should also be considered when selecting gloves. Static-free gloves may be needed when handling dry toxins. In general, hand exposure to infectious material or toxins can be managed effectively through the use of latex, nitrile, or vinyl gloves. Cut- or puncture-resistant gloves provide suitable hand protection for activities where there is a risk of accidental cuts, bites, punctures, or abrasions.

9.1.1.1 Double-Gloving

While typical glove materials create a suitable barrier against infectious material and toxins, they will not always remain impermeable. Gloves are susceptible to wear, punctures, rips, and tears, causing the glove barrier to fail with use. Wearing two pairs of disposable fluid-resistant gloves significantly reduces breaches to the innermost glove barrier.[8] Double-gloving may be recommended in certain situations, and indicated in higher **containment levels**, based on an LRA.

9.1.1.2 Protection from Physical Hazards

It is important to verify that the glove material selected provides an effective protective barrier against the hazards that will be encountered prior to handling the hazardous materials (i.e., compatible with disinfectants or solvents in use). For example, latex, nitrile, and vinyl gloves offer little to no protection from physical hazards such as temperature extremes (e.g., heat from an autoclave, cold from liquid nitrogen) or from sharp objects (e.g., needles, scalpels, animal teeth). In these situations, alternative or additional gloves would be more suitable. Insulated glove materials include terry cloth or wool to protect against high temperatures, and jersey- or cotton-lined nylon gloves to protect against low temperatures. When protection against sharps, cuts, or bites is needed, para-aramid fiber or stainless steel mesh gloves provide an effective barrier. For some activities, para-aramid fiber or stainless steel mesh gloves may need to be paired with fluid-resistant gloves for appropriate protection.

9.1.2 Foot Protection

Protective footwear is meant to provide an effective barrier against the pathogens and toxins encountered in the containment zone. This may include protection from spills of infectious material or toxins, or slipping, tripping, crushing, or puncture injuries, all of which have the potential of leading to or increasing the risk of an exposure to pathogens and toxins, and thereby to a risk of subsequent transmission to the public or the animal population. Completely enclosed footwear will protect the entire foot from exposure to hazardous liquids in the event of a spill. The risk of an accidental trip or fall can be reduced by wearing footwear with no heels or low heels, or footwear with a non-slip sole in areas where the walking surface is often wet or slippery. Steel-toe footwear can be worn to protect against crushing injuries when working with heavy objects or **large-sized animals**. For activities involving the use of sharp objects, footwear with puncture-resistant soles provides protection

against puncture injuries. Footwear made of non-absorbent materials will enable easy cleaning and **disinfection**. The use of dedicated footwear limits the **movement** of infectious or potentially infectious material out of the containment zone on contaminated footwear.

Disposable, fluid-resistant shoe covers provide an added layer of protection from liquid contamination. Reusable boot covers can be used, provided that appropriate **decontamination** procedures are in place. Alternatively, rubber boots may be used in conjunction with disinfectant footbaths to protect personnel whenever **large volumes** of water will be used (e.g., cubicle decontamination and cage washing). Additional protective footwear for work in **animal rooms**, **animal cubicles**, and **post mortem rooms (PM rooms)**, may include boot covers in animal containment zones housing **small-sized animals**, and rubber boots or other safety footwear in animal cubicles and PM rooms housing large-sized animals.

In addition to the requirements specified in Matrix 4.4 of the CBS, foot protection should comply with Canadian Standards Association (CSA) standard CSA-Z195, *Protective Footwear*, where applicable.[9]

9.1.3 Head Protection

Some activities involving infectious material and toxins pose a risk of exposure due to sprays, splashes, or airborne exposure. This can be mitigated through the use of head protectors or covers (e.g., liquid-repellent bouffant caps) to shield the hair and scalp from contamination. Where applicable, head protection should comply with standard CSA Z94.1, *Industrial protective headwear — Performance, selection, care, and use.*[10]

9.1.4 Eye and Face Protection

There are many different types of eye and face protection that can be used to shield the eyes, nose, or mouth from flying objects or splashes from infectious liquids or toxins. The type of eye and face protection selected will depend on the degree of coverage needed for the specific task at hand. Safety glasses protect the eyes from injuries associated with larger objects, including chips, fragments, sand, and dirt, as well as minor splashes. Safety goggles provide a higher level of protection due to the snug fit over and around the eyes, which creates a barrier to liquid hazards. Face shields provide coverage of the nose, mouth, and skin, in addition to the eyes. Depending on the type of protective eye and face equipment selected, prescription eye glasses may be worn underneath; safety glasses can also have prescription lenses. Some activities may pose additional risks for users of contact lenses in the event of an exposure or injury affecting the eye. A means of identifying contact lens wearers will help facilitate a timely and appropriate response in the event of an eye-related exposure or injury. Eye and face protection may be reused, provided that it is appropriately decontaminated after coming in contact with infectious material or toxins. Used disposable eye and face protection is considered contaminated **waste**.

Where applicable, eye and face protection should comply with standard CSA Z94.3, *Eye and Face Protectors* and CSA Z94.3.1, *Selection, Use, and Care of Protective Eyewear*. According to CSA Z94.3, face shields are considered secondary protectors only and provide adequate eye protection only when worn with safety glasses or goggles.[11,12]

9.1.5 Body Protection

A lab coat is the most common type of PPE used to protect an individual's body and personal clothes against contamination with infectious material. Lab coats that are approximately knee-length and cover the arms to the wrists protect the skin and personal clothing from exposure to hazardous materials. Lab coats that fit closely to the body and have cuffed sleeves help prevent dragging and catching of clothing during **laboratory** work. Snap closures are preferred over buttons to allow quick removal of the lab coat in the event of an emergency. Lab coats are commercially available in single-use (i.e., disposable) or reusable materials. Fire-resistant and fluid-resistant varieties are also available; these provide increased protection against flammable or liquid hazards. Restricting storage and use of lab coats and other protective clothing to designated areas within the containment zone helps prevent contamination of clean areas.

Wearing appropriate personal clothing also contributes to body protection. Wearing clothes that cover the legs down to the ankles will offer protection. Shorts, skirts, and other clothing that leaves the legs exposed below the lab coat should not be worn in the containment zone.

9.1.5.1 Additional Layer of Protective Clothing

An additional layer of dedicated PPE provides enhanced protection for work with infectious material, toxins, or animals infected with **zoonotic pathogens**. This additional layer may include a second pair of gloves; head covers; solid-front gowns with tight-fitting wrists worn over dedicated laboratory clothing; full-body suits and coveralls; or, disposable sleeve covers or waterproof aprons worn over a solid-front gown or lab coat. A solid-front, rear-closing gown, typically worn over dedicated clothing (e.g., scrubs) in place of a lab coat, provides protection to the torso and can be worn when working with open vessels of infectious material or toxins. Surgical scrubs can be worn under the outer layer of protective clothing to avoid potential contamination of personal attire in the event the outer layer of protection is breached. Surgical scrubs are commonly part of the dedicated PPE for **high containment zones** or animal rooms and cubicles as they can be sterilized and laundered for reuse. Surgical gowns designed for use in operating rooms are reinforced with impermeable fabric to provide a full fluid-resistant layer, and have back tape ties and an overlapping back for improved coverage. Aprons are commonly used in PM rooms and necropsy suites; they are worn over lab coats or gowns and offer additional protection against spills or splashes of infectious material or toxins. Full-body suits and coveralls provide further protection and are available in disposable or reusable materials. Individuals working with large-sized animals commonly wear coveralls to provide protection from organic material. Coverall materials

such as flashspun high-density polyethylene fibres, rubberized cloth, polyvinyl chloride (PVC), and neoprene provide a good barrier as they are hard to tear or puncture and will prevent penetration by biological, chemical, or particulate contaminants.

9.1.5.2 Positive-Pressure Suits

Positive-pressure suits provide the maximum full-body coverage (i.e., head-to-toe) to protect from the containment zone environment, and include integral boots, gloves, and headpiece. Breathable air is provided through a supplied air hose connected to the suit, which creates a positive pressurization within the suit. Integrity testing is conducted to demonstrate that suits are gas tight (i.e., no tears or leaks) and able to maintain a fixed positive pressure when inflated.

9.1.6 Masks and Respiratory Protection

Safe operational practices and the use of **primary containment devices** can limit the creation of, and exposure to, infectious **aerosols** or aerosolized toxins. Surgical masks and many types of dust masks offer little protection from **airborne pathogens**, infectious aerosols, or aerosolized toxin, but will protect mucous membranes of the nose and mouth from spills and splashes. Masks are not intended to be used more than once. Respirators are used when there is a risk of exposure to aerosolized toxins or infectious aerosols that can be transmitted through the inhalation route. Respirators are divided into two classes: air purifying respirators and atmosphere-supplying respirators. The type of respirator selected will depend on the hazard associated with the particular activity being carried out. Personnel education on airborne hazards and training on respirator selection, fit, inspection, and maintenance are some examples of elements of a workplace respiratory protection program, which is required for any workplace where respirators are used. Where applicable, respiratory protection should conform to standard CSA Z94.4, *Selection, Use and Care of Respirators*.[13]

9.1.6.1 Respirator Fit

All respirators need to fit properly in order to function as intended. Some types of respirators require a seal between the apparatus and the wearer's face in order to provide adequate protection. Using the wrong respirator or misusing one can be as dangerous as not wearing one at all. The respirator should be individually selected and fitted to the operator's face, and fit tested for its seal. Facial hair, imperfections of the skin, cosmetics, and changes in a person's weight can affect respirator fit. Most jurisdictions within Canada currently require qualitative or quantitative fit-testing to be conducted to demonstrate proper fit for the selected respirator(s) before an individual carries out any activities that require respiratory protection. In addition, standard CSA Z94.4, *Selection, Use, and Care of Respirators*, requires that an employer take reasonable precautions to verify that an individual is medically cleared to wear a respirator. Proper use and care of respiratory protection equipment is a core component of the training program in workplaces where respirators are used.

9.1.6.2 Air Purifying Respirators

Air purifying respirators help reduce the concentration of **microorganisms** and particulates in the air inhaled by the user to an acceptable exposure level by passing the air through a particulate filter or chemical cartridge. Half-mask air purifying respirators cover the nose and mouth but not the eyes, while full-face air purifying respirators cover the entire face. Disposable half-mask air purifying respirators, including the N95 and N100 type respirators, are designed for single use. Non-powered half-mask and full-face respirators can also use disposable filter cartridges to provide a similar level of protection. Non-powered respirators work through the creation of negative-pressure inside the respirator during inhalation. There are nine classifications of particulate filters used with non-powered respirators approved by the United States National Institute of Occupational Safety and Health (NIOSH).[14] These are the N-Series (N95, N99, N100; not resistant to oil), R-Series (R95, R99, R100; oil-resistant), and P-Series (P95, P99, P100; oil-proof). The associated numbers identify the efficiency in removing contaminants. Respirators rated at N95 or higher are adequate to protect personnel carrying out most activities with microorganisms.

9.1.6.3 Powered Air Purifying Respirators

Powered air purifying respirators (PAPRs) create a positive-pressure around the wearer's head. PAPRs are designed to be decontaminated and reused, and the disposable filter cartridges are replaced on a regular basis, as determined by an LRA. Particulate filters for PAPR units are all high efficiency (HE), which are certified to be 99.97% efficient at filtering the most penetrating particle size (0.3 μm). Due to the effects of impaction, diffusion, and interception, **high efficiency particulate air (HEPA) filters** are even more efficient for particles that are either smaller or larger than 0.3 μm.[15] Most PAPR filters are suitable for use against oil-based aerosols; however, this is not always the case and users should check the manufacturer instructions before use in oil environments.

9.1.6.4 Atmosphere-Supplying Respirators

Atmosphere-supplying respirators deliver clean, breathable air from a source such as a compressed air cylinder or tank. These are generally supplied-air respirators, but could be a self-contained breathing apparatus (SCBA). Supplied-air respirators deliver air through a small hose connected to an air compressor or a cylinder of compressed air, whereas SCBAs supply breathable air from a portable cylinder worn on the back.

9.2 Key Considerations for the Selection of Personal Protective Equipment

No single glove or respirator type can be expected to provide protection against all the different types of hazards in a work environment. Poorly chosen PPE can impair personnel performance (e.g., stiff, bulky or inappropriately sized gloves may reduce dexterity and control), creating the potential for **accidents** that can lead to the exposure to hazards. The first step to be taken prior to handling infectious material or toxins is to perform an LRA to develop safe work practices and select appropriate PPE. The selection of PPE will depend on the containment level, the amount and nature of the infectious material or toxins in use,

and the activities being performed. This type of evaluation should be conducted by the **biological safety officer (BSO)** or other appropriate personnel (i.e., infection control or industrial hygiene specialist) and the employee(s) concerned, possibly in consultation with the employer, the institutional biosafety committee (IBC), and the health and safety committee. Once the need for PPE has been identified, the correct PPE is chosen based on the degree of protection required and the suitability of the equipment for the situation (e.g., gloves that provide appropriate dexterity, clothing and footwear that provide adequate protection). For example, in **large animal containment zones (LA zones)**, where the animal cubicle provides **primary containment** and PPE is the worker's primary protection against exposure to pathogens and toxins, it is essential that personnel select their PPE accordingly. It is important to involve employees in the selection of the PPE to verify fit and comfort, and to encourage use. Once selected, employees should be adequately trained in the proper use of the PPE, including when it must be worn, the appropriate methods of donning and doffing PPE, limitations, proper care and maintenance, and decontamination and disposal of PPE.

Many factors related to the hazard must be taken into consideration when selecting proper PPE, but it is also important to take allergies and ergonomics into account. Allergies to certain materials (e.g., latex in gloves) can sometimes pose more of a health risk than the hazards themselves. Comfort and fit are key factors in addressing potential ergonomic issues; personnel may be inclined to remove PPE if it does not fit correctly or is uncomfortable. When selecting PPE that will be used when handling large-sized animals, consideration should be given to selecting PPE that is lightweight, non-encumbering, cool, and that is not prone to becoming snagged or entangled by animals or equipment.

9.3 Use of Personal Protective Equipment

9.3.1 Donning

Procedures to don, or put on, PPE describe the specific order to don each article. **Standard operating procedures (SOPs)** to don PPE prior to entering **laboratory work areas**, animal rooms, animal cubicles, PM rooms, and containment zones are developed based on an LRA and vary in complexity depending on the types of PPE used. It is important that these SOPs are understood and adhered to by all personnel. Storage of PPE at all points of routine entry enables easy access to PPE when preparing to enter the containment zone. Individuals should carefully inspect the articles for damage or breaches prior to donning PPE. For containment zones where only a lab coat and gloves are worn, the following donning procedure is recommended:

Donning Order ↓	Single Gloves and Lab Coat	Double Gloves and Lab Coat	Doffing Order ↑
	• Lab coat (properly fastened) • Gloves (fitted over cuffs of lab coat)	• Inner gloves • Lab coat (properly fastened) • Outer gloves (fitted over cuffs of lab coat)	

In containment level 2 (CL2) large animal containment zones (LA zones) (i.e., CL2-Ag zones) and high containment zones, clothing change areas are used to separate personal clothing and dedicated containment clothing, or to separate the dedicated protective clothing worn in the different areas of **containment** (i.e., animal containment zone versus animal cubicle). An example of a donning procedure when multiple layers of PPE are involved is shown below. Personal clothing and accessories, including jewelry and identification (ID) cards, are removed and stored in a dedicated area before donning PPE.

Donning Order ↓	Multiple Layers of PPE	Doffing Order ↑
	• Dedicated containment clothing, such as scrubs, dedicated footwear, shoe covers, and head covers • Inner gloves • Back-closing gown or equivalent protective layer • Mask or respirator • Eye protection, including safety glasses, goggles, or face shield • Outer gloves, fitted over gown cuffs	

Depending on the nature of the activities carried out in the containment zone, there may be special requirements posted at the point(s) of entry for personnel to follow prior to entering, including the use of specific PPE. The PPE and donning order could be different in other scenarios (e.g., working in a containment level 3 [CL3] LA zone [i.e., CL3-Ag] or PM room where a PAPR is worn based on the LRA).

9.3.2 Doffing

It is important to doff, or remove, PPE with care to minimize the risk of contamination of the skin and hair. The SOPs for doffing PPE when exiting laboratory work areas, animal rooms, animal cubicles, PM rooms, and containment zones describe the removal order and any specific instructions related to each article of PPE being removed. Doffing is generally done in the reverse order to the donning procedure, as outlined in Section 9.3.1 of this chapter. It is important to remember that the front and sleeves of the lab coat may be contaminated.

Considerations when doffing gloves are as follows:

- Gloves should be carefully removed by grasping the outside of the glove near the wrist with the opposite gloved hand and carefully peeling the glove off, turning it inside out.
- The removed glove should be held in the opposite gloved hand. A finger from the ungloved hand should slide under the wrist of the remaining glove to peel it off from the inside, creating a bag for both gloves that is carefully discarded in a designated biohazardous waste container. If two pairs of gloves are worn, these steps are repeated for the second pair.

- Hands are then to be washed, in accordance with exit procedures, before leaving the containment zone, animal room, animal cubicle, or PM room. It is recommended that hands be washed with soap under clean running water; the hands should be rubbed together to make a lather and thoroughly scrubbed, including the backs of the hands, between fingers, and under the fingernails, for a minimum of 15-30 seconds before rinsing.[16] Alcohol-based hand sanitizers are not as effective as handwashing with soap and water and cannot eliminate all types of pathogens.[17] A hand sanitizer that has been demonstrated to be effective against the pathogen(s) or toxin(s) in use in the containment zone may be an alternative where handwashing sinks are not easily accessible to avoid the spread of contamination. See Appendix B for proper handwashing technique.

Considerations for the doffing of other items and the recommended doffing sequence for all PPE are reflected in the following example:

- Gloves should be removed prior to removing hands from a **biological safety cabinet (BSC)** after handling infectious material or toxins, and they should be discarded as biohazardous waste within the BSC. This will help prevent the inadvertent spread of contamination outside the BSC. If two pairs of gloves are worn, it is the outermost layer of gloves that is removed prior to exiting the BSC. The inner layer of gloves will protect the user's hands from exposure to any residual infectious aerosols or aerosolized toxins prior to removal from the BSC. In order to prevent the spread of contamination outside of the BSC, gloved hands should be disinfected or decontaminated immediately after removal from the BSC; bare hands should be washed immediately after removal from the BSC.
- Gowns should next be removed, keeping in mind that the gown front and sleeves may be contaminated. The gown should be removed by unfastening the ties and peeling the gown away from the neck and shoulders, keeping the contaminated side away from the body and folding or rolling it into a bundle before discarding it in the designated waste container for decontamination.
- Face shields and protective eyewear should then be removed, keeping in mind that the outside of the eyepiece may be contaminated. These should be handled by the head band or ear pieces and pulled away from the face, then placed in a designated receptacle for decontamination.
- Masks and respirators can then be removed, keeping in mind that the front of the mask may be contaminated. Masks are removed as per the manufacturer directions and precautions should be taken to avoid transfer of contamination from the outside of the mask. The mask is then discarded.
- Hair covers and protective headgear can be removed and discarded or decontaminated.
- Protective footwear and shoe covers should be removed next and stored or discarded.
- Finally, the inner pair of gloves (when two pairs of gloves are worn), can be removed and discarded. Hands are immediately washed thoroughly with soap and water to remove and decontaminate any potential pathogens that may have penetrated the layers of PPE.

Doffing of PPE should always be immediately followed by handwashing at a sink dedicated for the purpose, after which personnel can change out of surgical scrubs and back into their personal clothes. Hand sanitizers may only be used as an alternative to handwashing where sinks are not easily accessible, provided the hand sanitizer has been demonstrated to be effective against the pathogen(s) or toxin(s) in use in the containment zone. This example does not represent the procedure when a walk-through body shower is required upon exit, but it does indicate the order in which PPE should be removed to minimize the risk of contamination. Personnel working in high containment zones are required to remove the additional layer of full-body coverage clothing when exiting across the **containment barrier**. Personnel exiting animal rooms, animal cubicle and PM rooms at any containment level, are to remove dedicated clothing (including footwear) or remove the additional layer of PPE and footwear (when worn), unless exiting to the dirty corridor. Where a clothing change is not required for exiting animal cubicles and PM rooms, it is good practice for personnel to use a disinfectant footbath appropriate for the pathogen in use to effectively decontaminate footwear when exiting the **animal work area**.[18]

9.3.3 General Use Tips

The following sections provide general information for the use of different types of PPE.

9.3.3.1 Gloves

- Nitrile or vinyl gloves can be worn instead of latex to provide fluid-resistance (e.g., if allergic to latex).
- Verify that gloves are intact; inspect for rips, tears and flaws before use.
- Change gloves often if wearing for long periods of time.
- Never reuse disposable gloves. Dispose of used gloves in an appropriate contaminated waste receptacle.
- Remove gloves and wash hands prior to exit from the containment zone, animal room, animal cubicle, or PM room.

9.3.3.2 Footwear

- Wear shoes that cover the entire foot with no heels or low heels.
- Footwear should protect from hazardous liquids and be easily cleaned and disinfected. Verify that disposable shoe covers are intact; inspect for rips and tears before use.
- Never reuse disposable shoe covers. Dispose of used shoe covers in an appropriate contaminated waste receptacle.
- Never wear dedicated footwear outside the containment zone.
- Wear waterproof boots in wet environments.

9.3.3.3 Head Protection

- Remove head protection prior to exiting from the containment zone.
- Decontaminate reusable head protection after use; collect disposable head covers and decontaminate prior to removal from the containment zone for disposal.

9.3.3.4 Eye and Face Protection

- Wear safety eyewear in environments where there is a chance of eye exposure.
- Wear safety goggles to protect eyes against splashes and spills.
- Wear a face shield to protect nose, mouth, and skin against splashes and spills.
- Decontaminate reusable eye and face protection after every use, even if stored in the containment zone.
- Decontaminate prescription eyeglasses at the containment barrier prior to exiting high containment zones, unless protected by additional PPE (e.g., PAPR or other full head cover, as determined by an LRA).
- Never wear dedicated eye or face protection outside the containment zone.

9.3.3.5 Body Protection

- Wear completely fastened body protection with sleeves covering arms.
- Remove, decontaminate, and launder protective clothing after it has become contaminated. In high containment zones, remove protective layer prior to exit from the containment barrier; all PPE (reusable or disposable) is decontaminated before removal from the containment zone. Reusable protective clothing is decontaminated before being sent to laundry; laundering **facilities** located inside the containment zone may be suitable for decontamination provided they have been demonstrated to be effective for decontamination of the pathogen(s) in use (i.e., validated).
- Never wear dedicated body protection outside the containment zone (e.g., in offices, cafeteria).

9.3.3.6 Masks and Respiratory Protection

- Complete respirator training and verify proper fit through qualitative or quantitative fit-testing prior to commencing any activities requiring respirator.
- Perform a seal check every time the respirator is donned.
- After every use, clean and sanitize or decontaminate the respirator according to the manufacturer's instructions or SOPs developed in consultation with the manufacturer, even if it is stored in the containment zone.
- Care must be taken to prevent the filters or cartridge media from becoming wet during decontamination. Replace cartridges that are near the end of their service life.
- Never reuse disposable respirators or masks; decontaminate used respirators and masks prior to disposal.
- Inspect the respirator after use; discard or repair any defective parts.
- Remove respiratory protection at the point at which a risk assessment deems it safe to do so upon exit from the containment zone.
- Reusable respirators should be stored in a manner that will protect them against any potential hazard that can have a detrimental effect (e.g., dust, sunlight, heat, extreme cold).

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CHAPTER 10

AIR HANDLING

CHAPTER 10 – AIR HANDLING

The heating, ventilation, and air conditioning (HVAC) system provides fresh air and maintains good indoor air quality. It provides general cleaning and filtration of air in the indoor environment and controls temperature, humidity, and odours from animals, as well as providing ventilation (e.g., for chemical use during **decontamination**). Guidelines on ventilation for **laboratory** environments are provided in several standards, including the American National Standards Institute (ANSI)/American Industrial Hygiene Association (AIHA) Z9.5, ANSI/American Society of Heating, Refrigerating and Air-Conditioning Engineers (ASHRAE) 62.1 and National Standard of Canada (CAN)/Canadian Standards Association (CSA)-Z317.2, although local regulations and building and fire codes should also be consulted.[1,2,3] The Canadian Council on Animal Care (CCAC) *Guidelines on: Laboratory Animal Facilities* provides further guidance on HVAC systems for activities involving animals.[4] The requirements for air handling in **containment zones** regulated by the Public Health Agency of Canada (PHAC) and the Canadian Food Inspection Agency (CFIA) are specified in Matrix 3.5 of the *Canadian Biosafety Standard* (CBS), 2nd Edition.[5]

10.1 Inward Directional Airflow

HVAC systems can be designed to maintain a negative differential air pressure within the containment zone so that air flows into the containment zone, across the **containment barrier**, from areas of lower **containment** to areas of higher containment. **Inward directional airflow (IDA)** helps to establish a buffer zone of air at the containment barrier that serves to reduce the potential for aerosolized **infectious materials** or **toxins** to be **released** from the work area. Where inward directional airflow (IDA) is provided, **anterooms** and door **interlocks** (or **standard operating procedures [SOPs]**) are often included to accommodate the entry of personnel, animals, and equipment through the containment barrier. HVAC systems that provide inward directional airflow (IDA) are critical **containment systems**. Further guidance and considerations regarding anterooms can be found in Chapter 3. The requirements for anterooms are specified in Matrix 3.3 of the CBS; the requirements for inward directional airflow (IDA) are specified in Matrix 3.5 of the CBS.

High containment zones are designed so that air pressure decreases when progressing deeper into the containment zone (e.g., through a sequence of air pressure differentials between the clean and dirty sides of anterooms and showers). In high containment zones, HVAC systems can be supported by emergency power, and at **containment level** 4 (CL4), building automation systems are supported by uninterruptible power supply (UPS) to maintain operation in case of a power failure. Interlocks, visual and audible alarms, and protocols can be used to prevent the opening of the **critical door** on the containment barrier simultaneously with the door leading into the anteroom from outside of the containment zone, and the door(s) leading from the anteroom into the **laboratory work area, animal room, animal cubicle**, or **post mortem room (PM room)**, which could disrupt the inward directional airflow (IDA) and the integrity of the containment barrier.

Sealing openings in the barrier (e.g., windows, doors, ductwork, conduits), and the correct use of appropriately designed equipment located on the containment barrier (e.g., barrier autoclaves, **pass-through chambers**, and **dunk tanks**), will help to maintain inward

directional airflow (IDA) and the integrity of the containment barrier (Matrices 3.2 and 4.8 of the CBS). Monitoring devices such as ball-in-tube monitors above doors that visually demonstrate inward directional airflow (IDA), or a differential pressure gauge to measure the pressure differential between rooms, are provided to allow containment zone personnel to verify that the inward directional airflow (IDA) is being maintained prior to entry (Matrices 3.5 and 4.5 of the CBS).

High efficiency particulate air (HEPA) filtration of exhaust air reduces the **risk** of releasing infectious material or toxins from high containment zones, while small in-line HEPA filters are used to protect the lines to pressure differential monitoring devices that penetrate the containment barrier. Supply air may also be HEPA filtered, depending on the containment level (CBS Matrix 3.5).

The following list highlights some requirements and recommendations for the installation of HVAC systems:

- Air should be exhausted from high containment zones to avoid re-introduction into the building, in accordance with applicable standards such as ANSI/ASHRAE 62.1.[2]
- Controls such as interlocked supply and exhaust air systems will prevent sustained pressurization of the laboratory during fan failures, and audible and visual alarms will notify personnel of such failures (CBS Matrix 3.5).
- Transfer air devices designed to provide controlled leakage into the containment zones should be designed to ensure directional airflow is maintained and that **backdraft protection** is provided. These are devices that can be mounted into walls or doors to allow air transfer to limit pressure differentials between rooms.
- The use of auxiliary localized humidifiers may contribute to personnel and animal well-being by providing additional moisture.
- Mechanical support services for HVAC systems should be located as close as possible to the containment barrier. Locating HEPA filter housings as close to the containment barrier as possible will reduce the length of potentially contaminated ductwork. Installing valves to isolate sections of the ductwork will facilitate decontamination.

10.1.1 Verifying Inward Directional Airflow and Containment Barrier Integrity

Visual demonstration of inward directional airflow (IDA) at all critical doors of the containment barrier will confirm that air flows toward areas of higher containment, according to design, and never the reverse. Pressurization across adjacent areas can be visually tested under normal HVAC system operations by holding a smoke pencil at each door with the door in its normal state (i.e. typically closed). Testing with a smoke pencil or other visual aid should be conducted under normal operating conditions, as well as under simulated failure scenarios.

Smoke testing is also used to detect leaks in the surfaces within a containment zone that form part of the containment barrier. All joints, corners, sealed penetrations (e.g., conduits, plumbing, wiring), as well as seals around doors, windows, autoclaves, and dunk tanks should be surveyed for leaks. Visual inspections of floors, walls and ceilings, as well as floor/wall and wall/ceiling joints can identify cracks, chips, or wear that need repair.

10.1.1.1 Pressure Decay Testing

In containment zones with **airtight doors** or **sealable doors, pressure decay testing** of the containment zone (whole room) provides an indication of the integrity of the room perimeter (i.e., the ability of gases and liquids to move through the perimeter membrane and service penetrations). The basic procedure for pressure decay testing under negative pressure is as follows:[6]

1. Isolate the area by closing and securing all doors, valves, and **isolation 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., differential pressure gauges).
2. Install a calibrated manometer across the containment barrier such that it is not affected by air distribution. The manometer should have a minimum accuracy of 10 Pa (i.e., 0.05 inches water gauge [in. w.g.]) and be capable of reading pressure up to 750 Pa (i.e., 3 in. w.g.).
3. Install a ball valve in the piping between the vacuum source (i.e., pump or fan) and the room to allow the room to be sealed once the test pressure has been attained.
4. Connect a vacuum source to the room and create a 500 Pa (i.e., 2 in. w.g.) negative pressure differential. Allow the room pressure to stabilize and close the valve between the vacuum source and the room to seal the room at 500 Pa (i.e., 2 in. w.g.).
5. Dynamically trend pressure loss starting at 500 Pa (i.e., 2 in. w.g.) negative pressure differential; record the differential pressure at 1 minute intervals for 20 minutes.
6. If repeat testing is required, allow a 20 minute wait period or longer if necessary for equilibration of the HVAC system.
7. Disconnect the vacuum source and open the ball valve slowly to allow room pressure to return to normal.

8. If the 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 (e.g., joints, corners, sealed penetrations); or if using audible leak location method, locate audible leaks (i.e., using electronic sound detection equipment);
    - identify places where bubbles are found; and
    - after repair of leak, retest as required.

10.2 High Efficiency Particulate Air Filters

HEPA filters are capable of filtering greater than 99.97% of airborne particles 0.3 µm in diameter, the most penetrating particle size. Due to the effects of impaction, diffusion, and interception, HEPA filters are even more efficient for particles that are either smaller or larger than 0.3 µm in diameter.[7] Although HEPA filters are factory rated at 99.97% efficient, they will typically achieve a much higher efficiency. HEPA filter performance of a minimum efficiency of 99.99% should be used for containment **facilities**. Consumers need to make the installed filtration requirements clear to the HEPA filter suppliers prior to purchase.[8]

Typical HEPA filters are fabricated from a single pleated sheet of fibres. The pleats are divided by separators (e.g., corrugated aluminum) to prevent the pleats from collapsing in the air stream (see Figure 10-1 inset). The filter medium is glued into a wood, metal, or plastic frame, which can be easily damaged or distorted if handled incorrectly. For this reason, it is important that filter integrity and performance be verified after installation or relocation, and regularly thereafter.

HEPA filters are typically installed in filter housings (Figure 10-1) by means of a gasket (e.g., neoprene) or fluid (e.g., gel) seal. A common problem with gaskets is that they can be compressed, torn, or may be incompatible with gaseous decontaminants. For example, some types of neoprene (e.g., open-celled black neoprene gaskets) are degraded by hydrogen peroxide (H2O2). Dense gasket materials can be more resistant to frequent decontamination than open-celled gaskets made of similar material. Gel seals establish an airtight seal between the filter and housing by means of a channel filled with gel that surrounds the filter perimeter. The housing knife-edge flange seals into this channel. Gel seals are not prone to the compression and compatibility problems associated with gasket seals. The integrity of HEPA filters is performance tested to confirm that there are no leaks in the filter media, the gaskets, 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 (i.e., "scan" testing).

Filters that are loaded should be replaced when airflow cannot be maintained within target range, or as per the manufacturer's instructions. Consideration should be given, especially in animal containment zones, to the installation of pre-filters to protect HEPA filters from dust and debris (e.g., hair, fur). The standard ASHRAE 52.2, *Gravimetric and Dust-Spot Procedures for Testing Air-Cleaning Devices Used in General Ventilation for Removing Particle Matter* can be consulted for more information on pre-filters.[9]

HEPA filters that can be decontaminated through ***in situ*** fumigation with a gas (e.g., formaldehyde or vaporized hydrogen peroxide [VHP], chlorine dioxide [ClO_2]) allows for them to be decontaminated prior to their removal. In containment zones where available **decontamination technologies** are not effective against the **pathogens** and toxins handled (e.g., **prions** are not completely inactivated by most common decontamination methods), an alternative mechanism for the safe removal of HEPA filters is required. Examples of suitable alternatives include using HEPA filters with a bag-in/bag-out capability or using procedures to contain the HEPA filter for removal followed by its subsequent decontamination.

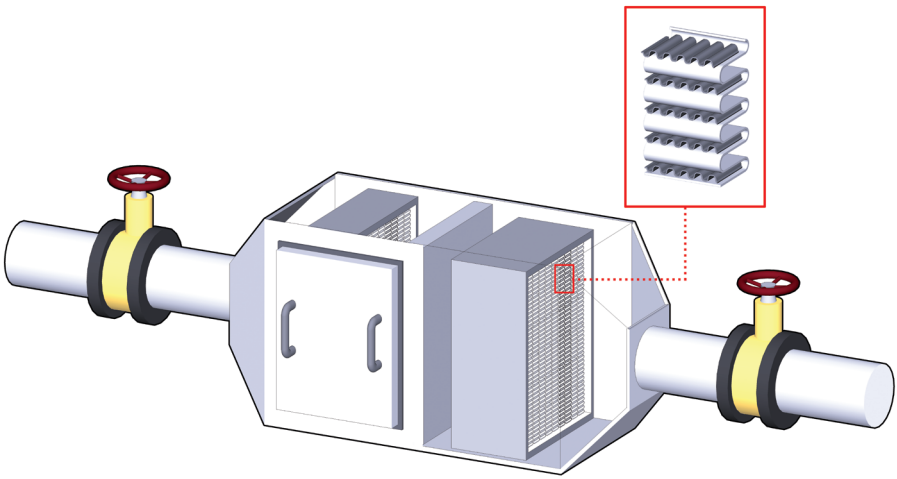

Figure 10–1:
Representative Diagram of a High Efficiency Particulate Air (HEPA) Filter Housing with Cut Away Showing HEPA Filters within the Housing.

The inset shows the filter media: a pleated sheet of fibres divided by separators.

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7. Richardson, A. W., Eshbaugh, J. P., Hofacre, K. C., & the Edgewood Chemical Biological Center United States Army Research, Development and Engineering Command. (2006). *ECBC-CR-085: Respirator Filter Efficiency Testing Against Particulate and Biological Aerosols Under Moderate to High Flow Rates*. Columbus, OH, USA: Battelle Memorial Institute.
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9. *ANSI/ASHRAE 52.2-2012, Method of Testing General Ventilation Air-Cleaning Devices for Removal Efficiency by Particle Size*. (2012). Atlanta, GA, USA: American National Standards Institute / American Society of Heating, Refrigerating and Air-Conditioning Engineers.

CHAPTER 11

BIOLOGICAL SAFETY CABINETS

CHAPTER 11 – BIOLOGICAL SAFETY CABINETS

Biological safety cabinets (BSCs) provide effective **primary containment** for work with **infectious material** or **toxins** when they are properly maintained and used in conjunction with **good microbiological laboratory practices**. The various classes and types of BSCs operate under the same basic principles. Personnel protection is provided through a continuous stream of inward air, known as inflow, which helps prevent **aerosols** from escaping through the front opening. The air that is exhausted into the surrounding **containment zone** or directly to the outside atmosphere is passed through **high efficiency particulate air (HEPA) filters** to protect the environment. Some classes of BSCs also offer product protection by using HEPA-filtered downflow to flush the cabinet interior of airborne contaminants and to prevent unfiltered inflow air from entering the work area. This chapter provides general descriptions of the different types and classes of BSCs. Different manufacturers may have unique design features and new technology in their BSCs. The **physical containment requirements**, **operational practice requirements**, and **performance and verification testing requirements** relating to BSCs in containment zones regulated by the Public Health Agency of Canada (PHAC) and the Canadian Food Inspection Agency (CFIA) are described in Matrices 3.7, 4.6, and 5.1 of the *Canadian Biosafety Standard* (CBS), 2nd Edition.[1]

11.1 Classes and Descriptions

11.1.1 Class I

Class I BSCs provide personnel and environmental protection, but offer no product protection (Figures 11-1a and 11-1b). This type of cabinet is commonly used to enclose equipment (e.g., fermenters, homogenizers) or for procedures where product protection is not a concern (e.g., cage changing). Room air is drawn into the cabinet through the front opening, moves directly across the workspace, and is then discharged from the BSC through a HEPA filter. Class I BSCs can recirculate exhaust air into the containment zone, or exhaust directly to the outside atmosphere when hard-ducted to the **facility**'s heating, ventilation, and air conditioning (HVAC) system. Since the air is never recirculated within the BSC, it is possible to work safely with minute quantities of volatile toxic chemicals if the BSC is hard-ducted. Class I BSCs are suitable for work with **Risk Group** 1 (RG1), Risk Group 2 (RG2), and Risk Group 3 (RG3) **biological material**. BSCs that are used as cage changing stations may require more frequent filter replacement due to filter loading.

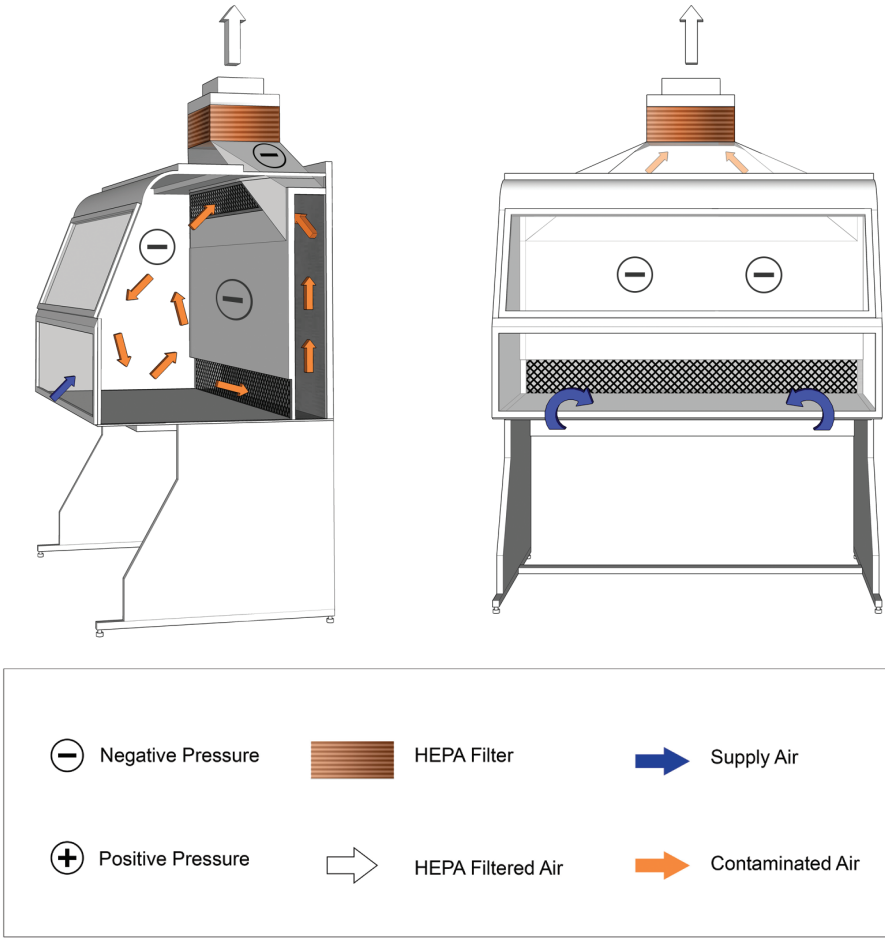


Figure 11–1a: Illustration of a Class I Biological Safety Cabinet (BSC).

Cabinet used in conjunction with building HVAC system. HEPA-filtered exhaust air is vented to the outside.

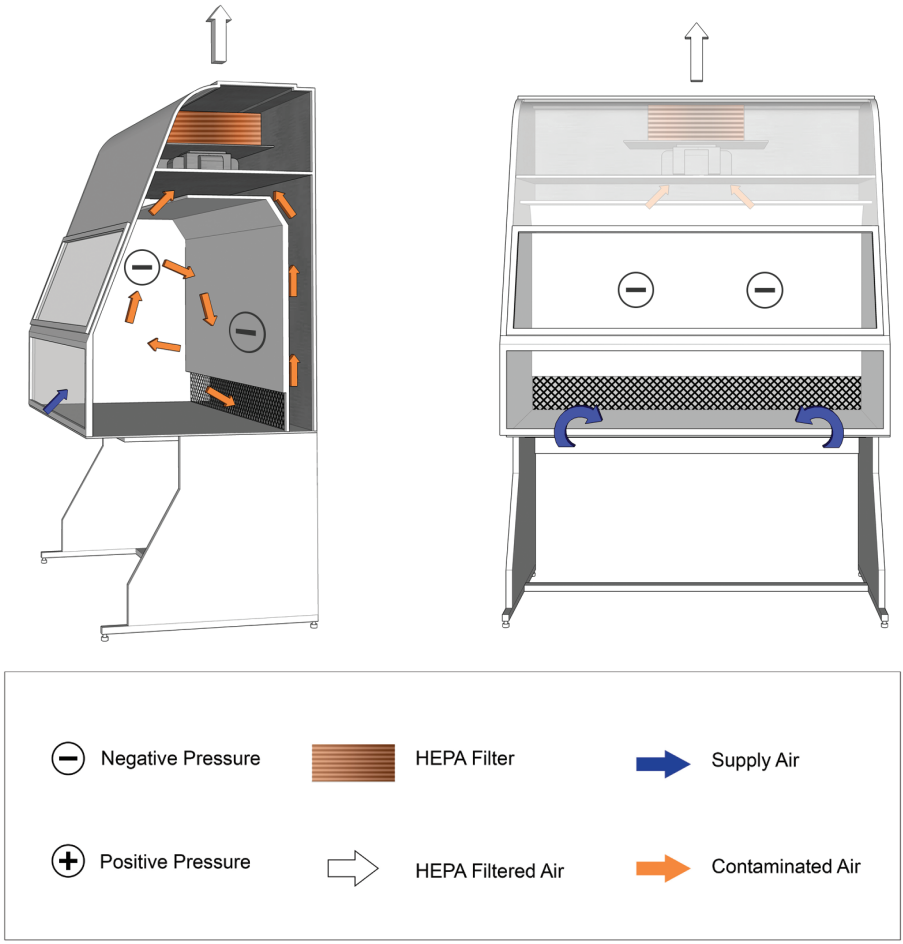


Figure 11–1b: Illustration of a Class I Biological Safety Cabinet (BSC).

Cabinet shown is complete with internal motor/blower assembly. HEPA-filtered exhaust air is vented into the room.

11.1.2 Class II

Class II BSCs provide personnel and environmental protection; however, unlike Class I BSCs, they also offer product protection. Class II BSCs are further divided into four types: A1, A2, B1, and B2. Newer models do exist that can be configured as either a type A or a type B BSC during installation. The main differences between the types are the ratio of air exhausted from the BSC to the air that is recirculated within the BSC, and the type of exhaust system present. Some BSCs may recirculate air within the containment zone, while others may exhaust air directly to the outside atmosphere through dedicated ductwork. Class II Type A are the most commonly encountered BSC in a microbiology laboratory. RG1, RG2, and RG3 biological material can be handled safely in a Class II BSC. Risk Group 4 (RG4) biological material may be handled in a Class II BSC provided that a positive-pressure suit is worn. Table 11-1 summarizes the technical differences between the Class II cabinets.

11.1.2.1 Type A1

In this type of BSC, the room air and a portion of the BSC's recirculated air are drawn into the front grille and then HEPA filtered before flowing downwards over the work area (Figure 11-2). At approximately 6-18 cm above the work area, the downflow air splits, with approximately 50% of the air passing through the front grille and the other 50% passing through the rear grille, which then combine within a contaminated plenum. The contaminated plenum is either negatively pressured, or positively pressured and may be surrounded by negatively pressured plenums or ducts (Figure 11-2 illustrates a model with a positively pressured contaminated plenum). From this contaminated plenum, approximately 30% of the air passes through a HEPA filter before being exhausted out of the cabinet. The remaining 70% is recirculated and passed through a HEPA filter before flowing once again towards the work area. Type A1 BSCs can be exhausted into the containment zone or directly to the outside atmosphere through a thimble connection. Type A1 BSCs are never hard-ducted. Absolutely no work with volatile toxic chemicals or radionuclides is performed within this type of BSC as the recirculated air could cause a dangerous buildup of the toxic materials inside the BSC, or inside the containment zone.

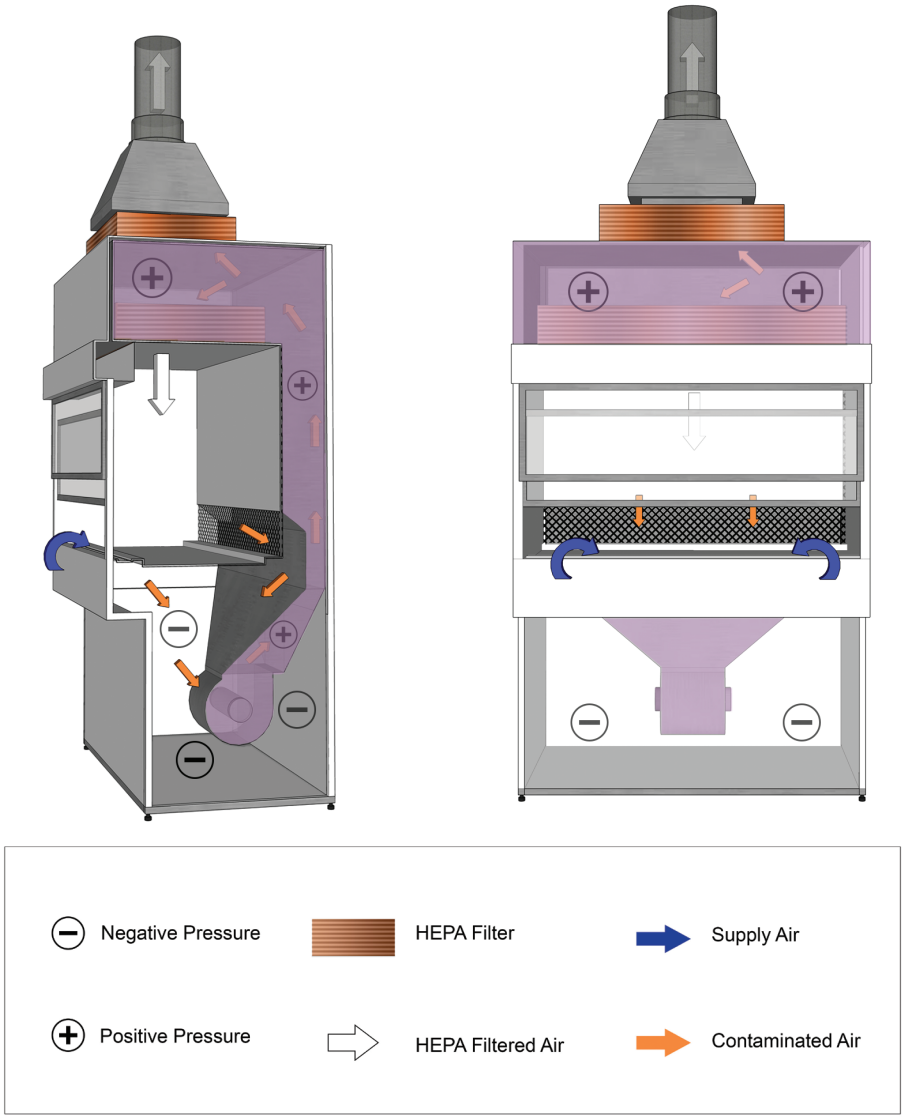


Figure 11–2:
Illustration of a Class II Type A1 Biological Safety Cabinet (BSC) (with a Positively Pressured Contaminated Plenum).

Cabinet exhaust may be recirculated into the room or vented to the outside atmosphere through an air gap type (thimble) connection, as shown. Purple shading indicates positively pressured contaminated plenum.

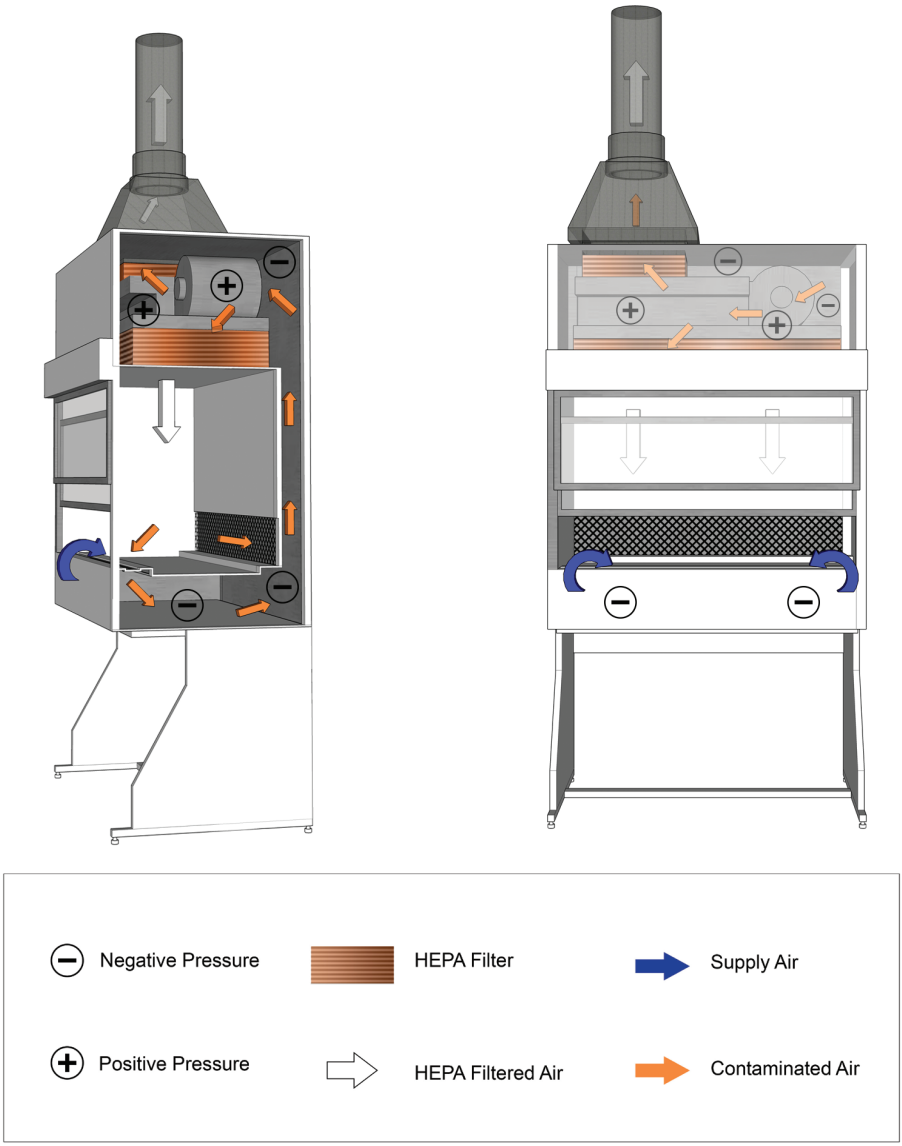


Figure 11–3: Illustration of a Class II Type A2 Biological Safety Cabinet (BSC).

Cabinet exhaust may be recirculated into the room or vented to the outside atmosphere through an air gap type (thimble) connection, as shown. Cabinet shown has a negatively pressured plenum.

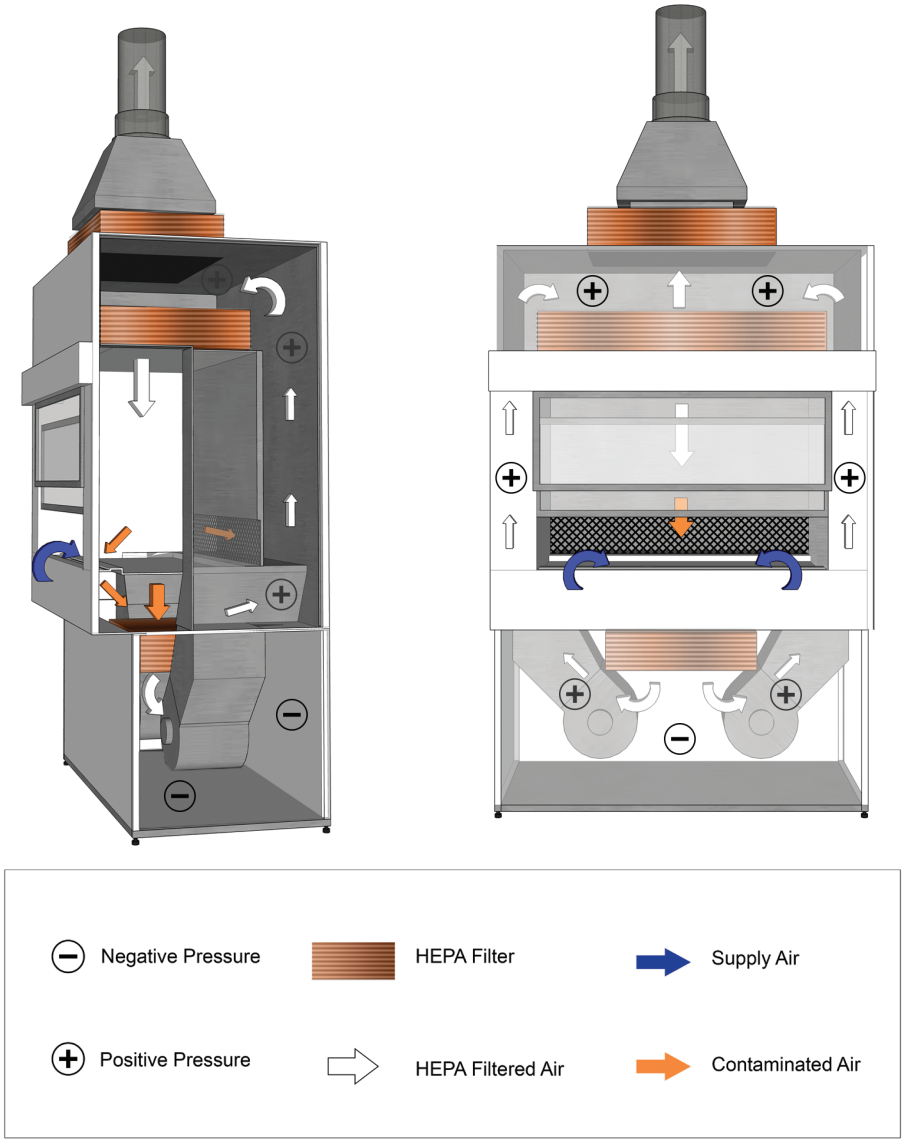


Figure 11–4: Illustration of a Class II Type B1 Biological Safety Cabinet (BSC).

Cabinet is vented to the outside atmosphere through a hard-ducted connection, as shown. The positively pressured plenum in this example is not contaminated, as the air is filtered before passing through the exhaust blowers.

11.1.2.2 Type A2

Type A2 cabinets are almost identical to type A1 cabinets; however, they have a greater inflow velocity and always have negatively pressured contaminated plenums or positively pressured contaminated ducts/plenums surrounded by negatively pressured ducts/plenums (Figure 11-3). In the event of a leak in the positively pressured ducts or plenums, this design feature draws air inward, thus preventing the contaminated air from escaping outward into the containment zone. This type of BSC is suitable for work with minute amounts of volatile toxic chemicals and radionuclides, if air is exhausted through a thimble connection.

11.1.2.3 Type B1

In this type of BSC, the room air and a portion of the BSC's recirculated air is drawn into the front grille and then directed through a HEPA filter located below the work surface (Figure 11-4). The air then flows upwards, through the side plenums and then through a second HEPA filter and downwards over the work area. Directly above the work surface and halfway between the front and rear grilles, the air splits and more than 50% of this contaminated air passes through the rear grille and through a HEPA filter before being exhausted out of the BSC directly to the outside atmosphere. The remaining air (less than 50%) passes through the front grille, mixes with the inflow air, and then passes through the HEPA filter located below the work surface. Type B1 BSCs are hard-ducted. Work with low levels of volatile toxic chemicals and trace amounts of radionuclides may be performed towards the rear of the work surface, where the air is discharged directly to the outside atmosphere.

11.1.2.4 Type B2

In this type of BSC, the supply blower draws room air into the top of the cabinet, through a HEPA filter, and then downwards over the work surface (Figure 11-5). The building exhaust system draws the air through the front and rear grilles into a contaminated plenum and then through a HEPA filter before being exhausted out of the cabinet directly to the outside atmosphere. Type B2 BSCs are hard-ducted. Work with volatile toxic chemicals and radionuclides may be performed in the BSC since the air is never recirculated within the BSC or within the containment zone. Reversal of airflow from the face of a BSC, also known as a **puff-back**, can occur in Class II type B2 BSCs, for example upon failure of the HVAC system, power, or the exhaust fan serving the BSC. Every effort is to be made to address puff-backs mechanically (CBS Matrix 3.7). When puff-backs occur in **high containment zones**, the laboratory is considered contaminated and full room **decontamination** may be necessary. Consideration should also be given to the amount of air required to operate this type of cabinet as it may lead to additional adjustments to balance the airflow in the containment zone.

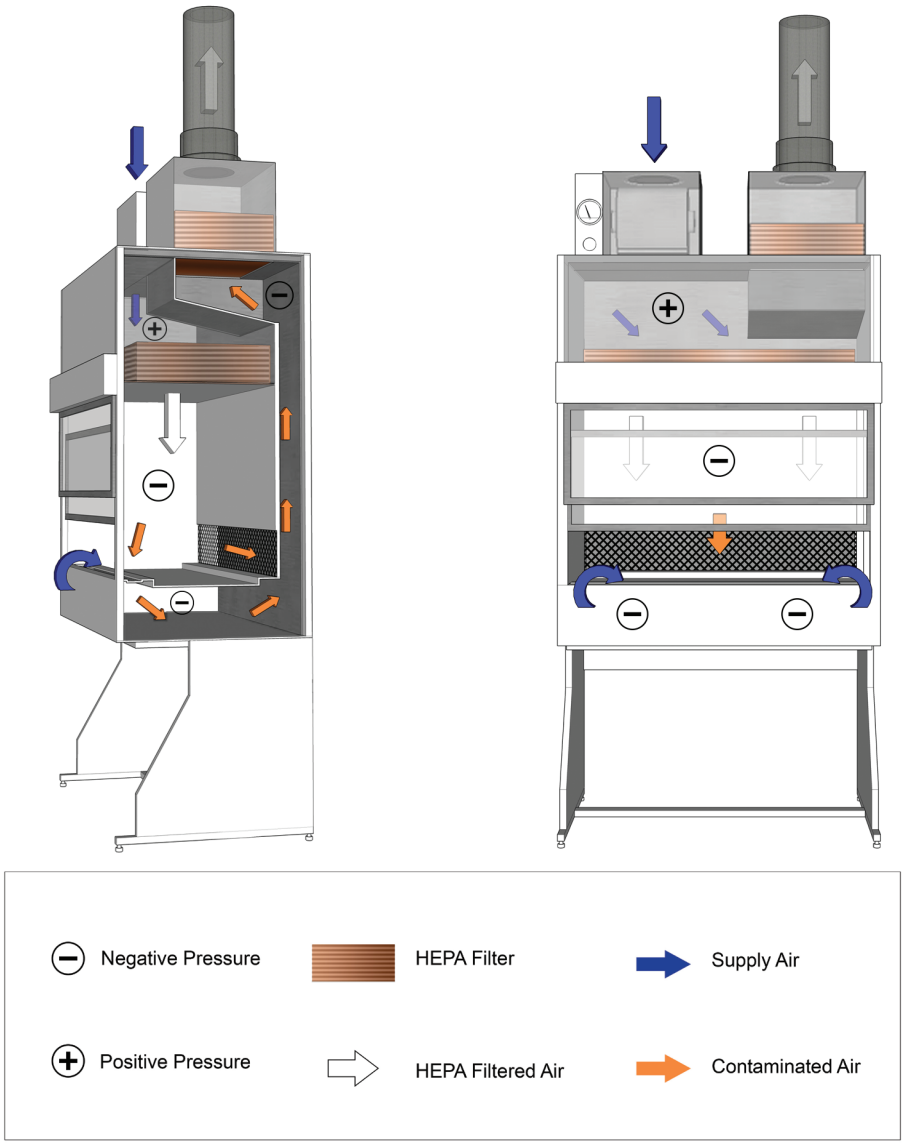


Figure 11–5: Illustration of a Class II Type B2 Biological Safety Cabinet (BSC).

Cabinet is vented to the outside atmosphere through a hard-ducted connection, as shown.

Table 11–1:
Summary Table of Key Characteristics of Class II Biological Safety Cabinets (BSCs).

	Type A1	Type A2	Type B1	Type B2
Minimum average inflow velocity through front opening	0.38 m/s [75 fpm]	0.51 m/s [100 fpm]	0.51 m/s [100 fpm]	0.51 m/s [100 fpm]
Air patterns	30% of the air is exhausted out of the BSC and 70% of the air is recirculated within the BSC	30% of the air is exhausted out of the BSC and 70% of the air is recirculated within the BSC	>50% of the air is exhausted out of the BSC and <50% of the air is recirculated within the BSC	100% of the air is exhausted out of the BSC
HEPA-filtered downflow air	Composed of mixed downflow and inflow from common plenum	Composed of mixed downflow and inflow from common plenum	Inflow air	Drawn from the containment zone or from the outside atmosphere
HEPA-filtered exhaust air	Recirculated to the containment zone or directly to the outside atmosphere	Recirculated to the containment zone or directly to the outside atmosphere	Exhausted through dedicated exhaust plenum to the outside atmosphere	Exhausted through dedicated exhaust plenum to the outside atmosphere
Type of exhaust	Can be thimble connected	Can be thimble connected	Hard-ducted	Hard-ducted

	Type A1	Type A2	Type B1	Type B2
Contaminated ducts and plenums	Negatively pressured or surrounded by negatively pressured ducts or plenums; plenum may be positively pressured in some models	Negatively pressured or surrounded by negatively pressured ducts or plenums	Negatively pressured or surrounded by negatively pressured ducts or plenums	Negatively pressured or surrounded by negatively pressured ducts or plenums
Work with volatile toxic chemicals and radionuclides	No	Minute amounts if exhausted through thimble connection	Low levels of volatile toxic chemicals and trace amounts of radionuclides	Yes

11.1.3 Class III

Class III BSCs provide product protection and maximum personnel and environmental protection (Figure 11-6). They are designed for work with RG4 **pathogens** and provide an alternative to the use of positive-pressure suits if the infectious material is exclusively handled within the Class III BSC. This type of BSC is completely enclosed; all penetrations are airtight and the BSC is kept under negative pressure (-200 Pa or lower, or as specified by the manufacturer) by a dedicated exhaust system. Manipulations are performed through attached heavy-duty long sleeved gloves that prevent direct contact with biological material. An **inward directional airflow (IDA)** of 0.7m/sec should be maintained when one glove is removed. The air from a Class III BSC is exhausted directly to the outside atmosphere through two consecutive HEPA filters or through a single HEPA filter followed by incineration. The introduction or removal of materials can be done in a variety of ways, including through a **dunk tank**, a double-door autoclave, a **pass-through chamber** that is decontaminated between uses, or a bag-in/bag-out system. **Interlocks** are used to prevent autoclave or pass-through chamber doors from being opened simultaneously (CBS Matrix 3.2). It is possible to join multiple Class III BSCs in a line to obtain a larger work area.

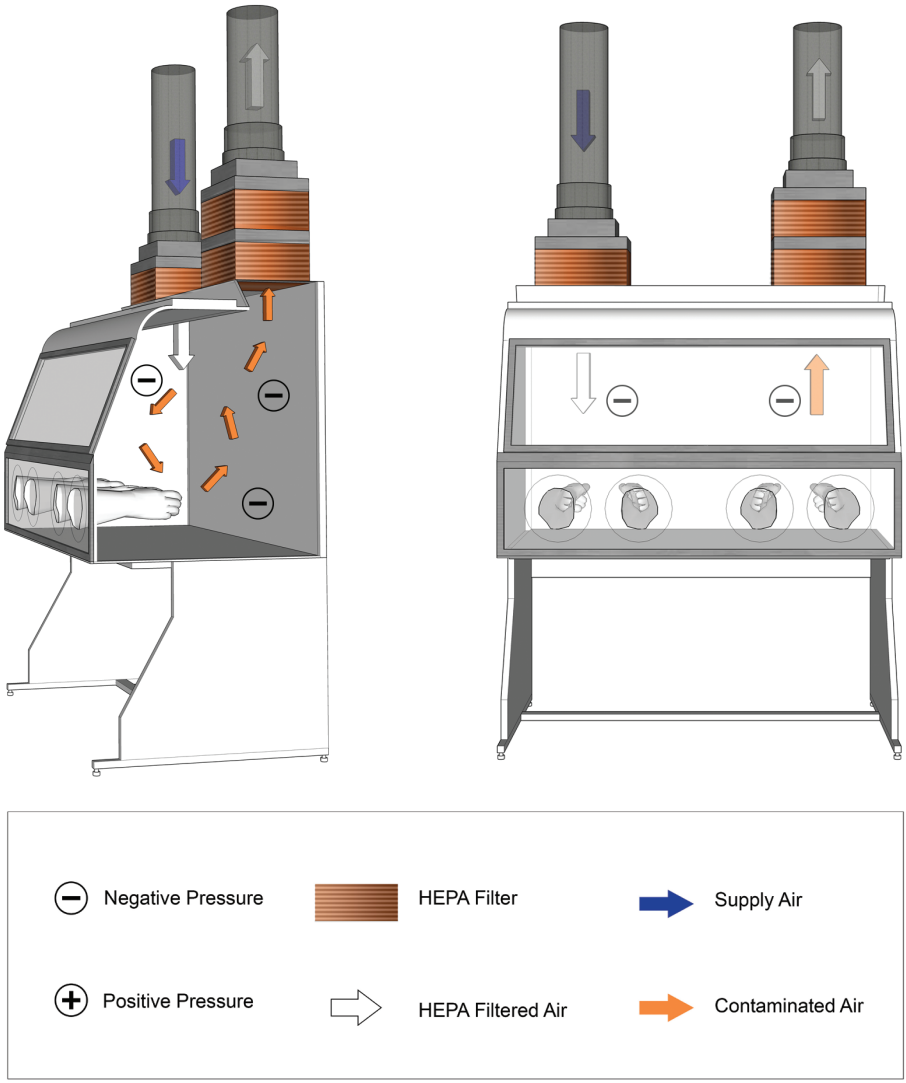
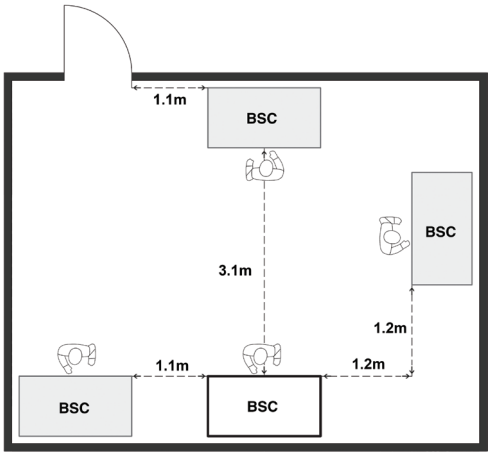


Figure 11–6: Illustration of a Class III Biological Safety Cabinet (BSC).

Cabinet is vented to the outside atmosphere through a hard-ducted connection, as shown.

(a) Well-located BSCs.



(b) Poorly-located BSCs.

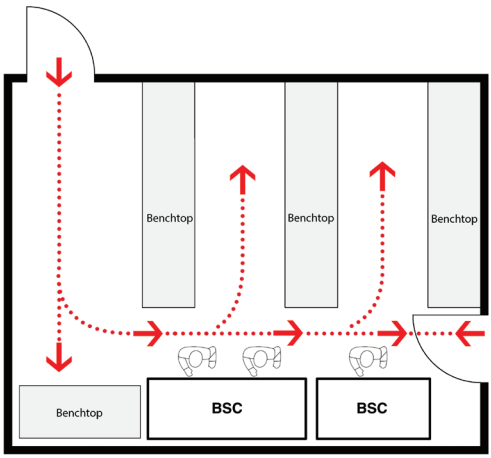


Figure 11–7:
Representative Diagram Illustrating Location Considerations for Biological Safety Cabinets (BSCs).

(a) Well-located BSCs; minimum recommended clearances from a doorway and between BSCs when more than one BSC is installed in the room are shown. Specific BSCs may have different recommended clearances to prevent airflows from a neighbouring BSC from interfering with the protective air curtain. (b) Poorly-located BSCs; traffic, doorway, and neighbouring BSC are likely to disrupt the protective air curtain, and compromise personal, environmental, and product protections. Class II BSCs are designed and certified for use by a single individual.

11.2 Installation of BSCs

Locating BSCs away from areas where airflow patterns may be disrupted (e.g., room air supply and exhaust grilles, doors, open windows, high traffic areas, and large pieces of equipment that generate heat) will help protect the fragile air curtain at the front of the cabinet (CBS Matrix 3.7). The following recommendations are to be considered with respect to the installation of BSCs:

- Consideration should be given to the use of bag-in/bag-out (or another procedure for the safe removal of filters) HEPA filters in situations where effective ***in situ*** decontamination is not feasible or possible. This allows for subsequent decontamination and disposal off-site (CBS Matrix 4.6).
- Adequate clearance should be provided between the exhaust outlet on top of the BSC and any overhead obstructions.
- Adequate clearance should be provided on each side of the BSC to allow access (Figure 11-7).
- BSCs should not be located directly opposite seated work stations, other BSCs, or **chemical fume hoods**. A reasonably safe distance, as determined by a **local risk assessment (LRA)**, should be maintained to avoid operator collision.
- The thimble should be removable or designed to allow proper certification of the BSC (e.g., **isolation damper** to seal off the cabinet for decontamination, access port to allow scan testing of the HEPA filter).
- Hard-ducted BSCs should have exhaust blowers located at the terminal end of the ductwork. Exhaust flow failure(s) should signal an alarm to the user and activate an interlock system to prevent the cabinet blower from operating whenever the exhaust flow is insufficient (e.g., flow/electrical control) to prevent pressurization of the cabinet. **Backdraft protection** (i.e., damper) in the ductwork may be necessary to prevent reversal of airflow through the HEPA filter in the cabinet.
- Supporting BSCs with emergency power will help to maintain containment during emergency situations.

11.3 Testing and Certification

The required elements for testing and certification of BSCs are described in Matrix 5.1 of the CBS. Testing BSCs upon initial installation, annually, and after any repairs, modifications or relocation demonstrates that they are operating as designed. These activities can impact the integrity of the HEPA filters and plenums which could result in the **release** of infectious material and toxins. Most types of BSCs are tested in accordance with National Sanitation Foundation (NSF)/American National Standards Institute (ANSI) 49; however, for certain types (i.e., all Class I BSCs, Class II A1 BSCs, Class III BSCs, and custom BSCs), NSF/ANSI 49 is not applicable and the BSCs are tested in accordance with manufacturer

specifications.[2] The following summarizes additional information to be considered for testing and certification of BSCs:

- On-site field testing should be performed by experienced and qualified individuals using test equipment with valid calibration certificates. The NSF accreditation program for BSC certifiers provides a list of individuals who have demonstrated their competencies by means of written and practical examinations.
- Interlocks (i.e., Class II Type B2 BSC internal cabinet supply fan and exhaust fan) should be tested in accordance with NSF/ANSI 49 to confirm that the internal supply fan shuts off whenever the exhaust air parameters fall outside of the setpoints.
- Alarms should be tested for detection of BSC or exhaust fan failure by simulation of alarm conditions.
- A label indicating the date of certification, the date when the cabinet is to be recertified, the standards or specifications to which the cabinet was tested, and the name of the certifier should be affixed to the cabinet exterior.
- During an exhaust fan failure, the time from the moment of alarm detection to the moment of airflow reversal from the face of the BSC (i.e., puff-back), if applicable, should be known for Class II B2 BSCs. If not conducted when installed, the cabinet alarm should be tested and adjusted to give the earliest possible warning to the user and to maximize the amount of time before the puff-back occurs.
- Positive **pressure decay testing** of Class III BSCs is done upon initial installation and when modifications have been made to the integrity of the cabinet, as per manufacturer's specifications. When modifications have not been made, annual integrity testing is done as well as any other tests recommended by the manufacturer. An example of an integrity test would be to smoke test the outside of the Class III BSC under normal operation. If no smoke is drawn into the cabinet from any of the seams, the integrity of the Class III BSC is acceptable.

Where a custom enclosure or the design of a BSC does not permit certification in accordance with NSF/ANSI 49, it is to be verified to meet the manufacturer's specifications, with minimum parameter values specified in Matrix 5.1 of the CBS.

11.4 Proper Use

Incorporating the elements outlined below into the applicable **standard operating procedures (SOPs)** to be followed by facility personnel is strongly recommended to encourage the proper and consistent use of a BSC by personnel to prevent **exposures** and the release of pathogens and toxins.

11.4.1 Start-Up Considerations

- Check that the sash is at the appropriate height. Adjust stool height so that the user's underarms are level with the bottom of the sash.
- Check the pressure gauges to verify that readings are within the acceptable range.
- If present, test the airflow alarm and ensure it is switched to the "on" position.
- Confirm inward airflow by holding a tissue at the middle of the edge of the sash to establish that it is drawn in.
- Disinfect the interior surfaces with a disinfectant effective against the infectious material and toxins used in the laboratory, allowing an appropriate contact time. If a corrosive disinfectant is used, the surface should be rinsed with water after **disinfection**.
- Assemble all materials required for manipulation and load into the BSC. Care should be taken not to overcrowd or block the front or rear grilles to prevent the appropriate airflow patterns from being compromised.
- When there is significant potential for splatter or splashes to occur during manipulations of infectious material or toxins, the work area should be lined with a plastic-backed absorbent pad.
- Place aerosol generating equipment (e.g., vortex mixer, sonicator) towards the back of the BSC, without blocking the rear grille.
- After loading material in the BSC, allow sufficient time for the air to purge and the airflow to stabilize before initiating work. This will be specified in the manufacturer's instructions, and is generally 3-5 minutes.

11.4.2 Working in the BSC

- Perform operations as far to the rear of the work area as reasonable. Ensure that elbows and arms do not rest on the grille or work surface.
- Avoid excessive **movement** of hands and arms through the front opening. Such movements disrupt the air curtain at the front of the BSC, which can allow contaminants to enter or escape the BSC. Arms should enter and exit the BSC slowly and perpendicular to the front opening.
- Keep a bottle of an appropriate disinfectant in the BSC while work is performed to avoid having to move hands outside of the BSC.
- Segregate non-contaminated ("clean") items from contaminated ("dirty") items. Work should always flow from "clean" to "dirty" areas (Figure 11-8).
- Material should be discarded in a **waste** container located towards the rear of the cabinet workspace. Do not discard materials in containers outside of the cabinet.
- Decontaminate the surface of all objects in the BSC in the event of a spill. The work area, including the inside surface of the window, should be decontaminated while the BSC remains in operation.

- Natural gas and propane should not be used in a BSC; sustained open flames (e.g., Bunsen burner) in BSCs are prohibited. On-demand open flames (e.g., touch-plate microburners) are to be avoided as they create turbulence in the BSC, disrupt airflow patterns, and can damage the HEPA filter (CBS Matrix 4.6). Non-flame alternatives (e.g., microincinerator, or sterile disposable inoculation loops) should be used whenever possible.
- Work in a BSC should only be conducted by one person at a time.
- Equipment creating air movement (e.g., vacuum pumps, centrifuges) may affect the integrity of the airflow and should not be used within the BSC.
- Windows that open should be kept closed when the BSC is in use.

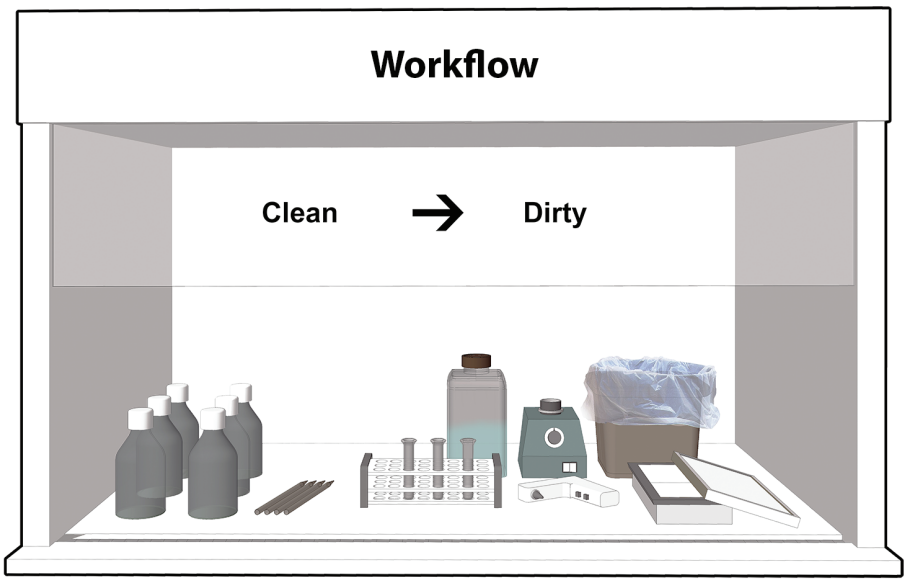


Figure 11–8:
Representative Diagram of a Recommended Layout of Materials and Workflow inside a Biological Safety Cabinet (BSC).

The direction of workflow from "clean" (i.e., less contaminated) to "dirty" (i.e., higher contamination) is indicated.

11.4.3 Completion of Work in the BSC

- Upon completion of work, allow sufficient time for the air in the BSC to purge (i.e., pass through the filter) before disrupting the air curtain by removing hands or unloading material from the BSC. The purge time will vary by model and can be up to several minutes.
- Close or cover all containers.
- Surface decontaminate items before removing them from the BSC.
- Disinfect the interior surfaces of the BSC, including sides, back, lights, and interior of the glass, with a disinfectant effective against the pathogens in use, allowing an appropriate contact time (CBS Matrix 4.6). If a corrosive disinfectant is used, the surface should be rinsed with water after disinfection to avoid corrosion of the stainless steel surfaces.
- Routinely remove the work surface and disinfect the tray beneath it.
- Routinely wipe the surface of the lights within the BSC with a suitable cleaner or disinfectant (e.g., ethanol).

11.4.4 Ultraviolet Light Considerations

The use of ultraviolet (UV) germicidal lamps is strongly discouraged due to their limited effectiveness at disinfecting the inside of BSCs.[3,4] Personnel wishing to use UV irradiation in BSCs should receive training on the safe work practices required and the hazards of UV radiation beforehand, including the following elements:

- UV irradiation of the work area should only be used as a secondary method of disinfection in the cabinet. Never rely on UV irradiation alone to disinfect a contaminated work area.
- UV irradiation is ineffective if a **microorganism** is protected by dust, dirt, or organic matter.[4] A liquid chemical disinfectant should be the primary method of cleaning and disinfecting the interior of a BSC.
- UV irradiation does not penetrate into cracks or through the grilles of a BSC.
- UV irradiation can cause deterioration of various materials, including certain plastics and tubing.
- Never touch a UV bulb with bare hands as the natural oils from hands may leave a fingerprint and create dead space on the bulb's surface.
- UV bulbs should be cleaned frequently with an appropriate disinfectant.
- The UV lamp should be routinely tested with a UV meter to verify that the proper intensity (i.e., 40 $\mu W/cm^2$) is being delivered at the appropriate wavelength (i.e., 254 nm) in the centre of the work area.[5]

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CHAPTER 12

SAFETY CONSIDERATIONS FOR EQUIPMENT USED FOR BIOLOGICAL WORK

CHAPTER 12 – SAFETY CONSIDERATIONS FOR EQUIPMENT USED FOR BIOLOGICAL WORK

In both **laboratory work areas** and animal **containment zones**, a wide variety of equipment can be used when handling **infectious material** or **toxins**. Equipment that is operated and maintained properly minimizes the **risk** of **exposure** and prevents the **release** of **pathogens** and toxins into the environment. An equipment maintenance program facilitates tracking and scheduling of inspections and repairs, and is an important component of a **facility**'s **Biosafety Manual**. In addition, training of personnel on standard operating procedures (SOPs) pertaining to all containment zone equipment, such as centrifuges, microtomes, pipetting aids, vacuum systems, and **pass-through chambers** is critical to the provision of a safe work environment. This chapter provides guidance on the safe use of select equipment used in **laboratory** and animal containment zones for activities with **biological material**. **Local risk assessments (LRAs)** are conducted to identify risks, examine procedures, and develop safe work practices for all equipment that, in turn, can be developed into SOPs. The minimum **biosafety** requirements for the equipment described in this chapter are specified in Chapters 3, 4, and 5 of the *Canadian Biosafety Standard* (CBS), 2nd Edition; the specific matrices referencing the equipment type being described are provided in each of the following sections.[1]

12.1 Centrifuges

There is a risk of infectious **aerosol** generation when a centrifuge is used (e.g., tube breakage, improper use of safety cups or rotors, or lack of proper maintenance). The following points highlight some requirements and recommendations for centrifuge use when working with infectious material or toxins:

- The outside surface of cups and rotors should be decontaminated, as required.
- Equipment should be used in accordance with the manufacturer's instructions, which includes the balancing of rotors to prevent rotor damage or explosion.
- Plastic tubes that are suitable for centrifugation should be used (e.g., thick wall external thread plastic tubes with screw caps).
- Sealed centrifuge cups or rotors are to be used to prevent the release of aerosols during centrifugation, and the integrity of the cup or rotor seal regularly inspected (CBS Matrix 4.6).
- Cups and rotors with samples of infectious material or toxins are to be unloaded inside a **biological safety cabinet (BSC)** to protect against the release of infectious aerosols or aerosolized toxins (CBS Matrix 4.6).
- Sufficient time for aerosols to settle should be allowed prior to opening cups and rotors.
- The use of centrifuges inside a Class II BSC will disrupt the airflows and compromise the protection provided by the BSC, and should be avoided.

12.2 Microtomes

Microtome work with infectious material or toxins that may not have been inactivated by fixation should be performed in a low traffic dedicated area (e.g., taped off) to prevent tracking of wax shavings within or out of the containment zone. Care should be taken as the floors in histopathology areas tend to be quite slippery from the wax. Disposable shoe covers, dedicated to this area, should be worn; slip-resistant shoe covers are recommended for such areas. Respiratory protection should also be worn if deemed necessary by an LRA. Troughs may be installed on the edge of the work bench to contain excess shavings. Care should be taken when installing or removing microtome blades; non-disposable blades can be cleaned with an instrument, rather than by hand, to prevent contact with the blade. When manipulating tissue potentially infected with pathogens or **prions**, additional **personal protective equipment (PPE)** such as cut-resistant gloves can be worn to reduce the risk of exposure or injury.

12.3 Blenders, Sonicators, Homogenizers, Shaking Incubators, and Mixers

The operation of blenders, sonicators, homogenizers, mixers, shaking incubators, and other similar equipment can generate aerosols. The following points highlight some requirements and recommendations when using these types of equipment:

- Laboratory equipment and associated accessories specially-designed to contain infectious aerosols can be used for manipulations of pathogens and toxins. For example, cup horn sonicators allow sonication of samples within a contained vessel without direct contact with the material being processed.
- When equipment designed to contain infectious aerosols is not available, equipment should be operated in a BSC (only if the equipment does not disrupt airflow patterns) (CBS Matrix 4.6) or another **primary containment device**.
- Time for aerosols to settle should be allowed before opening or removing the covers.

12.4 Bunsen Burners

Bunsen burners are commonly used for heating (e.g., fixing cells onto slides) and **sterilization** (e.g., inoculation loops). Aerosolization of infectious material can occur when inoculation loops are sterilized in the open flame of a Bunsen burner; microincinerators or disposable loops are recommended as alternatives. Sustained open flames are prohibited from use inside a BSC as they will disrupt the airflow patterns, decrease the user protection provided by the air curtain, and have the potential to damage the filters (CBS Matrix 4.6). When suitable non-flame alternatives are not available, touch-plate microburners that provide a flame on demand may be used. The correct operation of BSCs is discussed in Chapter 11.

12.5 Microincinerators

Microincinerators can be used as an alternative to Bunsen burners, especially for use in a BSC. They are often equipped with shields to minimize the dispersal of infectious aerosols. When used in a BSC, the microincinerator should be placed at the rear of the working area inside the cabinet to help minimize disruption of the air curtain at the front of the cabinet.

12.6 Disposable Loops

Single-use disposable loops are sterile, and can be used in a BSC as an alternative to reusable loops that require sterilization with a burner or microincinerator; however, they will add to the amount of **waste** requiring **decontamination**. Disposable loops should be placed in a leak-proof, puncture-resistant waste container immediately after use.

12.7 Pipetting Aids

Pipetting aids minimize the risk of aerosol generation when used properly; they also eliminate the risk of ingestion of infectious material through oral pipetting, which is prohibited at all **containment levels** (CBS Matrix 4.6). Discharging liquid from a pipette and the aspirate/expel action used to mix **cultures** can create aerosols. The following points highlight some requirements and recommendations for the safe use of pipetting aids:

- use a BSC when pipetting infectious material or toxins (CBS Matrix 4.6);
- work over plastic-backed absorbent material; the droplets will be absorbed rather than "splash";
- use pipettes calibrated "to deliver", which reduces the risk of creating aerosols by retaining the last drop in the tip;
- use plastic pipettes instead of glass pipettes whenever possible;
- use filtered serological pipettes with pipette aids and filtered pipette tips with micropipettors, as these will prevent **contamination** of the pipetting device;
- use appropriate decontamination procedures for pipette aids and micropipettors when non-filtered tips are used or when the pore size of the pipette filter is insufficient for filtering the pathogen(s) or toxin(s) in use;
- don't mix liquids by bubbling air from a pipette through the fluid or by alternate suction and forceful expulsion through the pipette;
- discharge liquids as close as possible to the wall of the tubes or to the surface of media;
- avoid forcefully aspirating or expelling liquids from the pipette;
- pipet tips can be ejected directly into a container (e.g., bottle, beaker) for subsequent decontamination or bag for autoclaving; and,

- pipettes should be decontaminated with a suitable disinfectant immediately after use;
  - serological pipettes can be laid horizontally in a pan and completely immersed in a disinfectant (care should be taken when moving the pan to avoid a spill hazard); or
  - serological pipettes can be filled with disinfectant and left to drain by gravity into an oversized waxed cup in an autoclave bag (the bag can be closed over the pipettes and this can be autoclaved as a whole in an upright position before reuse).

12.8 Vacuum Pumps and Systems

Vacuum systems are used to create a void in filtration units and to aspirate liquids. The most common laboratory vacuum systems are centralized vacuum (void) systems, vacuum pumps, or a faucet aspirator vacuum pump attached to the water supply. The primary concern with vacuum pumps is that the process of aspiration can cause the aerosolization of infectious material or toxins, and subsequent contamination of the vacuum line and pump or system. A device (e.g., in-line **high efficiency particulate air [HEPA] filter** or 0.2 µm filter with disinfectant traps) is used to protect the vacuum system from internal contamination (CBS Matrix 3.7). This is visually demonstrated in Figure 12-1. A maintenance program for the regular inspection and replacement of in-line filters (CBS Matrix 4.6) will help prevent a breach in filter integrity and **containment**. For **high containment zones**, the use of portable vacuum systems instead of centralized vacuum systems will minimize the risk of a containment breach. Decontamination methods and selection of chemical disinfectants are discussed in Chapter 15.

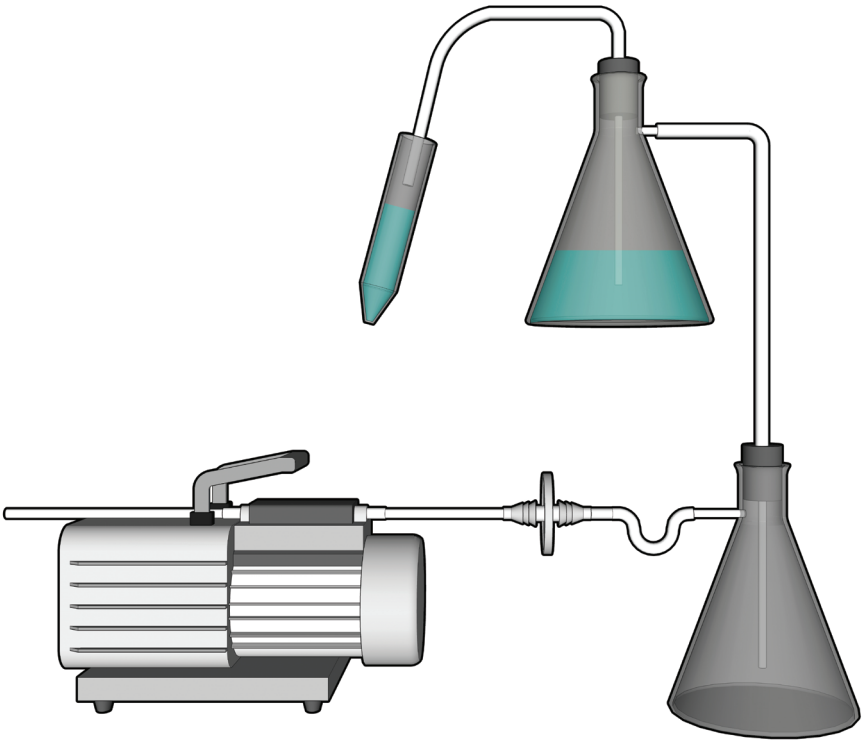

Figure 12–1:
Representative Diagram of a Vacuum System Set-up for the Aspiration of Infectious Liquids.

Liquid from a conical centrifuge tube is aspirated into a flask containing a disinfectant solution used for the collection and decontamination of liquid waste. This flask is connected via a hose to a second flask (also containing disinfectant) that is used to collect any overflow and trap aerosols. The vacuum source (in this illustration, a portable vacuum pump) is protected against infectious aerosols or aerosolized toxins through the use of an in-line filter (0.2 µm filter is illustrated) connected between the overflow flask and the vacuum source.

12.9 Chemical Fume Hoods

Chemical fume hoods are designed for the manipulation of chemical substances, particularly volatile substances. Materials exhausted from chemical fume hoods are filtered with recirculation of the remaining air stream, or exhausted directly to the outside atmosphere. If required, filters are selected according to the type of contaminant to be removed, the efficiency required to meet occupational and environmental exposure limits, and the required residence time. Locating filters upstream of the exhaust fan, and in such a way as to allow replacement without contaminating the surrounding area, keeps contaminated ducts under negative pressure and prevents the release of chemical substances. Testing and replacement should be more frequent for filters used to trap chemicals that are capable of degrading the filter. It is the responsibility of the facility to determine the compatibility of specific chemicals with various filters, and to determine the appropriate replacement frequency. The inclusion of exhaust air treatment devices (e.g., activated carbon filters) are to be consistent with applicable local regulations.

Chemical fume hoods are not designed for the manipulation of infectious material or toxins, and consideration should be given to minimizing the placement of chemical fume hoods in high containment zones; instead, Class II B2 BSCs, which are designed to handle infectious material or toxins as well as volatile chemicals and radionuclides, should be considered. Fume hoods that are located in high containment zones are to comply with the requirements for HEPA filtration of exhaust (CBS Matrix 3.5). Chemical fume hoods should not be located directly opposite or in close proximity to BSCs in order to prevent disruption of the protective air curtain. The installation of a HEPA filter upstream of the charcoal filter is recommended as a measure to protect the charcoal filter from contamination with infectious material and toxins. Class II B2 BSCs are further discussed in Chapter 11.

12.10 Pass-Through Chambers

Pass-through chambers allow for the safe **movement** of materials into and out of containment zones and are available in a variety of sizes and configurations, including wall-mounted types, floor-mounted types with built-in ramps, as well as the type integrated into a Class III BSC. Various methods of decontamination are available for different types of pass-through chambers, including moist-heat (autoclave), dry-heat (hot-box), and gas or vapour (fumigation). The choice of decontamination method will depend upon the nature of the items requiring sterilization, as well as the type of infectious material or toxins in use. Additional features, such as HEPA-filtered pass-through chambers, are also available. To prevent pass-through chamber doors from being opened simultaneously, they are usually equipped with door **interlocks** or visual and audible alarms (CBS Matrix 3.2). Decontamination methods and selection of chemical disinfectants are discussed in Chapter 15.

12.11 Cell Sorters

Cell sorters are used to physically separate a defined subpopulation of cells from a heterogeneous cell population.[2,3] The risk associated with cell sorters can be attributed to both the nature of the sample (i.e., the presence and type of the infectious material or toxins in the sample), and to the equipment itself (e.g., the use of droplet-based cell sorting, which uses jet-in-air technology that can produce aerosolized droplets). Droplet-based cell sorting involves the injection of a liquid stream carrying the cells through a narrow nozzle that vibrates at a high frequency. High-speed cell sorters with jet-in-air technology use even higher pressures and nozzle vibration frequencies, and consequently produce a large amount of aerosolized material. An LRA can be conducted to determine the physical containment and operational practices necessary to safely work with infectious material or toxins in a cell sorter. A cell sorter may need to be housed inside a ventilated enclosure that is custom-built by the same manufacturer for use with pathogens and toxins if it is not able to be housed inside a BSC. Custom ventilated enclosures are required to be certified to the manufacturer's specifications and demonstrate integrity as described in Matrix 5.1 of the CBS.

12.12 Compressed Gas Cylinders

Compressed gas cylinders can leak, and difficulties can be encountered when maintaining, replacing, and decontaminating tanks. In containment level 4 (CL4) zones, there is the added concern that positive-pressure suits could be damaged when changing tanks or fitting regulators. For these reasons, it is recommended that compressed gas cylinders be located outside high containment and prion zones where possible. Fire extinguishers and backup air cylinders may be required in high containment zones for personnel protection in life-threatening emergencies. Some containment level 3 (CL3) zones using sophisticated equipment (e.g., mass spectrophotometer, high performance liquid chromatography) may necessitate the use of small cylinders of reference gases in the containment zone that would be impractical to pipe in from outside the zone.

12.13 Additional Equipment Considerations for Prions

The following are additional equipment considerations for containment zones dedicated to prion work:

- dedicated laboratory work areas and equipment should be used, where possible;
- disposable equipment and laboratory supplies should be used when handling material known to contain prions;
- blunt cannulas can be used in place of needles; the use of needles, syringes, and other sharp objects are to be strictly limited (CBS Matrix 4.6);
- plasticware can be used in place of glassware; and
- instruments should be kept moist until decontamination.

12.14 Additional Equipment Considerations for Toxins

The following are additional equipment considerations for toxin work:

- plasticware should be used in place of glassware;
- thin-walled glassware should be avoided; and
- glass chromatography columns should be enclosed in a secondary container.

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CHAPTER 13

ANIMAL WORK CONSIDERATIONS

CHAPTER 13 – ANIMAL WORK CONSIDERATIONS

Conducting ***in vivo*** work (i.e., working with live animals) with **pathogens** and **toxins** in a **containment zone** increases the **risk** substantially compared to ***in vitro*** work. Animals can behave unpredictably, especially if they are ill. In addition, infected animals may be symptomatic or asymptomatic, or may be carriers of **zoonotic pathogens** also capable of causing **disease** in humans. Pathogens or toxins may be present in the **large volumes** of **waste** produced by animals, and can also be shed from their bodies. **Exposure** to pathogens that animals may harbour can occur as a result of animal bites, scratches, **aerosols**, or through direct contact with animal waste and bodily fluids. The risk of exposure to pathogens that animals may harbour can be reduced through an **animal health surveillance program**, with an emphasis on the selection of disease-free animals and the identification and treatment of diseased animals.

Additionally, some personnel may develop allergies from repeated exposure to animal fur or hair, dander, bedding, feed, and animal waste. As documented in *Biological Safety Principles and Practices* (2004), at least one-fifth of people who work with **laboratory** rodents, guinea pigs, and rabbits develop allergies.[1] An allergic reaction may manifest itself immediately or become more severe with each additional exposure. Symptoms may range from mild rashes to severe asthma. Unnecessary exposure to these allergens can be minimized through engineering controls (e.g., **biological safety cabinets [BSCs]**, **ventilated cage changing station**, ventilation, use of isolators and **containment** caging systems), and appropriate use of respiratory protection and other **personal protective equipment (PPE)**.

Large-sized animals also have the potential to kick, trample, or cause crushing injuries. The potential for personnel exposure to other physical hazards through equipment use and to associated noises should also be considered. The requirements for animal containment zones are specified in Chapters 3, 4, and 5 of the *Canadian Biosafety Standard* (CBS); animal-specific **operational practice requirements** are highlighted in Matrix 4.7.[2]

The use of animals for experimental purposes is highly controlled and monitored. Whenever **scientific research**, teaching, or testing requires the use of animals, the institutional animal care committee reviews and assesses animal use protocols to ensure compliance with the Canadian Council on Animal Care (CCAC) guidelines, and, where applicable, provincial/territorial legislation specific to animals in research. The CCAC is a national peer review agency responsible for setting and maintaining standards for the ethical use and care of animals in science. The CCAC acts in the interests of Canadians to ensure that the use of animals for research, teaching, and testing employs optimal care according to acceptable scientific standards. The CCAC also promotes an increased level of knowledge, awareness, and sensitivity to relevant ethical principles. For more information on CCAC programs, please contact the CCAC or visit their website.

13.1 Animal Characteristics

Awareness and familiarity with the behavioural (i.e., instincts and mentality), psychological, and social needs of the animals by containment zone personnel, including veterinarians, scientists, and animal handlers, is fundamental in predicting how the animal(s) will act and mitigating the associated risks. For this reason, project design should include the needs of the animals with respect to their physical attributes, their susceptibility to adventitious pathogens, and the shedding and transmission of pathogens. Feeding, watering, and environmental requirements may also differ from species to species. Some animals are best housed in groups while others may require separation. Some animals will need to be observed closely to evaluate the compatibility and dynamics among the group in order to minimize fighting or injuries. In all cases, the safety of personnel is of the highest priority when evaluating animal housing options. An adaptation period for the animals (i.e., acclimatization to their new surroundings) is important to reduce initial stress and anxiety, and should, therefore, be incorporated into the experimental design. Researching the needs of the animal is essential; CCAC guidelines, literature reviews, peer-reviewed articles, and consulting with a veterinarian can provide personnel with vital information on a wide range of animal species. It is important that the animals' needs as well as the needs of the project are properly balanced in the design of the study.

The following recommendations and precautions are applicable to work with many animal species:

- Consideration should be given to the behavioural, emotional, and social needs of laboratory animals when planning their housing. For group caging, factors such as compatibility between individual animals and the population dynamics of the species should be considered in order to minimize fighting and other adverse events.
- Behavioural conditioning can be effectively used in combination with restraint procedures.
- Animal handlers should always be protected by PPE based on a **local risk assessment (LRA)**. When appropriate, arm-length reinforced leather gloves and long-sleeved gowns or coveralls should be worn to prevent scratches.
- Protective clothing that has been in contact with animals should be decontaminated before being sent to laundry; laundering equipment located inside the containment zone is only suitable for decontamination when it has been proven to be effective for **decontamination** of the pathogen(s) present or suspected (i.e., validated).
- Animal handlers should immediately and thoroughly cleanse all bites, scratches, and abraded skin, and rinse all splashes that result in contact with mucous membranes. Such exposures are to be reported without delay (CBS Matrix 4.9) and post-exposure procedures implemented in accordance with the established **emergency response plan (ERP)** and the **medical surveillance program**.

- Security locks and closing devices on caging should take into consideration the persistent, creative, destructive, and intellectual capacities of the animal species (e.g., non-human primates [NHPs], raccoons), as appropriate.
- Cages should be equipped with a mechanism to facilitate examination and immobilization. Transfer boxes and other special apparatus can be used to hold animals safely while primary cages are being cleaned or to move animals from one room to another.

13.2 Animal Containment Zone Designs

An animal containment zone refers to a series of co-located **animal rooms** or **animal cubicles**, as well as associated corridors and **support areas** (e.g., storage rooms and preparation areas, **post mortem rooms [PM rooms]**) of equal **containment level**. The CBS specifies two types of animal containment zones: **small animal containment zones (SA zones)** and **large animal containment zones (LA zones)**. It is important to note that the designation as an SA zone or LA zone is dependent on the way in which the animal is housed rather than the actual physical size of the animal. For example, if an animal containment zone houses **small-sized animals**, such as mice in **primary containment caging**, then it would be considered an SA zone. In contrast, where small-sized animals are housed in **open caging** only intended for the **confinement** of animals to an area (i.e., it does not include filtration to prevent the **release** of **infectious materials** and toxins, such as a wire cage), where aerosols generated by the animals can contaminate the room, it is then considered to be an LA zone, despite the actual size of the animal. In an LA zone, the rooms housing the animals provide **primary containment** (i.e., animal cubicles). Guinea pigs, rats, and mice are examples of small-sized animals that can be easily housed in filtered cages or cage rack systems in SA zones. NHPs and other large-sized animals (e.g., pigs, sheep, and raccoons) can be considered to be housed in SA zones when they are housed in primary containment caging or the caging has been completely housed inside a custom ventilated enclosure.

In addition to meeting the requirements specified in Chapters 3, 4, and 5 of the CBS, SA zones and LA zones should be designed and operated in accordance with the CCAC *Guidelines on Laboratory Animal Facilities*.[2,3] Institutions using animals for research, teaching, and testing should hold a CCAC Certificate of Good Animal Practice®, which is provided to facilities that have been assessed and found to have standards of experimental animal care and use that satisfy the CCAC's guidelines and policy statements.

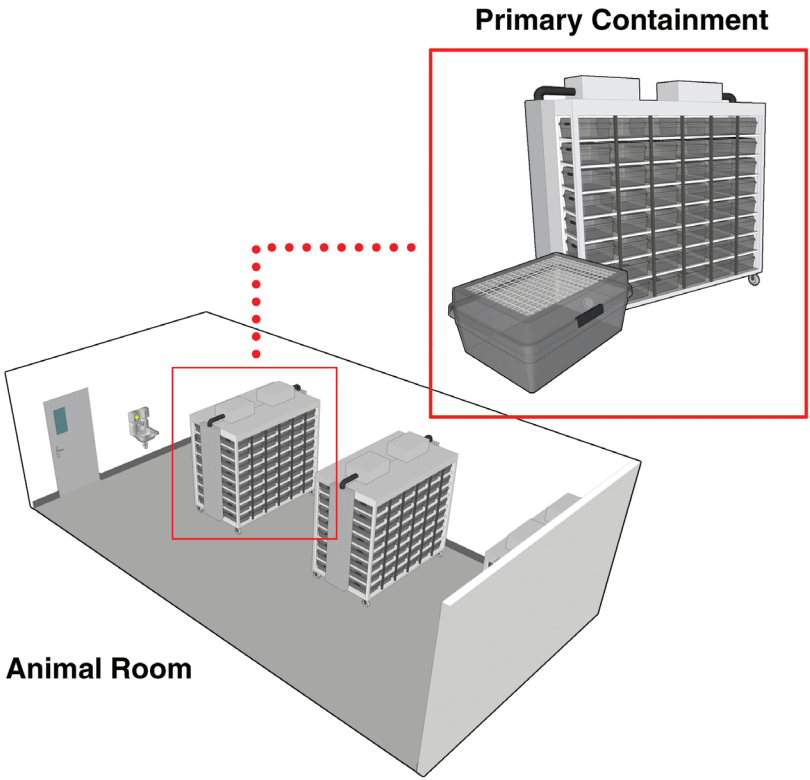


Figure 13–1: Representative Diagram of a Basic Animal Room.

Ventilated cage racks and primary containment cages are shown in the inset.

13.2.1 Small Animal Containment Zones

Animal containment zones where animal species are housed and handled in **primary containment devices** (i.e., filtered containment caging and BSCs) are referred to as "small animal containment zones" (or SA zones). The room where animals are housed in primary containment caging within an SA zone is referred to as an "animal room". Figure 13-1 illustrates a basic animal room.

Many different types of primary containment caging systems are available. These can range from microisolators, to more complex models that incorporate the use of **high efficiency particulate air (HEPA) filters**, to completely ventilated containment caging rack systems. The type of cage selected for a project should be compatible with the animal species and the planned method of decontamination. The caging requirements and operational activities are to reflect the containment level required for the pathogen in question. Matrix 3.7 of the CBS should be consulted directly to determine the minimum requirements for primary containment caging in SA zones. Advances in caging technologies have allowed better control of microenvironmental factors such as temperature, air exchange, and humidity. Containment zone design and support systems should take into consideration the type of caging system that will be used, in order to provide appropriate backup power, humidity, and ventilation.

Figure 13-2 illustrates a ventilated caging system, commonly used in SA zones as primary containment caging. Figure 13-2(a) depicts a ventilated cage rack (containing multiple microisolator cages) that supplies a source of filtered air into the individual cages. Exhaust air is either filtered and recirculated into the room, or discharged directly into the room exhaust system. Figure 13-2(b) illustrates a microisolator cage with a filter top and connected to a filtered exhaust that provides primary containment for small-sized animals (e.g., mouse). While filters are necessary, the need for HEPA filters will depend on the pathogen (i.e., LRAs).

13.2.2 Large Animal Containment Zones

An animal containment zone where the rooms housing the animals provide the primary containment is termed a "large animal containment zone" (LA zone). The room or space inside an LA zone where animals are housed is referred to as an "animal cubicle". Unlike a **laboratory work area** or SA zone, where the BSC or primary containment caging provides primary containment and the mechanical systems provide secondary containment, an animal cubicle in an LA zone provides both primary and secondary containment. Animals in an LA zone are not housed in primary containment caging (e.g., they are housed in stalls, pens, or non-filtered cages). Non-filtered cages (detailed in Figure 13-3) are a type of open caging that can be used to house small-sized animals (e.g., raccoons, NHPs).

The selection of animal housing and handling equipment should be specific to the species. For example, an LA zone can house mice, raccoons, NHPs, or dogs in non-filtered cages (i.e., caging only intended to restrict animals to an area and that does not include filtration to prevent the release of infectious material or toxins), chickens or pigs in pens, or livestock or deer housed in stalls inside a cubicle. Figure 13-4(a) illustrates an example of an animal cubicle equipped with open cages (i.e., non-filtered) suitable to house a number of animals such as dogs, cats, racoons, or NHPs; Figure 13-4(b) illustrates an animal cubicle designed with an alternative open caging system: three stalls suitable to house up to three large-sized animals such as cows, deer, horses, or sheep.

LA zones may accumulate a **high concentration** of pathogens in the animal cubicles, and the animals have the potential for the generation of high concentrations of infectious aerosols. Particular attention should be given to the use of PPE worn by personnel entering an animal cubicle in an LA zone, as PPE serves as the primary protection against exposure to pathogens. PM rooms are rooms inside LA zones where animal necropsies and dissections are performed, and there may be several PM rooms within an LA zone. In some cases (e.g., an LA zone where small-sized animals are housed in open caging systems), necropsies and dissections may be performed outside the LA zone in a BSC (i.e., not in a PM room). PM rooms are likely the area of greatest **contamination**; necropsy procedures are often associated with a high risk of generating infectious aerosols, splashes or spills of infectious material, and general **gross contamination**. In addition, exposures to pathogens and toxins in PM rooms may involve cutting instruments or the sharp ends of cracked bones. Similar to an animal cubicle, PPE is extremely important to protect personnel from exposure in a PM room and prevent the spread of contamination; therefore, PPE selection should take this into account. Engineering controls, such as a downdraft table, can be used to help reduce the spread of aerosols in the PM room, but will not fully contain infectious material and toxins. Entry and exit procedures in place for PM rooms include direction on sufficient time for aerosols to settle, prior to opening doors, especially where **anterooms** to the PM room are not provided.

Animal cubicles and PM rooms in LA zones require additional and sometimes unique physical containment and operational practices in order to contain the pathogens and toxins and protect personnel entering these spaces from exposure. Consequently, Chapters 3, 4, and 5 of the CBS specify the requirements for LA zones and distinguish the requirements for LA zones at containment level 2 (CL2) and containment level 3 (CL3) from other work areas of the same containment level by separating these requirements and labelling them as "CL2-Ag" and "CL3-Ag", respectively (i.e., "Ag" for "Agriculture").

(a) Rack containing multiple primary containment cages.

(b) A single primary containment cage.

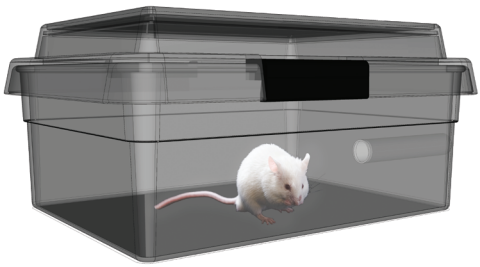


Figure 13–2: Representative Diagrams of Primary Containment Caging.

(a) A ventilated cage rack (containing multiple microisolators). Exhaust air is either filtered and recirculated into the room or discharged directly into the room exhaust system; (b) A single ventilated microisolator cage with a filter top. When connected to a ventilated cage rack system to exhaust and filter the air from the cage, this type of cage provides primary containment for small-sized animals.

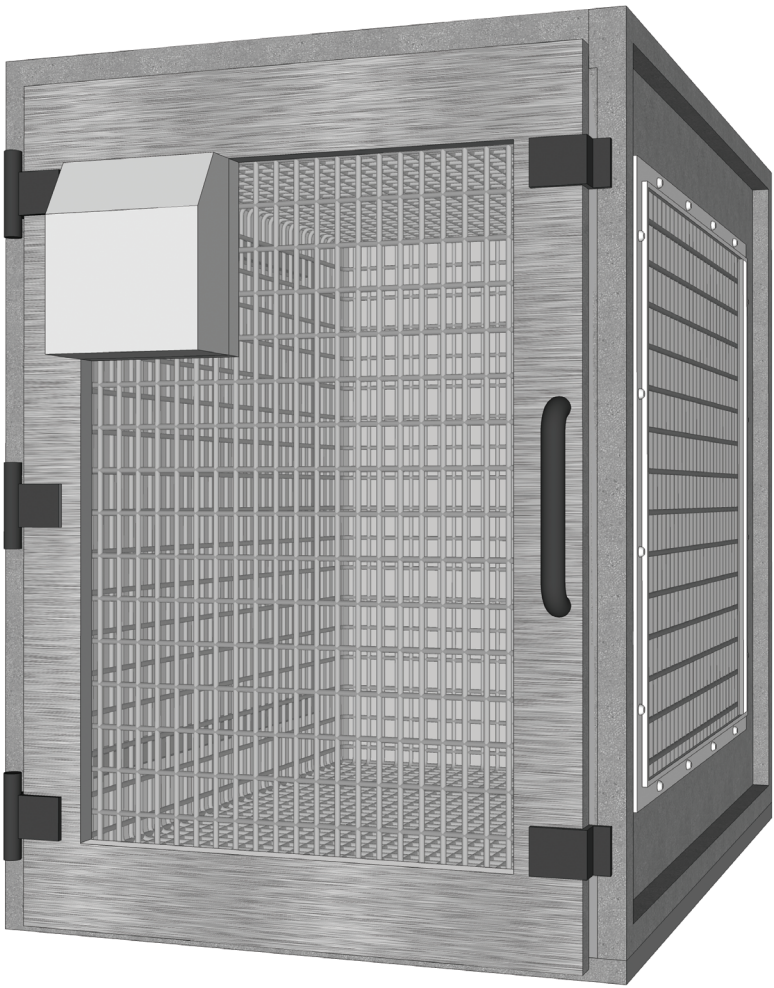
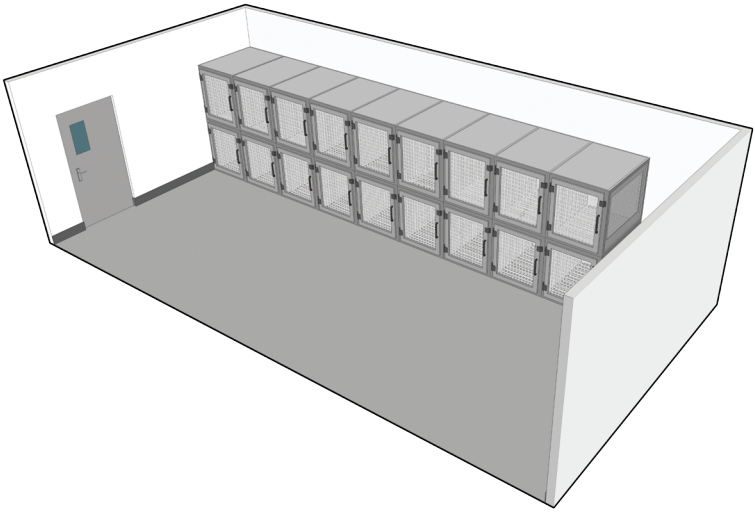


Figure 13–3: Representative Diagram of an Open Caging System.

Detailed illustration of a typical wire cage (non-filtered) used to house small-sized animals (e.g., NHPs or raccoons) in an animal cubicle. This type of cage confines the animal to a small space within the cubicle.

(a) Animal cubicle containing multiple open cages (non-filtered).



(b) Animal cubicle equipped with stalls and gating systems.

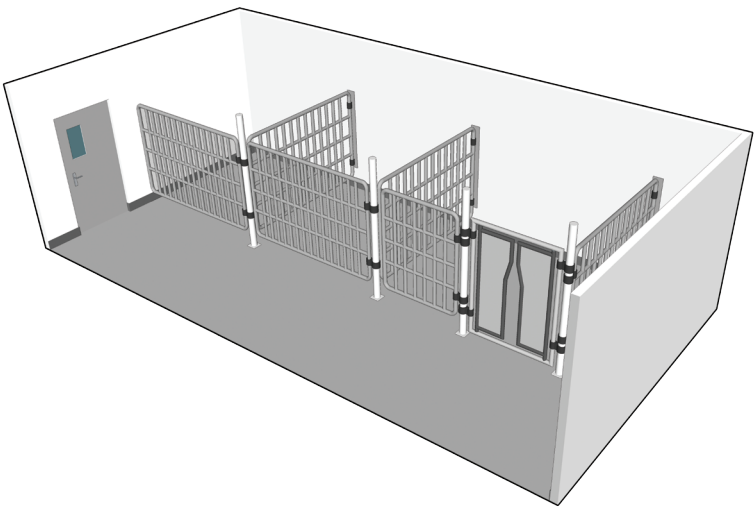


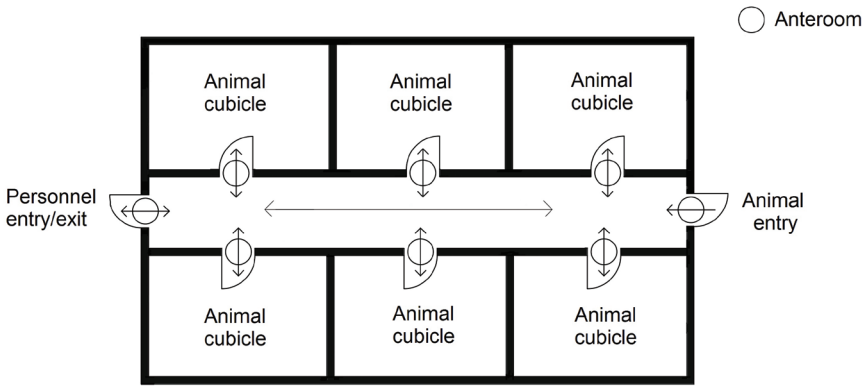
Figure 13–4: Representative Diagrams of an Animal Cubicle.

(a) Animal cubicle containing multiple open cages (non-filtered). This configuration is suitable to house animals such as dogs, cats, racoons, or NHPs. (b) Animal cubicle equipped with stalls and gating systems. This configuration is suitable to house up to three large-sized animals, such as cows, deer, horses, or sheep.

13.2.3 Animal Containment Zone Design Considerations

Design considerations relevant for the design of any containment zone, including animal containment zones, are discussed in Chapter 22; this section discusses several key concepts relevant only to the design of animal containment zones.

(a) Single corridor design, CL2 and CL3 LA zones



(b) Dual corridor design, CL2 and CL3 LA zones

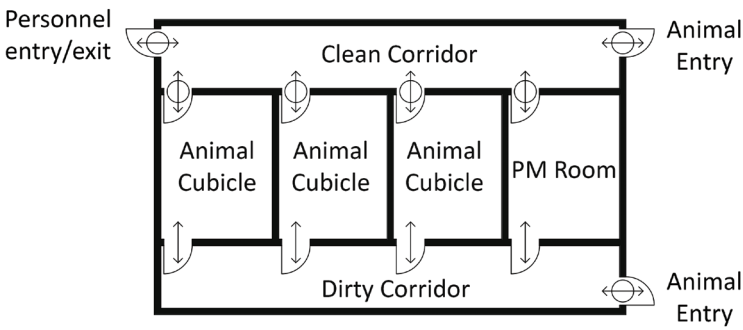


Figure 13–5:
Representative Diagram of Single Corridor and Dual Corridor Designs for Animal Containment Zones.

(a) Single corridor design for a CL2 or CL3 LA zone, (i.e., CL2-Ag or CL3-Ag); (b) Dual corridor design for a CL2 or CL3 LA zone, (i.e., CL2-Ag or CL3-Ag). Note that for CL2 LA zones, an anteroom is only required at the entry/exit to the containment zone <u>or</u> into each animal cubicle or PM room. For more detail, refer to Section 13.2.3.

13.2.3.1 Single Corridor versus Dual Corridor Designs

Where there are numerous animal rooms or cubicles within a more complex containment zone, the inclusion of separate "clean" (i.e., uncontaminated) and "dirty" (i.e., contaminated or potentially contaminated) corridors may ease personnel **movement** from one room or cubicle to the next. LA zones incorporating this dual corridor design (i.e., separate "clean" and "dirty" corridors) connecting the animal cubicles and PM rooms can offer advantages over LA zones designed with a single corridor connecting the animal cubicles and PM rooms ("single corridor design"). The dual corridor design facilitates traffic flow of animal handlers, staff, animals, feed, equipment, and samples/specimens. This design can also minimize the risk of cross-contamination between animal cubicles. The flow of animals and personnel in animal containment zones designed with a single corridor layout is considerably different from that in zones with a dual corridor layout. It is critical that traffic flow for animals and personnel be well defined in the **standard operating procedures (SOPs)** for single and dual corridor designs. Examples of single corridor and dual corridor designs in CL2 or CL3 LA zones (i.e., CL2-Ag or CL3-Ag) are provided in Figure 13-5.

A single corridor design **facility** may be operated in a manner that designates the corridor as a "clean" corridor (i.e., uncontaminated), in which case, anterooms for the entry to/exit from each animal cubicle and PM room are important and the strict adherence to operational procedures (especially entry/exit protocols) is critical to prevent the spread of contamination in the containment zone. Alternatively, where the single corridor is operated as the "dirty" corridor, the presence of an anteroom at each animal cubicle and operational protocols at these points is not highly emphasized since it does not separate clean and dirty areas. In this case, strict adherence to entry/exit procedures at the containment zone entry/exit is essential to prevent the release of pathogens from the zone. In the single corridor design depicted in Figure 13-5(a), the access corridor is considered "dirty" (i.e., contaminated). The containment zone is accessed by personnel through an anteroom located off the corridor (entry/exit). Each animal cubicle and PM room is accessed by personnel from the corridor via separate anterooms (entry/exit). In facilities where anterooms and showers are not available at each cubicle (e.g., CL2 LA zones), procedural means to limit contamination may be an option if supported by an LRA. Proper training of personnel in the movement between cubicles (e.g., handling uninfected animals first, using boot baths and chemical **disinfection** of outer PPE layers after exiting a cubicle into a "dirty" corridor) can be effective in preventing contamination.

In contrast, in the dual corridor design (Figure 13-5[b]), there are separate "clean" and "dirty" corridors to minimize the spread of contamination from infected animals to specific areas of the zone. The containment zone is accessed by personnel through an anteroom located off of the "clean" corridor (entry/exit). The "dirty" corridor allows for the movement of infected animals between cubicles and PM rooms. Animal entry to the containment zone is through the "clean" corridor. Entering more than one animal room or cubicle from the "clean" corridor, without a change of PPE, is generally not acceptable; however, in some cases it may not be necessary to change PPE when moving from uninfected animals to infected animals. Entering more than one animal cubicle from the "dirty" corridor may be

acceptable, provided that the same pathogen is handled in all rooms and cubicles and depending on the nature of the work. After personnel are finished working in animal cubicles or PM rooms, dedicated PPE is doffed in the connecting anteroom(s) before re-entering the "clean" corridor. The containment zone is exited through an anteroom located off the "clean" corridor.

13.2.3.2 Access and Anterooms

Access to animal cubicles in LA zones is provided through one or more anterooms. Depending on the design (described in Section 13.2.3.1) and containment level of the containment zone, anterooms may also be located at the entry to/exit from individual animal cubicles and PM rooms. Anterooms create an added buffer space to protect the outer environment from the infectious material and toxins handled within; they allow for the separation of personal clothing from dedicated animal cubicle clothing and PPE, and help to maintain the **inward directional airflow (IDA)** in animal containment zones to protect containment integrity. An LRA may be conducted to determine when a shower is needed prior to exiting from a CL2 LA zone (i.e., CL2-Ag) for example, if there is substantial contact with infected animals on a day-to-day basis, or when working with animals that harbour, as part of their normal flora, pathogens that may infect humans. Anterooms are further discussed in Chapter 3.

Restricting access to the animal containment zone increases the safety of personnel and increases the security of the animals and pathogens and toxins handled and stored inside the containment zone. In certain cases, **limited access** or **restricted access** to areas within the containment zone (e.g., individual animal rooms, animal cubicles, and PM rooms) may also be necessary and is determined by the pathogens, toxins, and activities in the zone. **Controlled access systems**, such as electronic access card systems, keypads, or key-locks with non-reproducible keys, restrict access to **authorized personnel**. Restricting access to animal rooms, animal cubicles, or PM rooms may be achieved through a controlled access system, or where determined to be acceptable, through other mechanisms such as signage (e.g., "authorized personnel only"). Observation windows on the doors accessing animal rooms and cubicles are generally recommended to allow personnel to view the interior of the animal room or cubicle prior to entry and to verify that animals are not loose.

13.2.3.3 Cold Storage

If tissues and carcasses are not disposed of immediately following euthanasia or necropsy procedures, refrigeration will be required to delay putrefaction and to minimize odours. Consideration should be given to the size of the animals in use and the quantity of carcasses that will need to be stored prior to disposal to confirm that the cold storage area or equipment needed is sufficient in size; this equipment could be an integral cold room or refrigeration equipment, such as a freezer or refrigerator, of adequate size, dependent on the size of the animals in use. Locating cold storage in or adjacent to the PM room in an LA zone will minimize the distance to move potentially heavy carcasses and limit the spread of contamination.

13.2.3.4 Unique Physical Requirements

Animals are curious by nature and have the ability to chew on or pull objects, and as such, protruding obstructions (e.g., lighting, electrical fixtures, exposed plumbing) in these spaces should be minimized and appropriately shielded. Locking mechanisms should be carefully selected to be sufficiently complex to prevent animal escape, as determined by the animal's ability to manipulate objects. In animal containment zones, floors are to be impact resistant and able to withstand the weight of animals and associated equipment without becoming gouged or cracked (CBS Matrix 3.4). They should also be designed to withstand prolonged contact with urine. Gates, rubber mats, and cages should have sufficient strength to resist the damage and abuse caused by the animal(s). Building and surface coverings of an animal containment zone should be selected knowing that animal rooms, animal cubicles, and PM rooms are subjected to frequent cleaning, decontamination, and high pressure washing.

Floors should be textured and slip-resistant so animals and animal handlers can maintain traction, even when the surface is wet. Personnel should also wear footwear that provides traction on wet, slippery floors. Due to the large volume of water that is needed for the cleaning of these spaces, it is recommended that floors slope directly towards the floor drains to avoid pooling of contaminated water. In CL2 LA zones (i.e., CL2-Ag) where **prions** are handled, CL3 zones where **non-indigenous animal pathogens** are handled, and CL3 LA zones (i.e., CL3-Ag), floors drains are to be separated from those of lower containment areas and directly connected to an **effluent decontamination system** to decontaminate all liquid waste prior to release into the sanitary sewer.

13.3 Equipment

The carcasses of livestock and other large-sized animals (e.g., deer, moose) can be quite difficult to move around in the containment zone. It may be necessary to use an overhead rail and hoist system to move large carcasses to the necropsy room or disposal unit. Consideration should be given to including a rail, chain fall, motorized operation, and adequate lift clearance when planning the height of an LA zone where work with large-sized animals will be conducted. In LA zones (including PM rooms), the operation of the electrical hoist/monorail should be limited to trained personnel wearing protective headwear.

It is recommended that surgical procedures and necropsies be carried out in dedicated laboratory work areas (i.e., procedure rooms), necropsy rooms, or PM rooms located inside the animal containment zone but separate from animal rooms or cubicles, wherever possible. To preserve personnel safety and promote proper animal care, adequate preparation is crucial; all necessary tools and equipment should be available inside the containment zone. The selection of tools and equipment for use in surgical procedures and necropsies should consider the potential to cause injury to personnel and the creation of potentially infectious aerosols. For example, it may be prudent to use manual equipment (e.g., hand saw) instead of electrical equipment (e.g., Stryker saw) during these procedures so that the amount of gross contamination and aerosols is minimized. Skilful technique is required to prevent the

excessive spread of contamination and the formation of aerosols originating from fluids and tissues. When performing surgical procedures and necropsies, every effort should be made to limit the spread of contamination.

13.4 Personnel Training

Personnel working with animals, facility maintenance employees, and other staff that may need to enter the facility are to have specific training in animal facility procedures (the requirements are specified in Matrix 4.3 of the CBS). Training plans are to be developed for each individual, accounting for their duties in the facility, and should include the physical and biological hazards associated with the animals themselves, restraint techniques, the characteristics of the pathogens or toxins in use, and all relevant SOPs. The relevant SOPs describe every aspect of the proposed work, including, but not limited to, entry and exit, PPE, communication between personnel, feeding, sampling, animal handling, animal escape (prevention and capture), signs of disease, daily cleaning, decontamination, surgical and necropsy procedures, and any other protocols specific to the work. The training will also include the procedures that are relevant to emergency situations, described in Chapter 17.

Development of the training program should take into consideration the applicable CCAC guidelines. Trainees may benefit from visits to other containment facilities and discussions with personnel who have extensive experience working with the animal species of interest. It is recommended that the training include mock scenarios and pre-task practice prior to the actual infection of animals. Consideration should be given to posting the contact information of experienced animal handlers throughout the animal containment zone. The training program is discussed in greater detail in Chapter 8.

13.5 Handling and Restraint

The use of proper handling and restraint techniques helps prevent injury to the handler, reducing the potential for a bite, scratch, or other exposure to infectious material, and therefore protecting against secondary transmission in the community. In addition, proper handling and restraining techniques will minimize animal stress; stressed animals are more likely to react in an unexpected manner, and from a scientific perspective, may lead to physiological changes that influence findings. Close attention should always be paid to the restraint of animals during inoculation procedures to avoid self-inoculation. Different species can have very different handling requirements. The selection of animal housing and handling equipment should be specific to the species. There are many resources available on proper handling techniques for various types of animals, including the CCAC and international resources.[4,5,6,7,8,9,10]

When handling animals that are in caging systems, a chemical restraint or a squeeze mechanism (e.g., squeeze-back cages) may be utilized to immobilize the animals, depending on the species. Transfer boxes may be an option to move animals within the

containment zone. When handling large-sized animals, special care should be taken to avoid serious injuries (e.g., crushing) that could occur. Methods such as gating systems, chutes, tunnels, squeeze mechanisms, and chemical restraint methods, can be used to restrain and move animals to other rooms or cubicles in LA zones.

Using the least amount of restraint necessary is the best way to provide a safe environment for both the animal and the handler and, when possible, it is good practice to habituate the animals to the restraint method before initiating project manipulations. For example, with positive reinforcement, pigs will habituate to a sling, and horses and cattle can be trained to accept a halter and lead rope.

13.6 Decontamination and Waste Management

The safe and effective decontamination of all waste, including animal waste, is critical to containment. In high containment LA zones (i.e., CL3-Ag and containment level 4 [CL4]), as well as in CL2 LA zones (i.e., CL2-Ag) where prions are handled, floor drains are connected to an effluent decontamination system. In contrast, in CL3 SA zones where only human or indigenous animal pathogens are handled, contaminated liquid effluent (e.g., from cage washing) is prevented from entering the floor drains procedurally. It is recommended that floor drains only be installed when necessary to minimize the potential for contaminated liquids to be released into the sanitary sewers. Where existing floor drains are not to be used, they should be sealed or capped. In all containment zones, contaminated liquids are to be decontaminated prior to release to sanitary sewers; therefore, procedures that reduce the amount of liquid waste produced (e.g., use of footbaths rather than rinsing boots, capturing liquids in bedding) are encouraged.

In animal cubicles connected to the effluent decontamination system, the drains can potentially become clogged by bedding or litter. Therefore, effluent decontamination systems are to include a mechanism to prevent blockage. This mechanism may be physical or operational, such as regularly removing bedding or litter from the drains and having them autoclaved or incinerated, provided that **validation** and efficacy testing demonstrate the approach to be effective. Decontamination of animal carcasses and anatomical waste is further described in Chapter 15.

A variety of equipment and processes can be used for cage cleaning. In SA zones, cage manipulations and bedding disposal are performed in a BSC or ventilated cage changing station designed to contain aerosols and provide user and environmental protection. Non-ventilated animal transfer stations should not be used when handling infected animals as they offer no environmental or user protection. The cages should be closed and surface decontaminated before removal from the BSC, and then sent for autoclaving before final cleaning. Various types of caging and bedding disposal systems exist and thorough research is essential to select an appropriate system.

Cage washers can only be used as the **primary decontamination technology** if the method has been validated to be effective against the pathogens or toxins in use. Often, cages and bedding are decontaminated inside the containment zone, before they are sent for cage washing. In **high containment zones**, cage washing areas can be located outside of the containment zone only if cages are decontaminated prior to removal from the containment zone. The use of disposable caging is an alternative to having cage washing areas.

The use of pressure washers to clean animal cubicles should be minimized and used only when necessary in order to prevent the generation of aerosols. Cubicles can first be cleaned with low pressure hoses and then sprayed with a pressure washer. Cleaning cubicles on a daily basis is recommended in order to reduce the accumulation of contaminants, with complete decontamination of the cubicle being carried out at the end of the experiment.

13.7 Confinement

Confinement is the term used when only certain containment components are required. During specific periods of time subsequent to inoculation with certain pathogens, natural excretions and casual contact with infected animals would not pose a risk for pathogen transmission (e.g., during the incubation period for bovine spongiform encephalopathy [BSE] in cattle). Thus, while the infected animals should always remain adequately confined, they do not have to be housed and maintained inside a containment zone as specified in the CBS. Confinement components will vary depending on the pathogens used and the study design. Many factors need to be taken into consideration, including disease transmission, potential for shedding, and endemicity in Canada. Due to recent evidence that scrapie can be detected in the feces and oral secretions of pre-clinical sheep through sensitive techniques, it is not acceptable to house scrapie-infected sheep in confinement at any time post-infection or exposure; they must be housed in an appropriate containment zone.[11,12,13]

Approval by the Public Health Agency of Canada (PHAC) and/or the Canadian Food Inspection Agency (CFIA) is required before experimentally infected animals are permitted to be housed in confinement conditions. The basic and minimal confinement components needed are based on the results of an LRA. Some additional considerations when working in confinement are as follows:

- Observation and counting of animals in confinement should be performed and recorded daily. Single-point access control is recommended in order to prohibit unauthorized movement of personnel or animals into or out of the confinement area. Access control should be verified to make sure it functions as intended.
- A method to limit the access of wildlife/scavengers to the confinement area should be put in place.
- A double identification (ID) system for animals should be in place and **verification** of ID should be conducted daily. If an ID tag or other device is missing it should be replaced immediately.

- Materials (e.g., manure and bedding) from pens may be treated by normal composting and disposal. Composting parameters require validation testing to demonstrate efficacy against the infectious material in use.
- Animals that have been inoculated with human or **animal pathogens**, or other experimental **biological material**, are not eligible for use in the human food or animal feed chain.

13.8 Special Considerations for Work with Prion-Infected Animals

Prions associated with **transmissible spongiform encephalopathies (TSEs)** can infect a wide range of animal species, including cattle, sheep, deer, and mink, as well as humans. While there do not appear to be any confirmed cases of **laboratory acquired infections (LAIs)** of TSEs in humans, precautions are required when working with prions and animals infected with prions to avoid worker exposure to contaminated materials and release of infectious prions into the environment. The most likely routes of prion transmission to humans are through accidental inoculation with contaminated instruments, bites or scratches from infected animals, or ingestion of material containing prions. There is little evidence to suggest that prion diseases are transmissible by inhalation; however, transmission through exposure to aerosols or splashes may be possible, especially if there is contact with the mucous membranes.[14,15,16,17]

To date, little evidence has been found to suggest that either animal to animal or maternal transmission of BSE occurs amongst cattle in a herd; rather, transmission appears to occur through the ingestion of contaminated feed (meat and bone meal).[18,19,20,21,22] In contrast, maternal transmission as well as animal to animal transmission of scrapie have been documented amongst sheep of the same herd.[14,23,24] Transmission of chronic wasting disease (CWD) in deer and elk has been documented to occur through both animal to animal contact as well as by contact with a heavily contaminated environment. Prions have been detected in many types of cervid tissues (e.g., central nervous system tissue, blood, cardiac and skeletal muscles, pancreas) as well as in bodily secretions and excretions (e.g., feces, urine, saliva) of CWD-infected cervids.[25,26]

Given the knowledge of prion transmission and the lengthy incubation period of TSEs in hosts, some animals inoculated with prions only present a significant risk for pathogen transmission during certain periods (i.e., immediately after inoculation, parturition) when prions may be shed in bodily secretions and excretions. The following operational practices are considerations when working with animals infected with prions and disposing of wastes:

- For BSE in cattle: Waste and bedding should be collected and treated accordingly for potential prion contamination for at least 4 weeks post-inoculation and again once clinical signs are observed. During the periods of incubation (i.e., more than 4 weeks post-inoculation and prior to the onset of clinical signs), natural excretions and casual contact with infected animals is not considered

to pose a risk for prion transmission. Consequently, during these non-shedding periods, it may be acceptable (i.e., with prior approval from the PHAC or the CFIA) to house the animals outside of the CL2 LA zone (i.e., CL2-Ag), provided that they are adequately confined, based on an LRA of specific experiments. During confinement, bedding and waste may be dealt with by normal composting and disposal.

- For scrapie in sheep: Bedding, placental fluids, and any other waste should be collected and decontaminated appropriately to inactivate prions for at least 4 weeks post-inoculation and again once clinical signs are observed. Experiments should be conducted in a CL2 LA zone (i.e., CL2-Ag) suitable for work with prions while lambing. Pregnant ewes should be contained so that placental materials may be collected and incinerated and birthing fluids appropriately decontaminated. Lambing areas should be decontaminated upon removal of the ewe and lamb.
- For CWD in cervids (e.g., deer, elk, and moose): CWD-infected animals must remain in an appropriate containment zone at all times post-inoculation. All waste and bedding must be collected and decontaminated throughout the entire study period.
- For uncharacterized TSEs (e.g., Feline Spongiform Encephalopathy, Exotic Ungulate Spongiform Encephalopathy) and non-host species inoculated with a prion disease agent (e.g., CWD in cattle or BSE in deer): In cases where it may be unclear whether or not prions are shed in animal waste, all animal waste and bedding (at all times post-inoculation) is to be collected and decontaminated throughout the entire study period. Incineration at 850°C is recommended for decontamination of solid animal waste and bedding that contains prions. An acceptable method to decontaminate liquid wastes that contain prions is heat treatment at 134 °C for 1 hour.

Additional details on the recommended methods of decontamination of wastes and other prion-contaminated materials are discussed in Chapter 15.

13.9 Working with Non-Human Primates

Work with NHPs presents unique hazards to animal handlers and containment zone personnel. Not only may NHPs harbour pathogens (i.e., normal flora) that can affect the health and safety of personnel, but the animals themselves also pose a risk. For example, the physical characteristics of NHPs (e.g., canine teeth, powerful jaws, sharp fingernails and toenails), make them capable of causing serious injury to animal handlers that may also result in an exposure to pathogens. These characteristics should be considered when designing animal rooms or cubicles to house them. The CCAC Guidelines provide information on housing and handling requirements specific to NHPs.[6]

Pathogens that may be naturally carried by NHPs and pose a hazard to personnel handling them include, but are not limited to, bacteria (e.g., *Salmonella, Shigella, Campylobacter, Mycobacterium tuberculosis*), viruses (e.g., hepatitis A virus, simian immunodeficiency virus, Macacine herpesvirus 1 [formerly known as herpes B virus or cercopithecine herpes virus 1]), and protozoan and metazoan parasites (e.g., *Entamoeba, Blastocystis, Trichomonas, Balantidium*). Macacine herpesvirus 1 is an **enzootic** virus present in up to 70% of captive macaques, including rhesus and cynomolgus macaques.[27] Although the virus causes oral lesions in its natural simian host, asymptomatic shedding from the buccal mucosa and urogenital tract, although rare, or the presence of the virus in conjunctival fluid, can occur without clinical signs of the disease. Human infection has been documented in at least 50 instances, resulting in either severe disease or death.[28] Except for one case of person-to-person transmission, all have occurred in people exposed to NHPs or NHP tissues. Transmission to humans is believed to occur primarily by exposure to contaminated NHP saliva through bites and scratches, although one fatal case following mucocutaneous exposure without injury has been reported.[27] Guidelines are available for working safely with macaques, for the prevention of Macacine herpesvirus 1 infection, and for the treatment of such infections in exposed people.[5] Consequently, it is strongly encouraged that all macaque colonies are handled with caution and as potential carriers of Macacine herpesvirus 1, even if they have been tested seronegative for the antibody.

Except when an NHP has been experimentally infected with or known to be naturally harbouring an infectious organism requiring high containment (i.e., CL3 or higher), it is acceptable and considered safe to handle NHPs in a CL2 animal containment zone, provided that the additional precautions outlined in Section 13.1 and the following additional practices and precautions outlined below are also implemented:

- any NHP that has caused a bite or scratch wound should be immediately immobilized and have its oral cavity examined for lesions characteristic of Macacine herpesvirus 1;
- protection against aerosol exposure and splashes onto mucous membranes (e.g., with surgical mask, face shield, eye goggles) should be provided for handlers and anyone entering animal cubicles where NHPs are housed; and
- an emergency medical contact card must be issued to containment zone personnel handling NHPs; details are provided in Chapter 7.

REFERENCES

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CHAPTER 14

LARGE SCALE WORK

CHAPTER 14 – LARGE SCALE WORK

Large scale production **facilities** such as industrial fermentation and vaccine production plants pose an increased **risk** to personnel and the environment due to the large quantities of **infectious material** or **toxins** being handled. As such, there are sometimes unique or more stringent requirements and additional considerations when compared to **laboratory work areas** at the same **containment level**. This chapter provides specific guidance to assist **large scale production areas** in developing a comprehensive **biosafety** program.

14.1 Scope

There is currently no universally accepted definition of "large scale". The United States National Institutes of Health (NIH) *Guidelines for Research Involving Recombinant DNA Molecules* considers anything greater than 10 litres as large scale.[1] The United States Centers for Disease Control and Prevention (CDC)/NIH *Biosafety in Microbiology and Biomedical Laboratories*, 4th Edition, 1999 defines "production quantities" as a volume or concentration of infectious organisms considerably in excess of those used for identification and typing.[2] In contrast, the United Kingdom Advisory Committee on Dangerous Pathogens states that it is not the volume but the intent of the work that determines the scale.[3]

The Public Health Agency of Canada (PHAC) and the Canadian Food Inspection Agency (CFIA) generally consider activities involving volumes of toxins or the ***in vitro* culture** of infectious material on a scale of 10 litres or greater to be large scale. This could be a single vessel with a volume of 10 litres or greater, or in some cases, multiple vessels with a total volume of 10 litres or greater. Determination of cut-off values for **laboratory** and large scale volumes can be made in consultation with the PHAC or the CFIA.

14.2 Considerations for Large Scale Work

When working in a large scale environment, a **local risk assessment (LRA)** is conducted to identify and examine the hazards associated with the infectious material or toxins, processes, and equipment in use. This analysis is used to develop safe work practices. Once an LRA has been completed, exemptions from certain large scale requirements may be determined in consultation with the PHAC or the CFIA. In addition, elements specific to large scale activities may apply to **licences** and **animal pathogen import permits**, for certain containment level 2 (CL2) large scale production areas; this is determined based on the processes and type of **pathogens** used, and in consultation with the PHAC or the CFIA.

Some factors that should be considered when conducting an LRA are as follows:

- the infectious material or toxins handled (e.g., properties, **risk group**, containment level);
- the nature of the final biological product (e.g., live versus, attenuated virus, or inactivated **pathogen** component);
- the volume (i.e., total volume; single vessel versus multiple vessels);

- the concentration;
- the manipulations to be performed (e.g., in-process sampling, harvesting of cultures, concentrating, blending, interventions prior to inactivation);
- the type of process used (i.e., batch versus continuous);
- equipment characteristics (e.g., type, open or **closed system** for production and processing, stationary versus movable, **aerosol** generating); and
- **facility** features (e.g., climatic conditions, air supply intake and exhaust, maintenance of differential air pressures, physical security).

14.3 Fermenters

Fermenters can vary significantly in size, design, instrumentation and features, such as automation and capacity for ***in situ*** cleaning and **decontamination**. Infectious material or toxins could potentially be released from many areas in large scale fermentation equipment (e.g., motor shaft, exhaust gas vents, sampling ports). Fermentation processes also have the potential to generate aerosols, thereby increasing the risk associated with **exposure** to aerosols from infectious material or toxins. To minimize the probability of leaks and the **release** of aerosols when using large scale fermentation equipment, the following should be considered:

- double mechanical seals on the motor shaft should be used, or alternatively, a top-mounted agitator;
- exhaust vents should be equipped with a **high efficiency particulate air (HEPA) filter**, incinerator, or equivalent method of preventing pathogen release;
- sampling ports should be fitted to a sterilizable closed sampling system;
- **validation** of the relief system should be conducted and consideration should be made to the consequences of discharge; and
- anti-foam product is recommended to prevent blockage of the exhaust air vent.

14.4 Regulatory Considerations

The production of regulated biological products, such as vaccines and biopharmaceuticals, for human and veterinary use may require higher standards than those specified in the *Canadian Biosafety Standard* (CBS), 2nd Edition, 2015 to achieve the necessary product quality.[4,5] For example, additional requirements may apply to work involving veterinary biologics, including vaccines and *in vitro* diagnostic test kits for the detection of **animal pathogens**. The CFIA's Canadian Centre for Veterinary Biologics (CFIA-CCVB) is the national authority responsible for regulating veterinary biologics in Canada. In addition, Health Canada's Biologics and Genetic Therapies Directorate (BGTD) is the Canadian federal authority that regulates biological drugs and radiopharmaceuticals intended for human use. The CFIA-CCVB and Health Canada's BGTD should be consulted for any large

scale exemptions for veterinary biologics intended for animal use and biological drugs/ radiopharmaceuticals intended for human use, respectively. For more information regarding the regulatory requirements for veterinary biologics intended for animal use and biological drugs and radiopharmaceuticals intended for human use, please contact the CFIA- CCVB or Health Canada's BGTD directly, or visit their website.

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CHAPTER 15

DECONTAMINATION

It is a basic **biosafety** principle and a critical component of **containment** that all contaminated material is decontaminated prior to disposal. The principles of **sterilization**, **disinfection**, and **decontamination** are critical for reducing the **risk** of **pathogen release** within **containment zones**, to the environment, and within the **community**. Examples of decontamination systems that may be used to decontaminate materials leaving the containment zone include, but are not limited to, autoclaves, **effluent decontamination systems**, incinerators, irradiators, **dunk tanks**, tissue digesters, and chemical showers. The requirements for decontamination procedures are described in Matrix 4.8 of the *Canadian Biosafety Standard* (CBS), 2nd Edition.[1]

Regulated parties that properly package and label biohazardous **waste** for off-site decontamination by a third party biohazardous waste disposal **facility** remain accountable for the waste until its decontamination, including **validation** and **verification** of the decontamination process.

15.1 Principles of Sterilization, Disinfection, and Decontamination

Sterilization is a process that completely eliminates all living **microorganisms**, including bacterial spores. The probability of a microorganism surviving a sterilization process is considered to be less than one in one million (i.e., $1:10^6$), and is referred to as "sterility assurance".[2] Sterilization is considered to be absolute (i.e., there is no middle range of sterility). Given that **toxins** and **prions** are not living microorganisms, the concept of sterilization does not apply. Decontamination of toxins and prions is discussed in Sections 15.11 and 15.12 of this chapter, respectively.

Disinfection is a process that eliminates most forms of living microorganisms but is less lethal than sterilization. The effectiveness of the disinfection process is affected by a number of factors, including the nature and quantity of microorganisms, the amount of organic matter present, the type and state of items being disinfected, and the temperature.

Decontamination is the process by which materials and surfaces are rendered safe to handle and reasonably free of microorganisms, toxins, or prions. The primary objective of decontamination is to protect containment zone personnel and the community from **exposure** to viable pathogens and toxins that may cause **disease**. Depending on the situation (i.e., pathogen or toxin in use), effective decontamination may require disinfection, inactivation, or sterilization to be considered safe and reasonably free of microorganisms, toxins, or prions. Decontamination procedures represent a critical element of containment; failure in the procedures can result in occupational exposure to, or the unintentional release of, **infectious material** or toxins.[3,4,5] The following are considerations for containment zone personnel responsible for developing decontamination processes and methods:

- Disinfectants effective against the infectious material in use, and neutralizing chemicals effective against the toxins and prions in use, are to be available in the containment zone and used for contaminated or potentially contaminated material, including equipment, specimen and sample containers, surfaces, rooms, and spills.

- Decontamination parameters (e.g., time, temperature, chemical concentration, humidity) consistent with the technology or method used are to be validated to demonstrate they are effective against the infectious material and toxins of concern under the conditions present.
- Prions and toxins can be resistant to the chemical disinfectants commonly used to effectively decontaminate microorganisms due to their proteinaceous nature. When working with prions and toxins, a neutralizing chemical capable of denaturing and inactivating the toxins or prions is needed for effective decontamination in the containment zone.
- Clear and strict procedures are to be in place to support routine decontamination and routine verification of the decontamination process.
- Decontamination processes and methods are to be conducted in accordance with applicable federal, provincial or territorial, and municipal regulations.
- Decontamination procedures are to be included in personnel training on the hazards and exposure/release mitigation strategies associated with the work being done. This includes information on the products used, and the factors influencing their effectiveness.

15.2 Validation and Verification of Decontamination Technologies and Processes

15.2.1 Validation

Autoclaves, effluent decontamination systems, and other **decontamination technologies** and processes are validated prior to implementation of the procedure. Validation demonstrates that the equipment and method are effective at decontaminating, inactivating, or removing the specific pathogen(s) or toxin(s) to be handled and stored. It is inferred that a validated method is suitable for its intended purpose.

Biological indicators or parametric monitoring devices (e.g., thermocouples, for heat-based technologies and processes only) can be used to confirm that treatment parameters have been achieved throughout a **representative load**. Placing thermocouples or indicators at various locations throughout the representative load or decontamination vessel will enable conditions in different parts of the load to be monitored. In autoclaves, they can be used to confirm that the centre of the load has achieved the temperature and time parameters necessary for successful decontamination.

The selection of an appropriate biological indicator is critical so that the resistance of the test organism adequately represents the resistance of the pathogens handled in the containment zone. In general, *Geobacillus stearothermophilus* spores are adequate for heat-based technologies and processes, whereas *Bacillus subtilis* spores can be used to validate chemical-based technologies and processes. In cases where biological or chemical indicators are not appropriate (e.g., prions), parametric monitoring devices, such as

thermocouples or gauges that capture cycle time, temperature, and pressure, can be used to accurately monitor the performance of the decontamination equipment.

Validation of all decontamination technologies and processes is required prior to initial use and whenever significant changes are implemented or new pathogens are introduced so that decontamination procedures and **standard operating procedures (SOPs)** can be established, amended, or updated as necessary (CBS Matrix 4.8). Validation through the use of **representative loads** is required annually (CBS Matrix 5.1). Performing validation tests on non-contaminated representative loads that simulate a batch of materials of similar type (e.g., gloves, plastics, liquids, reusable **personal protective equipment [PPE]**) and quantity (i.e., number of items or size) that will be regularly processed allows an operator to place indicators safely to demonstrate that appropriate decontamination parameters are achieved throughout the load (e.g., in the bottom, middle, and top of the batch of materials). By demonstrating this with a representative load, it can be extrapolated that similar conditions are achieved in a routine load (i.e., contaminated waste) of similar type and quantity.

15.2.2 Verification

Once effective decontamination parameters have been established through validation, it is important that decontamination processes and procedures be monitored (verified) on a regular basis to confirm that established parameters have been met.

Verification is the routine monitoring of equipment and processes to ensure they are functioning properly and continue to meet the parameters established during validation. This can be accomplished using parametric monitoring devices, biological indicators, chemical indicators, or chemical integrators. The information captured during verification should include the cycle parameters (e.g., temperature, time, and chemical concentration), a description of the size and type of load (e.g., reusable PPE, solid waste, and liquid waste), and a short description of the procedure. For each run performed, the parameters captured may include time and temperature charts and biological indicator results. If a biological indicator is used, results of a positive control from the same lot should also be captured. A **local risk assessment (LRA)** will help determine the procedures for routine monitoring (e.g., daily, weekly, monthly), taking into consideration the frequency of use.

15.2.3 Indicators, Integrators, and Parametric Monitoring Devices

A biological indicator is a standardized population of bacterial spores used to demonstrate effective sterilization conditions in a waste load. Achieving the target level of reduction in viable spores indicates that the decontamination process was effective. Attention must be paid to appropriate selection of indicators, as their design and construction vary depending on the intended use (e.g., liquid versus dry load, self-contained system, enzyme-based rapid method); the indicator should be representative of the pathogen or toxin being decontaminated.

Chemical indicators are meant to be used in conjunction with biological indicators and physical monitors (i.e., pressure and temperature gauge readings). They are used to monitor one or more parameters, but not all parameters needed for effective decontamination. Chemical indicators include autoclave tape, labels, and pouches embedded with a thermochromic ink (e.g., Bowie-Dick test packs). They provide instant results for day-to-day monitoring indicating that a certain parameter (e.g., temperature, steam, gas exposure) has been reached, but they are not an indicator of decontamination efficacy.

Chemical integrators are a form of chemical indicator used to confirm that all decontamination parameters have been met (e.g., temperature, pressure, and time for an autoclave cycle).

A comprehensive overview of biological and chemical/physical indicators and their recommended use can be found in *Developing Indicators for Monitoring Sterilization* in W.A. Rutala's *Disinfection, Sterilization and Antisepsis in Health Care*.[6] Where biological or chemical indicators are not appropriate, parametric monitoring devices can be used to capture cycle parameter (e.g., time, temperature, and pressure) to confirm that the conditions have been met for effective decontamination. A thermocouple is an application-specific parametric device used for the validation and verification of heat-based decontamination technologies.

15.3 Chemical Disinfectants

Chemical disinfectants are generally used for the decontamination of surfaces and equipment that cannot be autoclaved, specimen and sample containers to be removed from the containment zone or **biological safety cabinet (BSC)**, spills of infectious materials, and rooms and **animal cubicles**. The use of disinfectants can impact worker safety directly (e.g., direct exposure to a hazardous chemical) or indirectly (e.g., exposure to viable pathogens when an inappropriate disinfectant is selected). It is important that containment zone personnel are knowledgeable about the products required for the disinfection of the infectious material and toxins with which they will be working, including the recommended directions for use (e.g., application method, concentration, contact time, PPE, first aid, disposal) and chemical characteristics (e.g., toxicity, chemical compatibility, storage stability, active ingredient, identity, concentration).

Product effectiveness depends on the active ingredient(s) and the identity and concentration of other ingredients in the formulation. There are usually striking differences between the activities of disinfectants when used under actual **laboratory** conditions as opposed to the controlled, standardized testing methods (e.g., Association of Analytical Communities [AOAC International], American Society for Testing and Materials [ASTM]) used to generate efficacy data for product registration. The evaluation of disinfectant efficacy may be quantitative, semi-quantitative, or qualitative in nature. These differences are usually due to the variability in resistance to disinfection between surrogate strains used for standardized testing methods, and strains used in the laboratory. Also, environmental conditions such

as temperature, relative humidity, and even water hardness are variable in laboratory settings but controlled in standardized disinfectant tests. It is therefore difficult to make generalizations about contact times and concentrations needed to kill specific pathogens. It is advisable for laboratories to conduct in-use disinfectant efficacy testing to evaluate a product's performance under their specific conditions of use.

A number of standardized tests exist to evaluate the efficacy of liquid disinfectants. Both ASTM International and AOAC International have approved disinfectant efficacy test methods. ASTM standard E2197-11, in particular, describes a basic method involving the artificial **contamination** of a surface (carrier disk) with test microorganisms, and subsequent exposure to the liquid disinfectant at different concentrations or for different contact times.[7] In general these tests comprise four basic steps which can be adopted for laboratory in-use disinfectant testing.

1) Apply a known quantity of the microorganism in use in the laboratory to a carrier material or vessel. This quantity should be representative of the concentrations typically encountered in the laboratory.
2) Apply the test disinfectant to the carrier material or vessel for the contact time used in the laboratory.
3) Neutralize the disinfectant to halt its action. This can be accomplished by dilution or addition of growth media or other suitable reagent known to neutralize the active ingredients of the disinfectant.
4) Assess the viability of the microorganism in a suitable growth medium.

If the microorganism survives, altering the contact time or concentration of the disinfectant, or both, may be required to achieve the desired level of disinfection. Factors that may affect the efficacy of the disinfectant are outlined in Section 15.3.1.

15.3.1 Selection of Chemical Disinfectants

The selection of an appropriate chemical disinfectant is dependent on a variety of factors, including the resistance of the infectious material or toxin, the method of application (e.g., liquid or gaseous), and the nature of the material to be disinfected (e.g., hard surface, porous materials). **Organic load**, concentration, contact time, temperature, relative humidity, pH, and stability can also impact the efficacy of a chemical disinfectant. Table 15-1 describes the susceptibility of pathogens to chemical disinfectants and those reported to be effective against them.

Table 15–1:
Pathogens Ranked According to Relative Susceptibility to Chemical Disinfectants.

Susceptibility	Pathogen	Disinfectants reported to be effective
Extremely resistant	Prions	• **High concentrations** of sodium hypochlorite (NaOCl) or heated strong solutions of sodium hydroxide (NaOH) (see Section 15.12).
Highly resistant	Protozoal oocysts	• Ammonium hydroxide, halogens (high concentrations), halogenated phenols.
	Bacterial endospores	• Some acids, aldehydes, halogens (high concentrations), peroxygen compounds.
Resistant	Mycobacteria	• Alcohols, aldehydes, some alkalis, halogens, some peroxygen compounds, some phenols.
	Non-enveloped viruses	• Aldehydes, halogens, peroxygen compounds.
Susceptible	Fungal spores	• Some alcohols, aldehydes, biguanides, halogens, peroxygen compounds, some phenols.
	Gram-negative bacteria	• Alcohols, aldehydes, alkalis, biguanides, halogens, peroxygen compounds, some phenols, some quaternary ammonium compounds (QACs).
	Gram-positive bacteria	
	Enveloped viruses	
Highly susceptible	Mycoplasma	• Acids, alcohols, aldehydes, alkalis, biguanides, halogens, peroxygen compounds, phenols, QACs.

Adapted from: Quinn, P. J., & Markey, B. K. (1991). *Disinfection and Disease Prevention in Veterinary Medicine.*[8]

15.3.1.1 Organic Load

Organic matter (e.g., tissue, blood, bedding, feces) protects microorganisms, toxins, and prions from contact with disinfectants and can neutralize many germicides (e.g., NaOCl). Pre-cleaning with a detergent to remove bedding, litter, and feed prior to disinfection reduces organic load and achieves proper disinfection. It is important that pre-cleaning be carried out in a manner that avoids personnel exposure to infectious or potentially infectious material, and all cleaning materials are decontaminated prior to disposal. Pre-cleaning prior to disinfection may not always be appropriate and, in these cases, disinfectants that remain active in the presence of considerable amounts of organic material are a suitable alternative (e.g., phenolic disinfectants). It may be appropriate to saturate the contaminated material with a disinfectant, allowing it to remain wet for a long contact time (e.g., 30 minutes), then dispose of **gross contamination** and thoroughly clean surfaces before reapplying the disinfectant.[6,8,9]

15.3.1.2 Concentration

The disinfection process is generally quicker when a higher concentration is used. High concentrations of certain chemicals may cause damage to surfaces or tissues; however, if the concentration is reduced to avoid damage, the disinfectant may no longer possess sufficient germicidal activity to be effective. It is therefore important to determine the concentration at which the disinfectant can inactivate the organism, but will not damage other materials.[6,8,9]

15.3.1.3 Contact Time

The contact time is the period of time during which the treated surface remains saturated with the disinfectant. An effective contact time will depend on the disinfectant and the microorganisms, toxins, or prions that are present. Fast-acting disinfectants are preferable because longer contact times may be difficult to achieve. Although alcohols may have bactericidal and fungicidal activity after an extended contact time (e.g., 10 minutes), they are unlikely to remain on surfaces this long because they evaporate.[6,8,9]

15.3.1.4 Temperature

Elevated temperatures generally enhance germicidal action; however, elevated temperatures may accelerate evaporation, thus reducing contact time.[8,9] Lower temperatures are also a concern as the efficacy of disinfectants can be markedly reduced. This should be considered when decontaminating materials in a refrigerator, freezer, or low-temperature centrifuge.

15.3.1.5 Relative Humidity

Relative humidity can influence the activity of some disinfectants, particularly formaldehyde. The antimicrobial activity of formaldehyde gas fumigation is maximized at a relative humidity in excess of 70%.[6,8,9]

15.3.1.6 pH

The activity of some disinfectants may be affected by pH. It is important to carefully read the directions for use and notifications regarding incompatible chemicals to ensure efficacy as well as personnel safety.[8,9]

15.3.1.7 Stability/Storage

Dilutions of some disinfectants (e.g., sodium hypochlorite [NaOCl], alkaline glutaraldehyde) may not be stable over long periods, especially in the presence of heat or light. Products should therefore be stored in a cool, dark location. Prepare only enough disinfectant for daily or weekly use (depending on shelf life).[8,9]

15.3.2 Classes of Chemical Disinfectants

Numerous types of disinfectants are available; however, the active components of disinfectants belong to relatively few classes of chemicals, and understanding the capabilities and limitations of each class will allow selection of a product based on relative effectiveness.

Table 15-2 summarizes the susceptibility of different types of microorganisms to several chemical disinfectants, including their effectiveness and the contact time required to achieve disinfection. Toxins and prions are resistant to many chemical disinfectants; the specific considerations for decontamination of toxins and prions are discussed in Sections 15.11 and 15.12, respectively. Table 15-3 describes the disadvantages of the same chemical disinfectants.

Table 15–2: Susceptibility of Microorganisms to Chemical Disinfectants. [6,8,9,10,11,12,13,14]

Chemical Disinfectant	Commonly Available Form	Effective Against							Contact Time
		Bacteria			Viruses		Fungi		
		Vegetative	Mycobacteria	Spores	Enveloped	Non-enveloped	Fungi	Fungal Spores	
Chlorine	Liquid, powder and tablet	+	+	+	+	+	+	+	Generally short; longer for bacterial spores (≥ 30 min)
Iodine	Aqueous solutions, tinctures and iodophors	+	L	L	+	L	+	L	Generally short for vegetative bacteria and enveloped viruses; contact time for other organisms is product-specific
Alcohol	Ethyl or isopropyl alcohol; 70% in water is most effective	+	+	–	+	L	+	L	Generally short for vegetative bacteria and enveloped viruses; longer for fungi and mycobacteria
Phenolics	Wide variety; generally used as substituted phenols in combination with detergents	+	V	–	+	–	V	V	
Quaternary ammonium compounds	Wide variety available with built-in detergent action	+	–	–	+	–	+	–	

Chemical Disinfectant	Commonly Available Form	Effective Against							Contact Time
		Bacteria			Viruses		Fungi		
		Vegetative	Mycobacteria	Spores	Enveloped	Non-enveloped	Fungi	Fungal Spores	
Glutaraldehyde	2% acidic solution supplied with a bicarbonate compound	+	+	+	+	+	+	+	≥ 20 min required for non-enveloped viruses and mycobacteria; >3 hours required for bacterial spores
Formaldehyde	Available as solid paraformaldehyde and liquid formalin	+	+	+	+	+	+	+	
Hydrogen peroxide (H_2O_2)	Accelerated formulations and 30% solutions in water	+	+	+	+	+	+	+	Short contact time with 6% H_2O_2, for all viruses, vegetative bacteria, fungi, mycobacteria, and some bacterial spores; higher concentrations and longer contact times required for sporicidal activity
Chlorhexidine	4% solution of chlorhexidine gluconate in a detergent base and concentrated alcohol-based solutions	+ /L*	–	–	+	–	L	–	

+: effective; L: limited activity; V: variable activity; –: no activity
* Effective against Gram-positive bacteria; limited activity against Gram-negative bacteria.

Table 15–3: Disadvantages of Chemical Disinfectants.[6,8,9]

Chemical Disinfectant	Disadvantages
Chlorine	• solutions are light sensitive and should be prepared fresh and stored in light-protected containers • highly corrosive to metals • neutralized by organic material • concentrated solutions may be toxic to humans • reaction of chlorine with some organic molecules may lead to the production of carcinogens • not suitable for autoclaving
Iodine	• staining of treated objects • corrosive • neutralized by organic material
Alcohol	• alcohol should generally not be used to disinfect large areas of the laboratory as it may be a fire hazard • longer contact times are difficult to achieve due to evaporation • variable compatibility with certain materials (e.g., may harden rubber and deteriorate glues and some plastics)
Phenolics	• toxicity • pungent unpleasant smell • neutralization by hard water
Quaternary ammonium compounds	• decreased activity in hard water • reduced effectiveness in the presence of organic matter • due to detergent-like properties, QACs may make surfaces (including floors) slippery, which can be a hazard to both personnel and animals
Glutaraldehyde	• limited shelf-life • highly irritating and toxic to skin and mucous membranes
Formaldehyde	• more susceptible to inactivation by organic material than glutaraldehyde • pungent odour • extremely toxic • a known carcinogen

Chemical Disinfectant	Disadvantages
Hydrogen peroxide	• may be unstable when exposed to heat and light (some stabilized products are now commercially available) • high concentrations can cause skin burns, irritation or damage to the mucous membranes (with direct exposure), and can pose a risk of explosion • equipment used in H_2O_2 disinfection may be expensive when compared with other methods
Chlorhexidine	• incompatible with anionic detergents

15.4 Autoclaves

Infectious material and toxins, together with associated waste (e.g., petri dishes, pipettes, **culture** tubes, and glassware), can be effectively decontaminated in either a gravity displacement autoclave or a pre-vacuum autoclave. Gravity displacement autoclaves allow air to escape through the bottom of the chamber as steam displaces it from above. In order for this system to function efficiently, care should be taken to ensure that the valves remain unobstructed and that the chamber is not overfilled. Pre-vacuum autoclaves remove air from the chamber by employing a vacuum before letting saturated steam enter the autoclave chamber (except during liquid cycles). Pre-vacuum autoclaves resolve the air entrapment problems that are often encountered in gravity displacement autoclaves.

Autoclaves can be designed with a single door or with double doors. Double-door autoclaves are installed on the **containment barrier**, typically in **high containment zones**, to facilitate the decontamination and **movement** of waste and other contaminated material out of the containment zone. The effectiveness of decontamination by steam autoclaving is dependent on the temperature to which the material is subjected as well as the length of time it is exposed. Proper operation, loading, and monitoring of autoclaves are critical to ensure decontamination is achieved. Particular attention should be given to packaging, including the size of containers and their distribution in the autoclave so that the innermost regions of bags and containers reach and maintain the temperature required for sterilization. Arranging items in a manner that allows the free circulation and penetration of steam will help achieve effective decontamination of waste.

15.4.1 Recommended Procedures for the Use of Autoclaves

Some points to consider or to include when developing the standard operating procedures (SOPs) for the safe and effective use of a specific autoclave within the containment zone are provided in the following sections.

15.4.1.1 Before Loading the Autoclave

1. Before opening the door of a double-door barrier autoclave, confirm that the door on the opposite side of the autoclave is closed (i.e., through visual and audible alarms).
2. Check inside the autoclave for any items left by the previous user that could pose a hazard (e.g., sharps).
3. Clean the drain strainer.
4. Confirm that any plastic materials used, including bags, containers, and trays, are compatible with autoclaving. Some bags can impede steam penetration while others may melt during the cycle.
5. Avoid overloading containers and bags (they should never be more than 3/4 full).
6. Autoclave bags should be closed loosely to allow adequate steam penetration.
7. Loosen the caps of liquid containers to prevent bottles from shattering during pressurization. This should be done immediately prior to loading in order to minimize the risk of exposure or contamination if the container is tipped. Vented caps may be a suitable alternative.

15.4.1.2 Loading the Autoclave

1. Load autoclave according to the manufacturer's recommendations.
2. Arrange containers, bags, and trays in a manner that allows steam to circulate freely around all items. Avoid stacking or crowding items.
3. Consider placing containers and bags in trays with a solid bottom and walls to contain spills.
4. Avoid placing individual containers on the floor of the autoclave.
5. Make sure the door of the autoclave is fully closed (i.e., latched) and that the correct cycle has been selected.

15.4.1.3 Unloading the Autoclave

1. Verify the autoclave cycle log to ensure decontamination parameters have been achieved.
2. Visually check the pressure gauge to ensure that the pressure has decreased inside the chamber.
3. Don PPE, including eye protection, heat resistant long cuff gloves, rubber apron, rubber sleeve protectors, and, when handling sharps, cut resistant gloves.
4. Materials removed from the autoclave after effective (i.e., verified) decontamination should be placed in disposal bags that clearly indicate that the waste has been decontaminated, and any biohazard symbols removed or defaced.

15.4.1.4 Verifying the Autoclave Run

1. After decontaminated material has been removed from the autoclave, and prior to disposal, it is important to verify that the run has been effective (i.e., that all validated parameters have been reached). Parametric monitoring devices, chemical indicators and integrators, and biological indicators can be used for routine monitoring of the decontamination process.
2. Remove the indicator or integrator from the autoclaved material and visually inspect. Chemical indicators and integrators provide immediate information on the parameters to which they react. If it was required to also include biological indicator, the material cannot be released for disposal or reuse until the results of the biological indicator are known.
3. Biological indicators require incubation for a pre-determined period of time before reading.

15.4.2 Recommended Procedures for Efficacy Monitoring of Autoclaves

Recommended procedures for efficacy monitoring (i.e., verification) of autoclave cycles at 121°C are described below, and should be performed at a frequency determined through an LRA, taking into consideration the frequency of use of the autoclave. For extended autoclave cycles up to 134°C, chemical integrators or independent temperature monitoring devices (e.g., thermocouples) may be used for verification.

1. Place biological indicators (e.g., ampoules containing 10^4-10^6 colony forming units [cfu] of *Geobacillus stearothermophilus* spores) in the centre of the load (i.e., the most difficult areas of the load to decontaminate). Different load types (e.g., reusable PPE, solid waste, liquid waste) should be tested separately.
2. Leave a positive control biological indicator of the same lot number outside of the autoclave.

3. Process the load according to the applicable SOPs, taking into account the lag time required for the temperature at the centre of the load to reach the sterilization temperature; this time will vary depending on the nature of the waste to be sterilized. For example, *G. stearothermophilus* spores exposed to 121°C are killed in 15 minutes; however, the total cycle time and the temperature required will depend on the contents of the load.
4. Retrieve the biological indicators after completion of the cycle.
5. Do not dispose of waste until it has been confirmed that it has been effectively decontaminated (i.e., absence of growth in the autoclaved biological indicators).
6. Incubate the biological indicators, including the positive control, for the appropriate amount of time and examine for growth. Growth in the autoclaved biological indicators indicates sterilization failure. The absence of growth indicates that sterilization, and hence a reduction equivalent to the initial concentration in the positive control of *G. stearothermophilus* spores, was achieved. Rapid readout biological indicators containing *G. stearothermophilus* spores can also be used. After incubating for 1-3 hours at 56°C in a fluorometer, the illumination of a red light indicates fluorescence and sterilization failure, whereas the illumination of a green light indicates non-fluorescence and successful sterilization.
7. Failure to achieve sterilization may be due to insufficient sterilization time, pressure, or temperature due to user error (e.g., use of wrong cycle program), instrument failure, improper loading, or overloading of the autoclave (i.e., the centre of the load failed to reach and maintain the temperature required for sterilization). Should this occur, the failure should be investigated and corrected. If the cause is found to be instrument failure, the autoclave will have to be repaired and the process re-validated before repeating. Once corrected, the waste is re-autoclaved (i.e., the autoclave process is repeated) prior to disposal.

15.5 Gaseous Decontamination

Gaseous decontamination is generally used in high containment zones under particular circumstances (e.g., after a spill or accidental release of infectious material or toxins, before the removal of large equipment, before maintenance work on contaminated systems, before retesting heating, ventilation, and air conditioning [HVAC] control systems). Gaseous decontamination of rooms usually requires the use of hazardous chemicals (e.g., formaldehyde, vaporized hydrogen peroxide [VHP], chlorine dioxide [ClO_2], ethylene oxide). For this reason, it is important that gaseous decontamination be performed by personnel who have been trained in the procedure and the use of appropriate PPE, including respiratory protection. The two-person rule (also commonly known as a "buddy system"), where two authorized and trained individuals are present at all times, applies to this procedure. It is recommended that, prior to gaseous decontamination, the room or laboratory is leak tested with a tracer gas, such as mint, in order to identify and mitigate leaks.

Formaldehyde gas is a colourless, corrosive, flammable gas that acts as an alkylating agent, binding to specific sites on proteins, RNA, or DNA; it is generated by the depolymerization of paraformaldehyde, and in the presence of water vapour used effectively as a decontaminant.[15] The typical protocol for decontamination using this bactericide involves a 12 hour exposure (6 hour exposure for BSCs) at a relative humidity of 60-90% and a temperature between 15°C and 32°C. This ensures a survival rate of less than one bacterial spore in a million for bacterial spores known to be most resistant to formaldehyde gas. Formaldehyde gas can be neutralized by ammonia gas, which is generated by the thermal decomposition of ammonium bicarbonate or ammonium carbonate.

VHP is an oxidizing agent that is effective against many different types of pathogens, including bacterial spores. It has been proposed as a safer alternative to gaseous decontamination with formaldehyde.[2] This decontamination method does not generate harmful by-products since VHP is broken down into non-toxic oxygen and water. VHP is compatible with a broad range of materials and finishes; however, it has been shown to be incompatible with some materials such as natural rubbers and some plastics and paints. Recent advances in VHP technology have permitted the decontamination of increasingly larger spaces, from small **pass-through chambers** to areas up to 280 m^3 (10, 000 cubic feet) and beyond.

Dry fog is not gaseous, but rather consists of ultrafine droplets of peracetic acid and hydrogen peroxide (H_2O_2). It is a powerful oxidizer that breaks down organic components of microorganisms, destroying their structure. As with VHP, dry fog also breaks down to harmless components and leaves no residue. It is considered compatible with most materials, including electronics; however, as the fog consists of particles (about 7.5μm), it does not penetrate materials and is not effective for the decontamination of **high efficiency particulate air (HEPA) filters**.

ClO_2 is a selective oxidant that reacts primarily with organic compounds that are highly reduced (e.g., alcohols, aldehydes, ketones, tertiary amines, and sulphur-containing amino acids). ClO_2 displays broad-spectrum bactericidal, fungicidal, and virucidal activity, and is effective against bacterial spores.[16] Unlike vapours, ClO_2 is a true gas at standard room temperatures and is, therefore, not affected by temperature gradients that can cause condensation and concentration inconsistencies. ClO_2 demonstrates superior distribution compared to VHP, and as a selective oxidant, it is compatible with many standard materials, including paper, plastic, stainless steel, polyvinyl chloride (PVC), anodized aluminum, and wood.[16,17]

Pre-cleaning of all surfaces to remove superficial organic matter and dirt prior to performing gaseous decontamination allows the gas to effectively contact all surfaces. H_2O_2 and dry fog decontamination in particular require a clean surface since they do not have any penetrating power. Placing biological indicators in various locations including areas difficult for gas to reach or penetrate (e.g., corners, drawers, crevices) provides a means to evaluate the effectiveness of the gaseous decontamination process. Chemical indicators can be used in conjunction with biological indicators to provide an immediate confirmation

that the gas has reached all areas targeted, but the area is not considered decontaminated until the results of the biological indicators are known. *Geobacillus stearothermophilus* is the preferred biological indicator organism for testing the efficacy of formaldehyde, VHP, and ClO_2. The target value for decontamination within the room spaces and BSCs is a 6-$\log_{10}$ (i.e., 99.9999%) reduction of viable spores.[18,19,20]

15.6 Effluent Decontamination Systems

Liquid waste treatment systems are designed to prevent the release of untreated materials into sanitary sewers, and ultimately, the environment. The requirements relating to effluent decontamination systems are listed in Matrix 3.8 of the CBS. Effluent decontamination systems may also be a design consideration for other containment zones depending on the activities planned and the pathogens being handled (e.g., **large scale production areas**). Where an effluent decontamination system is present, it usually serves as the **primary decontamination technology** to treat all liquid waste from sources within or serving the containment zone, including sinks, showers, toilets, autoclaves, washing machines, and floor drains. In some areas, liquid waste may be treated with a validated decontamination process or procedure prior to disposal to the effluent decontamination system; in this case, the effluent decontamination system acts as a secondary **decontamination technology** (i.e., backup system). Effluent decontamination systems are commonly heat-based; however, a chemical-based system may be practical on a smaller scale where small volumes of liquid effluent require treatment.

In traditional effluent decontamination systems, liquid waste is collected in a large tank. When the tank is full, the liquid is heated or chemically treated and, after allowing sufficient time to complete the decontamination, the tank is drained into the sanitary sewer. A uniform temperature or chemical concentration in a large tank can be a challenge to achieve, which can lead to inadequate decontamination. To mitigate this risk, some systems include features to help achieve and maintain a uniform temperature, such as paddles to ensure constant mixing of the effluent, or steam jackets that surround the effluent vessel shell. Continuous effluent decontamination systems have also been recently introduced. In this type of system, the effluent is collected in a large tank and continuously streamed through a retention pipe where the decontamination process takes place. As it flows through the retention pipe at a pre-determined rate, the effluent is treated to a specific parameter (e.g., heat, chemical) for a specific period of time to achieve effective decontamination.

The decontamination parameters (e.g., time, temperature, chemical concentration) of the effluent decontamination system are validated to confirm they will be effective against the infectious material or toxins of concern. Parameters such as internal temperature and pressure of the effluent and the decontamination time are recorded throughout the cycle to evaluate the effectiveness of the process. Alarms are connected to the system to permit the timely detection of a failure. A "fail-safe" configuration will help to prevent untreated waste from leaving the system in the event of a malfunction. Performance verification of effluent decontamination systems should include a brief description of the run criteria for the specific pathogen(s) in use, the procedures for microbiological challenge and verification, trending charts, digital printouts, and other data, as necessary.

The quality of the treated liquid waste released from the effluent decontamination system has to meet the standards specified in applicable environmental regulations and bylaws (provisions related to temperature, chemical and metal content, suspended solids, oil and grease, and biochemical oxygen demand). For example, when chemical residues (e.g., chlorine and ozone) are not neutralized prior to release, they can generate noxious fumes and waterborne residues or by-products (e.g., bromine in salt water). This can be harmful to aquatic animals, and to humans if inhaled, absorbed, or ingested. With other types of treatment, such as heat, post-treatment cooling of the decontaminated waste may be required before discharge into municipal drains or waterways.

Although not specifically aimed at containment zone waste decontamination, the principles and physical/chemical processes described in the *Manual of Diagnostic Tests for Aquatic Animals* published by the World Organisation for Animal Health (OIE; Office International des Épizooties) may be applicable to the design of a waste treatment system.[21] Please refer to the OIE website for more information (http://www.oie.int).

15.7 Irradiation

Gamma irradiation (e.g., Cobalt-60) can be used for the decontamination of heat-sensitive materials and is effective at decontaminating the chemicals and solvents that may be used in higher containment zones; however, it may not be capable of effectively decontaminating certain pathogens (e.g., bacterial spores).[22, 23] The efficacy of this process is dependent on the penetration of the materials by gamma irradiation, which is a function of the density of the treated substance and the strength of the irradiation source.

Microwave irradiation is not widely used as a means of decontamination in containment zones. Similar to autoclaving, this process is based on the use of heat to eliminate viable microorganisms and, for this reason, autoclaving is usually the technology of choice. The efficacy of microwave irradiation is dependent on the wavelength of the irradiation, the duration of exposure, and the moisture content of the material to be decontaminated.

Ultraviolet (UV) irradiation should never be used as the sole means of decontamination in containment zones. Since UV irradiation lacks penetrating capacity, it is only effective in reducing airborne and surface contamination. If UV irradiation is being used in conjunction with other decontamination processes, UV lights should be maintained (e.g., properly cleaned) and periodically verified for function (e.g., emitting appropriate intensity of light).

Validation methods for irradiation sterilization require the use of biological indicators such as *Bacillus pumilus* spore strips.[24] Several spore strips should be distributed throughout the sample being sterilized or the sterilization chamber, depending on the type of validation. A positive control (i.e., unprocessed spore strip) should be used along with the testing strips.

15.8 Incineration

Effective incineration depends on proper equipment design, time, temperature, turbulence, and air required for complete oxidation, as well as careful loading of the unit. Incinerators with a single combustion chamber are generally not effective for the disposal of animal carcasses and plastics since these materials may not be completely destroyed.[25] Modern incinerators that have two chambers, with an ideal temperature of at least 800°C in the primary chamber and at least 1000°C in the secondary chamber, may be effective. Loads with high moisture content may lower the processing temperature, and sawdust may be added to enhance stability.[26] There are no microbial standards for stack emissions, but there are for emission of particulate matter and selected chemical contaminants.[27] Provincial or territorial regulatory authorities should be consulted for additional requirements related to incinerator operations and emissions.

Autoclaving is the preferred method to decontaminate materials, equipment, and waste at the containment barrier prior to its removal from high containment zones for transporting to an incinerator. Material to be incinerated should be packaged in leak-proof plastic bags, even if previously decontaminated. Off-site **transportation** of this material is subject to provincial or territorial legislation. It is important that written protocols for the packaging, labelling, storage, and transportation of waste materials destined for the incinerator be developed and followed by all personnel.

Effective waste management training for containment zone personnel will ensure that they are aware of the types of materials that may be incinerated. The effective operation of the incinerator is highly dependent on the material and the volume of material that is being incinerated. Additional training for personnel responsible for loading, operating, and cleaning the incinerator is needed to ensure that protocols are understood and adhered to, and PPE, such as a respirator for cleaning out ashes and a harness for loading, is used properly. Generally, ash generated by incinerators can be handled as normal waste.

15.9 Dunk Tanks

Dunk tanks are located on the containment barrier to allow for the safe removal of material and samples from the containment zone via surface decontamination. It is critical to use a disinfectant that is effective against the infectious material or toxins in use, and to use the appropriate concentration of disinfectant with sufficient contact time to effectively surface decontaminate vessels that are immersed in the dunk tank. The dunk tank should be inspected regularly as many disinfectants can be corrosive and degrade the dunk tank surfaces. Using a dunk tank with a lining that protects against corrosion may be preferable.

It is important that an adequate volume of disinfectant is maintained in the tank; regular inspections of the disinfectant level will help to identity when additional disinfectant is needed. Visual or audible alarms included on dunk tanks to signal low level of disinfectant require routine testing to verify that they function as intended (CBS Matrix 5.1). Disinfectants have varying shelf lives and the dunk tank solution is to be replaced or replenished as necessary in order to maintain the required disinfectant concentration.

15.10 Animal Carcasses and Anatomical Waste

Animal waste, discarded surgery and necropsy tissues, and whole carcasses can be decontaminated by heat or chemical means. In general, specimens and tissues can be autoclaved effectively, as described in Section 15.4 of this chapter. Whole infected carcasses may require rendering at high temperature, incineration, or chemical decontamination (e.g., alkaline hydrolysis). A modified rendering process has been shown to be an effective alternative and has been successfully used to decontaminate infected animal carcasses.[28]

Alkaline hydrolysis is a process by which animal carcasses and tissues are subjected to a strong alkali, high temperature, and high pressure. A tissue digester would be one example of a decontamination technology that relies on an alkaline hydrolysis process. In general, this method involves a temperature of 150°C and a pressure of 483 kPa (70 PSI), with a total process time of 3-8 hours; however, the exact temperature and time required to achieve effective decontamination is dependent on factors such as the pathogen of concern, and the size/amount of the carcass/tissue to be decontaminated.[29] The final products of this process are amino acids, peptides, sugars, nutrients, soap, bones and teeth.

Rendering is a process by which animal carcasses and tissues are subjected to high temperature and pressure. The pressure vessel, typically designed with a shaft and paddles, uses steam to sterilize and render the waste non-infectious. When properly processed, the final product is somewhat dry and can be sent for disposal (i.e., to landfill). Rendering is most often used for larger animal carcasses and tissues.

Composting is a naturally occurring process that involves the aerobic decomposition of tissue by bacteria and fungi, and may be used to dispose of animal carcasses and anatomical waste. When proper techniques are employed, composting is a well-established pathogen reduction technology that has demonstrated the ability to reduce nearly all pathogenic viruses, bacteria, fungi, protozoa (including cysts), and helminths ova to acceptably low levels.[30] With the exceptions of endospore-forming bacteria (e.g., *Bacillus cereus*) and prions, composting of waste from CL2 LA zones (i.e., CL2-Ag) and from animals in **confinement** may be an acceptable method to decontaminate waste, provided that the method was appropriately validated. Composting procedures should be developed and followed in accordance with applicable provincial, territorial, and municipal legislation, and may not be an option in all jurisdictions.

15.11 Thermal and Chemical Decontamination of Biological Toxins

Given the wide variety of biological toxins and the considerable differences in their physical properties, it is impossible to provide a standardized set of thermal or chemical decontamination parameters that apply to all circumstances. It is the responsibility of the facility where the toxins are handled or stored to ascertain the risks and determine how best to mitigate them, including appropriate and effective inactivation methods.

In an effort to provide a general recommendation for toxin decontamination, more stringent times, temperatures, and concentrations have been outlined below that are considered to be effective against most toxins; however, exceptions to these recommendations do exist and are discussed accordingly.

15.11.1 Thermal Decontamination

Moist-heat (i.e., autoclaving) methods of inactivation with temperatures in excess of 121°C for 60 minutes will permit adequate inactivation of most biological toxins, including proteinaceous bacterial toxins; however, this approach is not suitable for the inactivation of low-molecular-weight, heat-stable toxins, including anthrax toxins, perfringolysin O, and mycotoxins.[31] Dry-heat methods (e.g., incineration), at sustained temperatures of at least 815°C for 10 minutes, are effective for the inactivation of most biological toxins.[31,32] For heat-stable toxins, effective chemical decontamination methods should be applied.

15.11.2 Chemical Decontamination

A solution containing 2.5% NaOCl and 0.25N NaOH with a contact time of at least 30 minutes will permit adequate inactivation of most biological toxins, including peptide toxins and mycotoxins.32 In addition, select toxins are susceptible to other chemicals such as formaldehyde, glutaraldehyde, and ethanol.

15.11.3 Decontamination Parameters

Examples of thermal or chemical inactivation methods for certain toxins are provided below:

- Toxins A and B (*Clostridium difficile*) are susceptible to treatment with 2% glutaraldehyde.[33]
- Listeriolysin O (*Listeria monocytogenes*) is inactivated by heating at 80°C for 3 minutes.[34]
- *Pasteurella multocida* toxins are inactivated by heating at 56°C for 30 minutes.[35]
- Items grossly contaminated with mycotoxins require treatment with a solution containing 2.5% NaOCl and 0.25N NaOH for 2-8 hours.[31,32]
- The treatment of aflatoxin B1 (derived from *Aspergillus flavus* and *Aspergillus parasiticus*) with NaOCl can lead to the formation of a potent carcinogen and mutagen. To prevent this, the treatment solution should be diluted such that the final concentration of NaOCl is between 1 and 5%, followed by the addition of acetone to a final concentration of 5% (vol/vol). [31,32,36]

15.12 Additional Considerations for Prion Decontamination

Prions are resistant to normal decontamination procedures and processes, including moist heat from conventional autoclaving, irradiation, and chemical inactivation (e.g., formalin, alcohols). These processes can marginally reduce infectivity, but few (e.g., incineration, repeated alkali autoclaving at pH 13) are highly effective at eliminating infectious prions.[37] Combinations of physical and chemical treatment processes are recommended to decontaminate equipment, reusable materials, and waste products that are not suitable for incineration, as they can achieve greater prion inactivation efficacy than treatment with chemical agents alone. Autoclaves can be used as a single-step or a two-step method of decontamination for containment zones where prions are handled. Autoclaves used as part of a two-step process (i.e., at 121°C) can be validated with biological indicators, while those used as part of a single-step process (i.e., 134°C) are validated using thermocouples/temperature probes to demonstrate that they are operating as specified. The following are considerations for the decontamination of prions:

- For animal carcasses, tissue waste, bedding, excrement, and some disposable laboratory materials: Incineration at 850°C or alkaline hydrolysis using a pressurized vessel at 150°C are acceptable treatment methods that have demonstrated complete inactivation of prions.[5, 38] In general, incineration at 850°C is the recommended method to achieve effective decontamination of all prion disease agents.[39]
- For reusable instruments that are not heat-sensitive: Autoclaving at 134°C for 1 hour (i.e., single-step decontamination process) or a chemical treatment with 1N NaOH or NaOCl followed by autoclaving at 121°C for 1 hour (i.e., two-step process) is acceptable for prion decontamination.[5,9]
- For heat-sensitive reusable instruments and surfaces: 2N NaOH or 2% available chlorine, with a contact time of 1 hour at 20°C, is effective.[5,26,40]
- For wastewater: Decontamination of liquid effluent at 134°C for 1 hour is the indicated method of decontamination for prion decontamination. Effluent decontamination systems should be designed to treat liquid waste at 134°C for 1 hour.[26] Where the animals necropsied are not known to be infected with prions (e.g., veterinary diagnostic facilities), an effluent decontamination system is not required in **post mortem rooms (PM rooms)**. However, in the event that a positive animal is detected, operational procedures should be in place to collect and treat liquid waste. For example, plastic or absorbent pads may be used to contain liquids during necropsy of animals that exhibit signs of neurological disease.
- For HEPA filters: In a BSC, bag-in/bag-out filters are recommended because formaldehyde fumigation is ineffective against prions. It has been demonstrated that VHP provides a significant reduction in infectivity; however, it is necessary to evaluate the efficacy of VHP decontamination, including establishment and validation of VHP concentration and exposure time, prior to implementing VHP as a decontamination method.[41, 42] Decontamination of filters with VHP followed by incineration is considered to be an acceptable option for safe removal and disposal of filters.

There are additional precautions that should be considered when autoclaving chemically treated (e.g., NaOH, NaOCl) waste. This material can be damaging to equipment; therefore, proper containers should be used. In addition, personnel should be cautious when handling hot NaOH (post autoclave) in order to prevent potential exposure to gaseous NaOH.

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CHAPTER 16

WASTE MANAGEMENT

CHAPTER 16 – WASTE MANAGEMENT

Waste management is an integral component of a **biosafety** program, and comprises policies, plans, and procedures to address all aspects of waste management, including **decontamination** and disposal. Waste leaving the **containment zone** may be destined for disposal, **movement** or **transportation** to a designated decontamination area outside of the containment zone, or transported off-site for decontamination via a third-party biohazardous waste disposal **facility** (e.g., incineration, steam **sterilization**). Even if the waste has been thoroughly and effectively decontaminated prior to removal from the containment zone, it may not be acceptable to simply direct it to the normal waste disposal stream for eventual transfer to a local landfill. Depending on the type of waste material, additional waste management considerations or requirements specified by the provincial, territorial, or local (i.e., municipal) authorities may also apply and should be consulted and complied with when establishing and implementing a waste management program. The requirements for waste management are specified in Matrix 4.8 of the *Canadian Biosafety Standard* (CBS), 2nd Edition.[1]

Canada-wide guidelines exist for the management of certain types of waste (e.g., the Canadian Council of Ministers of the Environment [CCME] *Guidelines for the Management of Biomedical Waste in Canada*); however, these are not enforceable unless they are adopted by provincial legislation or municipal by-laws.[2] Local by-laws may be more stringent than the guidelines recommended by CCME. Standards such as Canadian Standards Association (CSA) Standard CSA Z317.10, *Handling of Waste Materials in Health Care Facilities and Veterinary Health Care Facilities*, may also be reviewed and considered when developing and implementing a sound waste management program.[3]

Standard operating procedures (SOPs) for waste disposal are developed to support disposal of solid and liquid hazardous material in a manner that minimizes the **risk** of harm to personnel, the **community**, and the environment. The SOPs describe all aspects of waste disposal, including handling procedures, from the classification and segregation of infectious waste to decontamination method(s), to storage and disposal. Inclusion of waste disposal SOPs in the **Biosafety Manual** enables personnel to consult protocols as needed. Some aspects to consider when developing the waste management SOPs are the quantity and type of waste that will be generated, as well as the availability of decontamination systems. Decontaminating all contaminated or potentially contaminated waste prior to disposal minimizes the risk of introducing the infectious **pathogens** or **toxins** used in the containment zone into the environment. Failure to follow SOPs can result in the unintentional **release** of **infectious material** or toxins from the containment zone, or personnel **exposure**. It is the responsibility of containment zone personnel to ensure that proper procedures are followed and that **containment** is not breached. It is also important to note that containment zone personnel remain accountable for all waste transported off-site for decontamination, until the waste has been effectively decontaminated. Shipping records, **validation** reports, and records of **verification** of decontamination equipment used by third-party waste disposal companies can be maintained to demonstrate compliance with decontamination requirements specified in Matrices 4.8 and 5.1 of the CBS. Accountability considerations are discussed in Chapter 19.

The first step to improving a waste management program is to determine if there is a way to reduce the amount of waste generated. This can be as simple as minimizing the amount of packaging (e.g., cardboard boxes) brought into the containment zone. All manipulations and processes that will generate contaminated waste should be identified, and the waste categorized according to type. Developing specific handling procedures for each type of waste generated in the containment zone supports disposal of all waste materials in a safe manner. The choice of decontamination method is determined by the nature of the infectious material or toxin and the nature of the item being decontaminated. Decontamination methods are discussed in Chapter 15.

16.1 Biomedical Waste

Biomedical waste can be defined as waste generated in human and animal health care facilities, medical or veterinary research and training facilities, clinical testing or research **laboratories**, as well as vaccine production facilities. Biomedical waste is segregated from the general waste stream as it requires decontamination prior to disposal. Most Canadian jurisdictions have prepared or are preparing guidelines or regulations for the management of biomedical waste. The treatment procedures used at each facility are subject to the standards in place for that province or territory. CCME has also developed Canada-wide guidelines for defining, handling, treating, and disposing of biomedical waste: the *Guidelines for the Management of Biomedical Waste in Canada*. The intent of these guidelines is to promote uniform practices and set minimum standards for managing biomedical waste in Canada. Further considerations on the storage and disposal of biomedical waste can be found in Section 16.2.

Waste resulting from normal animal husbandry (e.g., bedding, litter, feed, manure) where the animals are not known to carry a pathogen, and waste that is controlled under the *Health of Animals Act* (HAA), are not considered to be biomedical in nature; however, the same principles of segregation and disposal can be applied.[4] CCME categorizes biomedical waste into five types, described below, which the provinces and territories can use to develop their own provincial/territorial requirements.

16.1.1 Human Anatomical Waste

Human anatomical waste consists of all human tissues, organs, and body parts, excluding hair, nails, and teeth. Even after **disinfection** or decontamination, human anatomical waste is still considered biomedical waste and may require special means of disposal depending on applicable provincial, territorial, and local legislation.

16.1.2 Animal Waste

Animal waste consists of all animal anatomical waste (carcasses, tissues, organs, body parts), bedding contaminated with infectious organisms, blood and blood products, items highly contaminated with blood, and body fluids removed for diagnosis or removed during surgery, treatment, or autopsy. Hair, nails, teeth, hooves, and feathers are not considered animal waste. Even after disinfection or decontamination, animal waste is still considered biomedical waste and may require special means of disposal depending on applicable provincial, territorial, and local legislation.

16.1.3 Microbiology Laboratory Waste

Microbiology laboratory waste consists of **cultures**, stocks, **microorganism** specimens, **prions**, toxins, live or attenuated vaccines, human and animal cell cultures, and any material that has come in contact with one of these. Inactivation of pathogens and toxins prior to disposal is a critical step in preventing release of harmful material into the environment. Microbiology laboratory waste is no longer considered biomedical waste once it has been effectively decontaminated.

16.1.4 Human Blood and Body Fluid Waste

Human blood and body fluid waste consist of all human blood or blood products, all items saturated with blood, any body fluid contaminated with blood, and body fluids removed for diagnosis during surgery, treatment, or autopsy. This does not include urine or feces, as per the CCME guideline. Human blood and body fluid waste is no longer considered biomedical waste once it has been effectively decontaminated.

16.1.5 Sharps Waste

Sharps waste consists of needles, syringes, blades, or glass contaminated with infectious material and capable of causing puncture wounds or cuts. This can include pipettes and pipette tips that have come into contact with infectious material or toxins, unless they have been decontaminated prior to disposal. Using puncture-resistant containers located close to the point of use minimizes the risk of injury during handling. Sharps waste may be reduced by product substitution for some applications. Sharps waste is no longer considered biomedical waste once it has been effectively decontaminated.

16.2 Storage and Disposal of Biomedical Waste

Decontamination of all biomedical waste prior to disposal in the regular waste stream is essential to the protection of public health, animal health, and the environment. It is important to segregate and dispose of biomedical waste near the point that the waste is generated. For example, it is recommended that unbreakable discard containers (e.g., pans, jars) be placed at every workstation to collect microbiological laboratory waste such as contaminated pipette tips. Some types of pathogens, such as prions, are not inactivated by decontamination processes that would be effective against most microorganisms;

therefore, prion-contaminated waste should be segregated from other types of infectious waste. In facilities where multiple types of biomedical waste are generated, colour-coded waste holding bags or containers can be used to differentiate between types of waste.

It is important that the waste container used is suitable for the type of infectious waste generated. Plastic bags, single-use containers (e.g., cardboard), or reusable containers have different applications. Human anatomical waste, blood and body fluids, and animal waste should be placed in impervious, leak- and tear-resistant waste bags. Removing sharp objects (e.g., needles, capillary tubes, pipette tips) from tissues before placing the tissues in waste bags will prevent the bags from being perforated. Waste bags should be sealed, placed in leak-proof containers, and stored in a freezer, refrigerator, or cold room to await decontamination. Reusable containers may be used, provided that they are decontaminated and cleaned after every use. Sharps waste is disposed of directly into a puncture-resistant container in accordance with National Standard of Canada (CAN)/CSA Standard CAN/CSA Z316.6, *Sharps Injury Protection - Requirements and Test Methods - Sharps Containers*.[5] Broken glassware should never be handled with gloved or bare hands. Forceps, tongs or a dustpan should be used to pick up broken glassware and a wet paper towel held in tongs should be used to pick up tiny glass particles.

If waste is not decontaminated and disposed of immediately, it may be stored temporarily provided that it is in a designated area that is separate from other storage areas and clearly marked with a biohazard symbol. Some types of waste (e.g., human anatomical waste, animal waste) need to be stored in a refrigerated area to prevent putrefaction. Once materials have been decontaminated on-site, the biohazard symbol on the receptacle is removed or defaced to indicate that the infectious material has been inactivated. Decontaminated material may be disposed of as regular waste in areas of heavy traffic or public areas, provided that the facility has specific labelling procedures in place. In other cases, it may be necessary to transport waste off-site for decontamination and disposal. Whether the waste will be decontaminated on-site or off-site, placing waste in appropriate disposal containers promptly and labelling the containers accordingly will keep all infectious waste segregated from regular waste until decontamination and disposal.

Limiting the movement of waste disposal containers to the point of use in the work area, storage (e.g., dedicated area, cold room) or disposal areas, and connecting corridors, will help minimize the risk of release of pathogens and toxins, and personnel exposure. More information on the movement and transport of **biological material** can be found in Chapter 20.

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CHAPTER 17

EMERGENCY RESPONSE PLAN

It is critical that all **containment zones** address situations where **biosafety** or **biosecurity** issues may arise as a result of an emergency. Emergency situations may include **incidents** or **accidents**, medical emergencies, fire, spills (e.g., chemical, biological, radiological), power failure, animal escape, discrepancy or violation of an **inventory** of **pathogens** or **toxins**, failure of **primary containment devices** (e.g., **biological safety cabinet [BSC]**), loss of containment (e.g., heating, ventilation, and air conditioning [HVAC] system failure), or natural disasters. The **emergency response plan (ERP)**, based on an **overarching risk assessment**, describes the procedures relevant to any emergency situation and is essential to protect lives, property, and the environment. The ERP will identify foreseeable emergency scenarios and describe response measures that are proportional to the scale and nature of the emergency. The plan should take into account the hazards in the geographical area (e.g., severe weather or natural disasters). The ERP may also include contingency plans to continue operations in a safe and secure manner. The minimum requirements for an ERP in regulated containment zones are specified in Matrix 4.9 of the *Canadian Biosafety Standard* (CBS), 2nd Edition.[1] There are many additional resources widely available to assist in the development of an ERP.[2,3,4,5]

17.1 Emergency Response Plan Development

When developing the ERP for containment zones, collaboration with experienced **facility** staff will ensure that the final plan is comprehensive and integrated with facility-wide plans, where appropriate. Personnel involved in ERP development may include facility administrators, scientific directors, principal investigators, **laboratory** personnel, maintenance and engineering support staff, **biological safety officers (BSOs)**, and facility security officials. Coordination with local first responder organizations, including police, fire department, and paramedics, is recommended.

The ERP should be specifically tailored to the organization, facility, and **containment level** and will address the safety of emergency personnel who may enter the containment zone, particularly at **high containment levels**. It may also be advisable to inform emergency personnel of the type of **infectious material** in use within the containment zone. Mitigation strategies to address the biosecurity issues that arise from emergency personnel who may have access to restricted infectious material, toxins, or sensitive information while responding to an emergency should be considered.

The ERP may include, but is not limited to, the following:

- personnel responsible for the development, implementation, and verification of the ERP;
- consultation plan for coordination with local emergency response organizations and local hospitals or health care facilities, as appropriate;
- **risk** assessment tools allowing the identification of emergency scenarios and mitigation strategies;

- emergency exit/evacuation routes, avoiding evacuation through higher containment zones;
- protocols for the safe removal, transport, and treatment of contaminated personnel and materials;
- consideration of emergencies that may take place during and outside of regular working hours;
- emergency access procedures, considering the need to override existing access controls when appropriate, and the need to keep a record of emergency response personnel who enter the containment zone; including any contingency plans or mitigation strategies to maintain biosecurity during these situations;
- contingency plans to be implemented to ensure essential operations continue safely and securely;
- emergency training programs, including education on the safe and effective use of emergency equipment;
- emergency exercise plans, including the type and frequency of exercises to be conducted specific to the facility's risks;
- emergency (i.e., incident/accident) reporting and investigation procedures;
- a description of the type of emergency equipment available in the containment zone (e.g., first aid kits, spill kits, eyewash and shower stations) and directions for proper use; and
- procedures for the notification of key personnel and the appropriate federal regulatory agencies.

17.2 Emergency Response Plan Implementation

Once developed, the ERP or a summary of the plan can be included in a facility's **Biosafety Manual** and communicated to all facility personnel appropriately. Training of personnel on the emergency procedures is essential to make certain that personnel, particularly new personnel, are aware of and familiar with the procedures to follow prior to an actual emergency event. This is to be incorporated into the facility's training program (CBS Matrix 4.3). Annual refresher training for existing personnel is considered appropriate to maintain knowledge of emergency procedures, although more frequent training may be indicated by a risk assessment or **training needs assessment**. Structured and realistic exercises are useful tools to verify that personnel demonstrate knowledge of the ERP, understand its importance, can provide assurance of its effectiveness, and identify any deficiencies or areas for improvement. Biosecurity-specific procedures and scenarios can be included (e.g., response procedures in case of theft or loss of a pathogen or toxin, or theft, loss, or sabotage of containment equipment or systems, or communication arrangements with local law enforcements or authorities). In addition, all aspects of the ERP (e.g., development, implementation, training, exercises) should be thoroughly documented for training purposes and for review during an audit or inspection.

It is always important to revise the ERP and keep it up to date with respect to any changes within the containment zone or the surrounding environment (e.g., use of a new pathogen in the containment zone, a catastrophic weather event). It is the responsibility of the facility to determine the frequency of ERP reviews, assessments, and updates. Following an emergency in which the ERP was activated, it is recommended that the ERP be reviewed to address any newly identified deficiencies.

17.3 Spill Response

Spills are the most common incidents with the potential for **exposure** of personnel to pathogens or toxins, or their **release** from containment. Spills can contaminate surfaces, equipment, samples, and workers. The **decontamination** protocol used depends on where the spill occurred and its size (volume).

When a spill occurs outside a BSC, the potential exists for all those present in the work area to be exposed to infectious **aerosols** or aerosolized toxins. Personal safety is the top priority, but it is also important to prevent the spread of **contamination** outside the immediate area and the containment zone. Having a pre-assembled biological spill kit on hand that contains all items needed to contain and clean up a spill (e.g., gloves, disposable gowns and shoe covers, respirator, effective disinfecting agent, paper towels or spill pillows, dustpan, broom, tongs, **waste** bags, and a waterproof copy of spill clean-up **standard operating procedures [SOPs]**) will facilitate timely and effective spill response. It is important that personnel are adequately trained to follow spill response procedures.

17.3.1 General Spill Clean-Up Procedure

After the risk of injury has been controlled, the following steps are recommended to contain a spill of infectious material and decontaminate the area affected by a spill:[6]

1. Remove any contaminated or potentially contaminated clothing and **personal protective equipment (PPE)**.
2. Contaminated personnel doff their outer layer of PPE and any contaminated or potentially contaminated clothing and follow normal exit procedure, including handwashing. In the case of a large spill, personnel remove the outer layer of protection in proximity to the spill. Depending on a **local risk assessment (LRA)** and SOPs, personnel may proceed to a change room to remove the inner layer of PPE, which is placed into an autoclave bag for decontamination. Personnel proceed to wash any other potentially contaminated parts of their body.
3. Notify all staff in the immediate vicinity that a spill has occurred and to leave the area.

4. Exposed persons should be referred for medical attention. The laboratory supervisor or responsible authority should be informed without delay.
5. Allow **aerosols** to settle (e.g., for 30 minutes) before re-entering the area. If the laboratory does not have a central air exhaust, entry should be delayed (e.g. for 24 hours) to allow sufficient air exchanges to exhaust any aerosols and to allow heavier particles to settle. Signs should be posted indicating that entry is forbidden.
6. Don fresh PPE appropriate to the risk, which may include gloves, protective clothing, face and eye protection, and a respirator.
7. Assemble required clean-up materials (e.g., biological spill kit) and bring them to the site of the spill.
8. Cover the spill with cloth or paper towels to contain it.
9. Pour an appropriate disinfectant (i.e., sufficient concentration, effective against the pathogen(s) spilled, freshly prepared) starting at the outer margin of the spill area, and concentrically working toward the center, over the cloth or paper towels and the immediately surrounding area.
10. After the appropriate contact time (i.e., for the pathogen and disinfectant), clear away the towels and debris. If there is broken glass or other sharps involved, use a dustpan or pieces of stiff cardboard to collect and deposit the material into a puncture resistant container for disposal. Glass fragments should be handled with forceps. Dustpans can be autoclaved or placed in an effective disinfectant.
11. Clean and disinfect the area of the spillage. If necessary, repeat the previous steps.
12. Dispose of contaminated materials in a leak-proof, puncture-resistant waste disposal container.
13. Once the spill clean-up is complete, as per the general spill clean-up procedure, personnel doff contaminated PPE and don clean PPE prior to returning to work in the laboratory.
14. After **disinfection**, inform the appropriate internal authority (e.g., containment zone supervisor, BSO) that the site has been decontaminated.
15. Depending on the nature and size of the spill, a complete room decontamination may be warranted.

17.3.2 Spill Inside a Biological Safety Cabinet

The size of the spill is determined by how far it spreads, and less by its volume. When a small spill occurs inside a BSC, the worker is not considered contaminated unless a splash or spillage has escaped the BSC; however, the gloves and sleeves may be contaminated. A large spill in a BSC may result in material escaping the BSC and the worker becoming contaminated. In this case, the outer layer of PPE is considered potentially contaminated and should be removed at the BSC. The following general procedure is recommended for spills inside a BSC:

1. Remove gloves and discard within the BSC. If two pairs are worn, discard the outermost layer. If sleeves are potentially contaminated, the lab coat or gown should also be removed. Fresh gloves should be donned and if necessary, also a fresh lab coat or gown.
2. Leave the BSC blower on and the sash at the appropriate level.
3. Follow the instructions outlined in Section 17.3.1 for general spill clean-up, keeping head outside the BSC at all times.
4. Surface disinfect all objects before removing them from the BSC, or place them into bags for autoclaving. Remove contaminated gloves and dispose of them inside the cabinet.
5. Place PPE into bags for autoclaving.
6. If material has spilled through the grill of the BSC, pour disinfectant through the grill to flood the catch tray underneath.
7. Wipe all inside surfaces with disinfectant.
8. Raise the work surface, clean the catch tray, and then replace the work surface.
9. Allow BSC to run for at least 10 minutes before resuming work or shutting down.

17.3.3 Spill Inside a Centrifuge

If a breakage occurs or is suspected while a centrifuge is running, the motor should be switched off and the centrifuge left closed (e.g., for 30 minutes) to allow aerosols to settle. Should a breakage be discovered only after the centrifuge has been opened, the lid should be replaced immediately and left closed (e.g., for 30 minutes).

1. Inform the appropriate internal authority (e.g., containment zone supervisor, BSO).
2. Follow the instructions outlined in Section 17.3.1 for general spill clean-up.
3. If possible, use a non-corrosive disinfectant known to be effective against the pathogen concerned. Whenever possible, consult the centrifuge manufacturer's specifications on the unit to confirm the chemical compatibilities.

4. All broken tubes, glass fragments, buckets, trunnions, and the rotor should be placed in a non-corrosive disinfectant (forceps are to be used to handle and retrieve glass and other sharps debris). Unbroken sealed safety cups may be placed in disinfectant and carried to a BSC to be unloaded.
5. The centrifuge bowl should be swabbed with the same disinfectant, at the appropriate dilution, and then swabbed again, washed with water, and dried.

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CHAPTER 18

INCIDENT REPORTING AND INVESTIGATION

CHAPTER 18 – INCIDENT REPORTING AND INVESTIGATION

Though the terms "**incident**" and "**accident**" are often used interchangeably when referring to reporting procedures, a distinction should be made between the two words. An accident is an unplanned event that results in injury, harm, or damage. An incident is an event with the potential to cause injury, harm, or damage. Incidents include accidents, as well as near misses and other dangerous occurrences. In the *Canadian Biosafety Standard* (CBS), 2nd Edition, as well as in this volume, the term "incident" refers to all possible occurrences, including accidents, **exposures** (that may cause **disease**), **laboratory acquired infections/ intoxications (LAIs)**, **containment** failures, environmental **releases** (e.g., improperly treated **waste** sent to the sewer system), and **biosecurity** breaches (e.g., theft or intentional misuse of an **infectious material** or **toxin**) (illustrated in Figure 18-1).[1] All incidents, even those seemingly minor, should set in motion the **facility**'s internal incident reporting procedures as described in the **Biosafety Manual** and other appropriate protocols (e.g., incident investigation and documentation). The minimum requirements for incident investigation and reporting in regulated **containment zones** are specified in Matrix 4.9 of the CBS.

Protocols for incident reporting and investigation are an integral component of a facility's **emergency response plan (ERP)**. Incidents need to be properly reported, documented, and investigated in order to learn from these events and to correct or address any problems or issues that may have caused the incident and prevent a recurrence, and to notify external authorities (i.e., the Public Health Agency of Canada [PHAC] or the Canadian Food Inspection Agency [CFIA]) when necessary. Exposures in a containment zone can occur via inhalation (e.g., breathing in infectious **aerosols** or aerosolized toxins), ingestion (e.g., contact of mouth with contaminated hands or materials), percutaneous inoculation (e.g., subcutaneous contamination by puncture, needlestick, or bite), or through absorption (e.g., entry through direct skin, eye, or mucous membrane contact). Incidents may be indicative of failures in **containment systems**, **biosafety**-related **standard operational procedures (SOPs)**, training programs, or biosecurity systems; subsequent investigation enables containment zone personnel to identify these failures and take corrective action. Reporting and investigation procedures should be developed to complement or integrate with existing facility-wide programs (e.g., occupational health and safety). The ERP is discussed in more detail in Chapter 17.

Several standards are currently available to assist facilities in the development of incident reporting and investigation procedures. They include, but are not limited to, the British Standards Institution Occupational Health and Safety Assessment Series (OHSAS) 18001, *Occupational Health and Safety Management Systems;* the National Standard of Canada/ Canadian Standards Association (*CAN/CSA) CAN/CSA* Z1000, *Occupational Health and Safety Management*; and CAN/CSA Z796, *Accident Information*.[2,3,4] The minimum requirements for incident reporting and investigation in regulated containment zones are specified in Matrix 4.9 of the CBS. Record retention requirements for incidents are specified in Matrix 4.10 of the CBS.

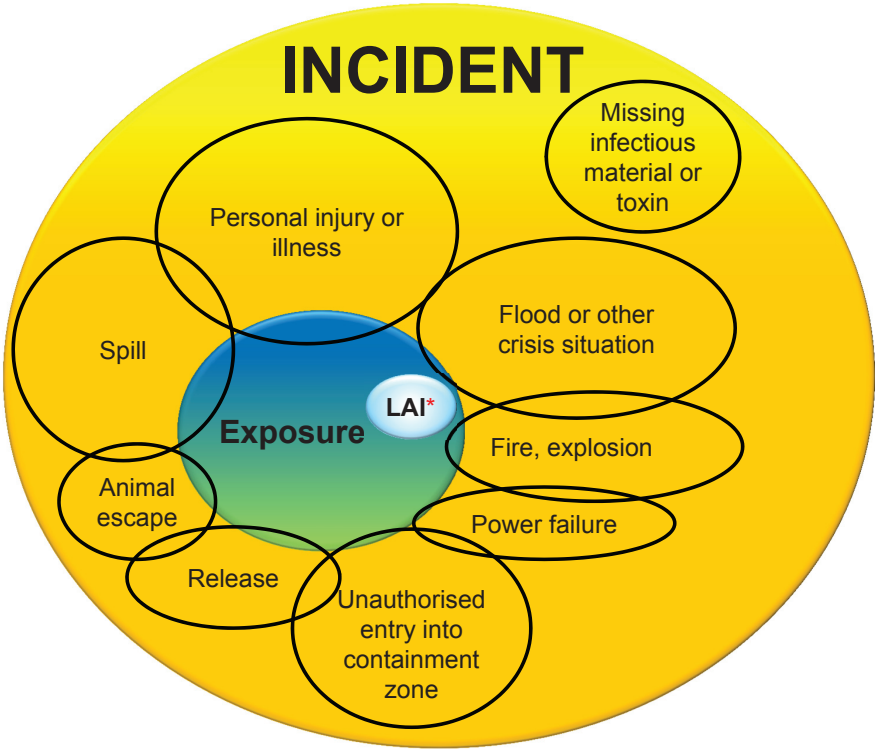

Figure 18–1:
Visual Representation of Incidents Involving Pathogens and Toxins, including Exposures and Laboratory Acquired Infections/Intoxications (LAIs).

18.1 Incident Reporting

All incidents involving infectious material, infected animals, or toxins, such as a containment systems failure, an exposure to a human **pathogen** or **toxin**, or release of an **animal pathogen**, must be reported immediately to the appropriate facility personnel (e.g., containment zone supervisor, **biological safety officer [BSO]**, **licence** holder in licensed facilities) (CBS Matrix 4.2). Moreover, all persons working under the authority of a licence are legally obligated to notify the appropriate facility personnel if they have reason to believe that an incident has occurred involving inadvertent release, inadvertent production, disease (i.e., any exposure incident), or a missing human pathogen or toxin (HPTA 15). It is important to promptly report incidents internally, within the facility, so that management (including the licence holder in licensed facilities) is made aware of the events, can implement immediate hazard reduction and mitigation strategies, and initiate a preliminary assessment to determine if an exposure has likely occurred (Figure 18-2 illustrates a decision chart to assist in the exposure assessment). As well, internal reporting initiates the facility's process to investigate the incident, determine the root cause(s), plan corrective actions, document outcomes (i.e., injury to personnel), and determine legislated notification requirements to regulatory authorities. In the event of certain types of incidents involving pathogens, infectious material, and toxins, personnel in facilities that are regulated by the PHAC and the CFIA are likewise obligated to notify or report to the appropriate regulatory authority. Regulated facilities are also required to develop and maintain documented procedures to define, record, and report incidents involving infectious material or toxins (CBS Matrix 4.1). These procedures should also comply with any additional applicable federal, provincial or territorial, and municipal regulations, as well as the organization's internal incident reporting and investigation requirements.

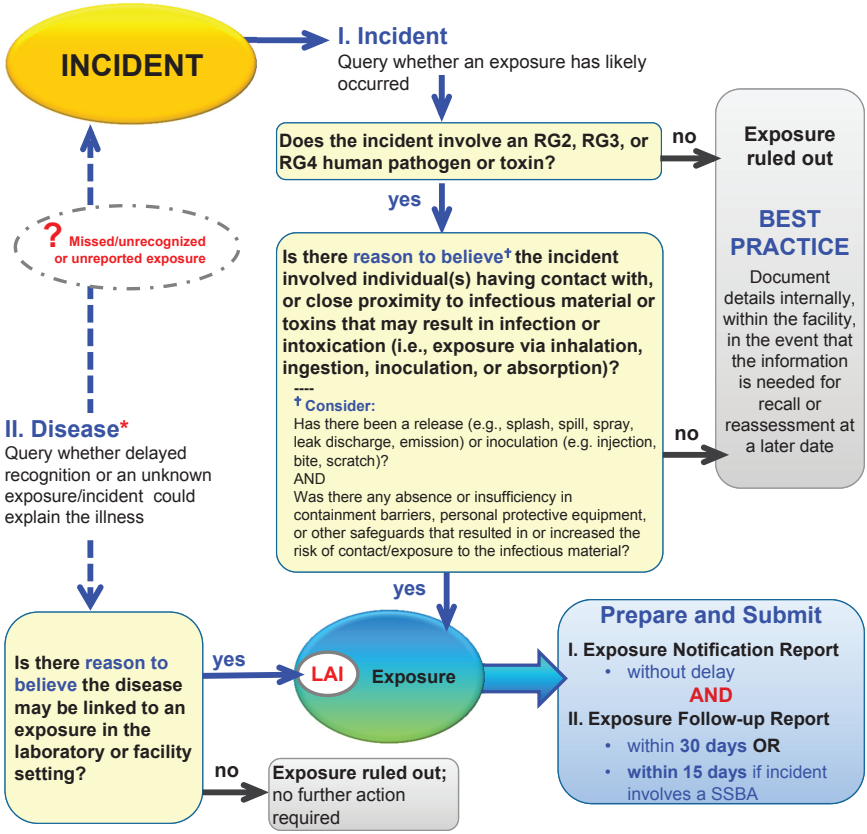

Figure 18–2:
Decision Chart to Assist in the Assessment of an Incident to Determine if an Exposure has Occurred and if Notification of the Public Health Agency of Canada (PHAC) is Required.

* In most cases disease state will include recognition of an illness, syndrome, or known disease; however, some facilities may employ medical surveillance practices that could identify a seroconversion, which may provide an additional source of information for recognition of infection or disease states.

18.1.1 Incident Reporting to the Public Health Agency of Canada

Facilities that hold a licence to conduct **controlled activities** with human pathogens and toxins are obligated to notify the PHAC in the event of incidents and exposures in accordance with the *Human Pathogens and Toxins Act* (HPTA), and *Human Pathogens and Toxins Regulations* (HPTR), as previously described.[5,6] The functions of the BSO include communicating with the PHAC on behalf of the licence holder (HPTR 9[1]), which includes the required reporting of incidents. In accordance with the HPTA and HPTR, a licence holder is obligated to notify the PHAC without delay in the following scenarios:

- when a licence holder has reason to believe that a human pathogen or toxin has been released inadvertently from a facility (HPTA 12[1]);
- when a human pathogen or toxin that a person is not authorized to possess is inadvertently produced or otherwise comes into their possession (HPTA 12[2]);
- when a **security sensitive biological agent (SSBA)** is not received within 24 hours of the date and time when it was expected to be received (HPTR 9[1]);
- when there is reason to believe that a human pathogen or toxin has been stolen or is otherwise missing (HPTA 14);
- when an incident involving a human pathogen or toxin has caused, or may have caused, disease in an individual (i.e., any exposure incident) (HPTA 13); and
- as specified by any additional licence condition set out on the licence itself that describes an incident scenario that requires notification of the PHAC (HPTA 18[4]).

18.1.1.1 Notification of Exposures Involving Human Pathogens and Toxins

Infections that result from an exposure to pathogens or infectious material being handled in the containment zone are referred to as LAIs; this term also includes a disease caused by exposure to a toxin (i.e., **intoxication**) that is being handled in the containment zone. Exposure incidents are more encompassing, and include any incident involving a pathogen or toxin where infection or intoxication is likely to have occurred, and thereby a potential for disease (whether or not overt disease actually does develop). Under the HPTA Section 13, any incident resulting in an LAI (i.e., recognized disease) or an exposure (i.e., probable inhalation, ingestion, inoculation, or absorption) involving a **Risk Group** 2 (RG2), Risk Group 3 (RG3), or Risk Group 4 (RG4) human pathogen or toxin that occurs in a licensed facility must be reported to the PHAC without delay (i.e., as soon as the situation is under control and sufficient information has been gathered to report the preliminary details of the incident). This information can be submitted to the PHAC electronically through the Biosecurity Portal, accessible through the PHAC website (www.publichealth.gc.ca/pathogens), in an **exposure notification report**. This initial notification of the exposure incident captures brief information to describe the incident, the name of the pathogen or toxin involved, and other preliminary information relating to the incident, such as immediate mitigation measures and current status of the affected individual(s), if known. Personal identification of the affected individual(s) is not normally required, but may be requested in exceptional circumstances

(e.g., to protect public health). An **exposure follow-up report** documenting the outcomes of the incident investigation is to be submitted to the PHAC within 15 or 30 days of the exposure notification report, depending on whether or not SSBAs were involved in the incident. The requirements regarding exposure reporting to the PHAC are specified in Matrix 4.9 of the CBS.

Figure 18-2 illustrates a decision chart to assist in the assessment of an incident to determine if an exposure is to be notified in accordance with HPTA Section 13. There are two scenarios: (I) a recognized incident where assessment is required to determine or rule out possible exposure(s) to human pathogens or toxins among one or more individuals involved in the incident; and, (II) a recognized disease in one or more facility personnel or other individuals (e.g., visitor, student) where assessment is appropriate to determine or rule out a possible missed, unrecognized, or unreported exposure incident that explains the illness.

Reporting to the PHAC is not necessary in the case of an incident in a licensed containment zone wherein the incident investigation and local assessment of facts has determined that exposure (i.e., infection or intoxication) is unlikely to have occurred as a result of the event. Reporting to the PHAC is also not necessary in the case of a recognized disease that is determined not likely to have been caused by an exposure incident in the containment zone. In this latter case, the incident investigation and local assessment of facts has ruled out exposure in the **laboratory** setting and determined that a **community**, travel-related, or other exposure setting is the most likely source of the disease. In addition, exposure incidents and LAIs that occur in containment zones or facilities that are exempt from the licence requirements under the HPTA and HPTR are not obligated to be reported to the PHAC, but may still be reported on a voluntary basis. Please contact the PHAC directly for more information on voluntary reporting of incidents.

18.1.1.2 Exposure Follow-Up Activity

The exposure follow-up report form, available from the PHAC, is an extension of the exposure notification report, wherein the preliminary incident details provided upon notification can be updated, added to and submitted electronically through the Biosecurity Portal in the exposure follow-up report. The design and format of the electronic report is intended to capture the outcomes of the incident investigation process. Ultimately, it serves to document key details on the incident, assist with conducting a root cause analysis, and record the corrective action plan aimed at mitigating the event and reducing the likelihood of a recurrence. An exposure follow-up report detailing the exposure incident investigation is to be submitted to the PHAC within 30 calendar days of submission of the initial exposure notification report for incidents, or within 15 calendar days if the incident involves an SSBA (CBS Matrix 4.9).

The PHAC reviews and analyzes information contained in exposure notification and follow-up reports to monitor for potential patterns, assess the potential significance for public health, and determine if any direct involvement by the PHAC is warranted. This decision may be based on consideration of factors such as risk group(s) of the human pathogens or toxins involved in the incident, the communicability of the human pathogens, the number of

affected individuals, and the likelihood of spread to the community. Where it is deemed appropriate, the PHAC will contact the licence holder promptly to follow up on actions taken. Upon request, the PHAC can also assist with the assessment, investigation, and reporting process by providing guidance, subject matter expertise, or related support.

18.1.1.3 Annual Reporting of Incidents Involving Security Sensitive Biological Agents

As an additional condition of licence, licence holders authorized to work with SSBAs may be required to submit an annual report to the PHAC summarizing all incidents involving SSBAs that occurred during the previous 12 months (or a report indicating that no incidents have occurred). The annual report is to include: a summary of any incidents that have occurred within the past year, the root cause analysis for each incident, a description of any systemic biosafety concerns, and details of the corrective measures that have been implemented to address them. The annual report builds upon the regular incident reporting requirements by providing the PHAC with a better understanding of the frequency and potential causes of incidents involving SSBAs. Since detailed information on any exposure incident involving an SSBA will be reported via the required initial exposure notification report, and the exposure follow-up report submitted 15 calendar days later, this information does not need to be repeated in the SSBA annual report.

18.1.1.4 Exposure Reporting Program

All information on exposure and LAI incidents involving RG2, RG3, and RG4 human pathogens and toxins in licensed containment zones are to be submitted to the PHAC and thereby captured within the PHAC's Exposure Reporting Program database. This allows the PHAC to monitor developing trends, and may prompt the issuance of biosafety advisories as well as contribute to updates of biosafety best practices and training. Under normal circumstances, the PHAC does not gather confidential business or personal information with respect to incidents involving the exposure of an individual to a human pathogen or toxin; however, should such information be required, it is protected in accordance with applicable federal laws.

Information collected from exposure notification and follow-up reports documenting exposures or LAIs involving human pathogens and toxins is analyzed by the PHAC to help shape current and future **biocontainment** and biosafety practices in Canada.

18.1.2 Reporting to the Canadian Food Inspection Agency

The *Health of Animals Act* (HAA) sets out requirements for the notification of the CFIA in the event that an animal is discovered to be infected with pathogens causing, or showing signs of, a reportable disease or toxic substance.[7] The Schedule to the *Reportable Diseases Regulations*, and Schedules VII and VIII of the *Health of Animals Regulations* (HAR) list the reportable and notifiable diseases that affect terrestrial animals.[8,9] This may be of concern in an animal containment zone where an experimental animal is discovered to inadvertently carry or develop clinical signs of one the indicated diseases. Please contact the CFIA directly for more information on reportable and notifiable diseases.

Based on the conditions included on an **animal pathogen import permit**, any incident involving an animal pathogen, toxin, or other regulated infectious material in a facility to which that material was imported or transferred using an animal pathogen import permit or transfer authorization issued by the CFIA, or in a facility that has been certified by the CFIA, may require reporting to the CFIA. The CFIA will review the information surrounding the incident to verify that a release has not occurred and to ensure continued compliance with the CBS. To this end, information on incidents involving animal pathogens received by the PHAC may also be shared with the CFIA. The animal pathogen import permit may include further conditions on reporting to the CFIA. Please refer to the conditions of permit for further information.

18.2 Incident Investigation

Incident investigation is necessary to determine the root cause(s) of the incident, in other words, to identify the most basic or underlying reasons why the incident took place. Equally important, results of a thorough incident investigation, serve to determine and direct appropriate corrective actions to mitigate the current problem(s) as well as prevent similar incidents from occurring in the future. As such, incident investigation provides a crucial feedback mechanism to assist in the improvement of existing incident mitigation and prevention strategies. Incident investigation and reporting procedures to uncover and document these findings may include the following:

- defining potential incidents and the triggers for reporting and investigation;
- identifying personnel roles and responsibilities;
- outlining the reporting chain of command;
- defining the sequence of events and the subsequent root cause(s) that led or contributed to the incident;
- documenting the incident and providing the types and content of incident reports and templates;
- identifying the frequency and distribution of incident reports;
- identifying the corrective actions to prevent a recurrence of the incident;
- identifying opportunities for improvement;
- assessing the effectiveness of the preventive and corrective actions taken; and
- communicating the investigation results and the corrective actions taken to the appropriate parties (e.g., facility personnel, health and safety committee, **senior management**, regulatory authorities, and local law enforcement).

The extent and depth of the incident investigation may vary, depending on the severity of the incident or the level of concern associated with the pathogen or toxin involved (e.g., SSBA). Prior to commencing an investigation, the personnel responsible for this duty should be selected and identified. Depending on the nature and severity of the incident, one individual may be assigned to conduct the investigation or a team may be assembled for more complex scenarios. The investigator(s) should conduct the investigation with an open mind

taking care to exclude any pre-conceived notions and opinions regarding the nature and cause of the incident. Incident investigation procedures should be reviewed and updated regularly so that they are current and effective. The investigation process is systematic and generally includes the stages outlined in the subsections below.

18.2.1 Initial Response

The initial response may include the provision of first aid or emergency services, assessing the severity of the incident (e.g., potential for loss of containment or infection), controlling the extent of the current hazard and preventing a secondary incident, identifying and preserving evidence, and notifying appropriate personnel. At this stage, an assessment is conducted to determine whether or not a pathogen or toxin has been released (e.g., as a result of a spill, splash, leak, spray, discharge, or emission) from a containment system or from the containment zone itself, and if it has, to implement measures to control and contain the released material and prevent any supplementary incidents. Incidents involving infectious materials require immediate reporting to the appropriate facility personnel (e.g., containment zone supervisor, BSO), and, depending on the type of incident, may also require external reporting as well (see Section 18.1). The extent and depth of the incident investigation may vary, depending on the severity of the incident.

18.2.2 Collection of Evidence and Information

The collection of evidence and accurate information is critical to any incident investigation. Photos, sketches, or videos may be used to capture a visual record of the evidence, including its location, and are useful for subsequent analysis. It is important to interview people who may have knowledge of what contributed to the incident and to do this as soon as possible to minimize any recall bias. Additionally, the collection of documentation pertaining to the incident may provide information relevant to the investigation. Documentation can be extensive and may include employee training records, maintenance logs, purchasing standards, SOPs, new employee and visitor orientation policies, and safe work practices.

18.2.3 Analysis and Identification of Root Causes

The analysis of the evidence and information to identify root causes is often achieved through an expanded version of the traditional questions: who, what, when, where, how, and why. Examples of these types of questions include the following:

- Who were the people involved in the incident (e.g., personnel, bystanders)?
- What infectious material or toxin was involved in the incident?
- When and where did the incident take place?
- How did the incident happen (i.e., what factors contributed to the incident)?

In the expanded use of the traditional questions, ask why each event in the incident scenario happened. Putting the "why" question in front of all the questions asked will help determine the basic causal factors and pathways that led to the incident. Through the cascading series of "why" questions, the underlying root cause(s) that led or contributed to the incident can be determined when there are no further answers. Considerations such as purchasing controls, training, and equipment operation should be taken into account when asking the "why" questions. It may also be advisable to question whether or not the incident was isolated or recurring, or accidental or intentional, especially where a breach of biosecurity has occurred (e.g., individual repeatedly removing SSBA samples without updating the sample **inventory**).

18.2.4 Development of Corrective and Preventive Action Plans

Developing corrective and preventive action plans assists in addressing the root causes of various incidents as well as identifying appropriate actions to prevent their recurrence. The investigation may identify biosafety or biosecurity elements that may have failed to prevent the incident and provide insight and opportunities that allow for their correction. Based on the investigation findings, the plans should identify actions to eliminate the immediate hazard (corrective plans) and mitigate the **risk** of the incident recurring (preventive plans). Both plans will also identify the personnel required to implement the actions and provide time frames for completion.

18.2.5 Evaluation and Continual Improvement

Once the corrective and preventive action plans have been implemented, it is important to review their effectiveness and confirm that the identified root causes are being effectively controlled.

The final stage of incident investigation involves ongoing program review to identify opportunities for improvement. This may be accomplished through the review of incident investigation reports, incident trends, and consultation with containment zone personnel or senior management. This should include a review of **local risk assessments** (LRAs) and SOPs to determine if revisions are required as a result of the incident. Where inadequate training was identified as a root cause, training materials should be updated accordingly and refresher training for personnel scheduled.

REFERENCES

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4. *CAN/CSA Z796-98 (R2013), Accident Information*. (1998). Mississauga, ON, Canada: Canadian Standards Association.
5. *Human Pathogens and Toxins Act (S.C. 2009, c.24)*. (2015).
6. *Human Pathogens and Toxins Regulations (SOR/2015-44)* (2015).
7. *Health of Animals Act (S.C. 1990, c. 21)*. (2015).
8. *Reportable Diseases Regulations (SOR/91-2)*. (2014).
9. *Health of Animals Regulations (C.R.C., c. 296)*. (2015).

CHAPTER 19

PATHOGEN AND TOXIN ACCOUNTABILITY AND INVENTORY CONTROL

CHAPTER 19 – PATHOGEN AND TOXIN ACCOUNTABILITY AND INVENTORY CONTROL

Good **biosafety** and **biosecurity** practices include provisions to adequately account for, protect, and safeguard **pathogens** and **toxins** against loss, theft, misuse, diversion, and **release**. This chapter describes measures used to create an environment that protects and prevents against **insider threats** while simultaneously incorporating best practices of material management systems.[1] The pathogens and toxins that should be subject to accountability and control measures can be identified by the **biosecurity risk assessment** (i.e., the **assets**, as described in Chapter 6). Control measures are used to confine the assets to designated locations and to limit their access to authorized individuals using physical means (i.e., **security barriers**) or by operational protocols (e.g., **standard operating procedures [SOPs]**). Accountability measures define the oversight and the responsibility of all authorized individuals for the safekeeping of the assets. The *Canadian Biosafety Standard* (CBS), 2nd Edition, requires that a regulated **containment zone** include pathogen and toxin accountability and inventory control as part of the **facility**'s biosecurity plan (Matrices 4.1 and 4.10 of the CBS).[2] Biosecurity, including the biosecurity risk assessment and biosecurity plan, is discussed in further detail in Chapter 6.

19.1 Pathogen and Toxin Accountability

Accountability measures aim to establish ownership of pathogens and toxins, and to describe the responsibility of each authorized individual.[3] In the context of a biosafety and biosecurity program as specified in the CBS and this volume, pathogen accountability measures can and should include any material that is known to contain a pathogen that necessitates such measures. This includes all material regulated under the *Human Pathogens and Toxins Act* (HPTA), *Human Pathogens and Toxins Regulations* (HPTR), *Health of Animals Act* (HAA), and *Health of Animals Regulation* (HAR), that contains pathogens (i.e., pure or isolated pathogen or toxin; infected or intoxicated animals, animal products or by-products that contain an **animal pathogen** or part of one, or other organism that contains an animal pathogen or part of one that has been imported under an **animal pathogen import permit**; experimentally or otherwise intentionally infected animals or specimens collected from such animals; **cell lines** or cell **cultures** containing a pathogen or part of one that retains **pathogenicity**).[4,5,6,7] Nonetheless, it is generally recommended that the pathogen and toxin accountability measures of a containment zone or facility also include any additional material that is known, presumed, or suspected to be infectious but may not be subject to regulation by the Public Health Agency of Canada (PHAC) and the Canadian Food Inspection Agency (CFIA). Domestically-acquired primary specimens or cell lines that are not well-characterized and have not been assessed for the presence of pathogens should be subject to the same level of accountability and control as a sample of the isolated pathogen to demonstrate due care.

An accountability system establishes a governance structure in an organization whereby relationships between individuals are determined on an account-giving basis. In brief, accountability creates a framework where Person A is accountable to Person B and is obliged to inform Person B of his/her actions and decisions, to justify them, and to be

answerable for these actions and decisions.[8] This type of relationship is present in many hierarchies and organizations, such: as a banking institution (i.e., "Person A") to a client or account holder (i.e., "Person B"); an employee to a manager; or a student to a teacher. In the context of pathogen and toxin accountability, this includes the assignment of qualified, **authorized personnel** to oversee the control of the pathogens, toxins, and regulated **infectious material**, the maintenance of accurate and timely records, and the routine **verification** of materials and records (i.e., auditing). These accountable authorized personnel are answerable for their actions and decisions involving pathogens, toxins, and regulated infectious material to their supervisors, the containment zone or facility directors, the **licence** holder or animal pathogen import permit holder, and may also be accountable to the PHAC and the CFIA.

The CBS specifies the minimum requirements for accountability and control of pathogens, toxins, and other regulated infectious material in regulated containment zones (CBS Matrix 4.10). Depending on the material in **long-term storage**, this can include a means to identify the pathogens and toxins (e.g., the genus, species, strain, where applicable), their **risk group(s)**, and their locations (e.g., room, fridge/freezer, shelf). Given the transitory nature of many **laboratory** specimens, only those in long-term storage, which is defined as storage for greater than 30 calendar days, need to be recorded in an inventory. It is expected that materials in long-term storage are not being actively manipulated (e.g., cryopreserved or otherwise stored for reference or future use). Materials in short-term storage (less than 30 days) will normally be captured elsewhere, such as laboratory notebooks, test requests, and entry logs. Active cultures of pathogens or infectious material that require more than 30 days of incubation (e.g., continuous cell cultures) may not need to be documented in the containment zone's formal inventory, depending on the **risk** associated with the material, and provided that the minimum required information is documented elsewhere (e.g., daily laboratory notebook entries, cell culture logbooks), and is of sufficient detail to determine immediately if a sample is missing or has been stolen.

19.1.1 Internal Accountability System

Development and implementation of an **internal accountability system** in facilities where pathogens, toxins, and regulated infectious material are handled and stored is needed so that all personnel are made aware of their individual responsibilities, the administrative controls in place, and the penalties for not following this system. This may be best achieved through a policy or code of conduct describing the responsibilities of all personnel, such as the biosafety policy described in Chapter 5. This should include a description of the roles and responsibilities of authorized personnel, **senior management**, the institutional biosafety committee, and the **biological safety officer (BSO)**, so that all personnel are made aware of their legal responsibilities and institutional expectations, as well as those of their co-workers. In licensed facilities authorized to conduct **controlled activities** with human pathogens and toxins, the licence holder is identified as the individual with the ultimate legal accountability under the HPTA and HPTR. In facilities that have imported an animal pathogen, the importer is identified as the individual with the ultimate legal accountability under the HAA. The disciplinary actions (i.e., penalties or punishments) for non-compliance with the internal accountability system should be clearly documented and communicated to all personnel.

When developing pathogen and toxin accountability procedures and tools, it is important to consider the material that is present within the containment zone and the associated biosafety and biosecurity risks. This is best determined in consultation with the facility's BSO. The level of accountability and control will be determined by the risk associated with the material. For example, maintaining information on the storage and use of **biological material** in laboratory notebooks may be suitable for low risk biological material and toxins (e.g., Risk Group 1 [RG1]); whereas, high risk pathogens (e.g., Risk Group 3 [RG3] or Risk Group 4 [RG4]) will necessitate detailed inventories of pathogens and toxins in storage, detailed records of **transfers** to other zones or facilities, documented access and authorization protocols, and regular verification of material and records (i.e., checks and audits). It is also encouraged that institutional pathogen and toxin accountability procedures and tools address accountability concerns that may arise in the event that the licence holder or animal pathogen import permit holder terminates their affiliation with the institution (e.g., change of employer, retirement) to ensure that the transfer or disposal of all regulated material is carried out in accordance with all legal requirements.

19.1.2 Accountability Measures during Movement and Transportation

The **movement** and **transportation** of biological material is a routine procedure in laboratories and other containment zones. **Good microbiological laboratory practices** will likely prevent **contamination** and inadvertent spills; nonetheless, accounting and control provisions for the movement or transportation of pathogens and toxins throughout their journey are important factors to reduce the risk of loss or theft by insider or **outsider threats**. For this reason, the biosecurity plan should include provisions and policies for transfer management outlining biosecurity-specific procedures for the shipping, receiving, monitoring, and storage of packages that contain pathogens, toxins, and other regulated infectious material. For example, a documented contingency plan for the receipt and security for unexpected shipments (i.e., parcels of pathogens or toxins that arrive at a facility that had not been requested nor arranged) should be considered. In addition, licensed facilities are required to notify the PHAC in the event that the facility has come into possession of a human pathogen or toxin that has not been authorized by the licence (HPTA 12[2]), when an expected shipment of a human pathogen or toxin is missing and has not been received (HPTA 14), or when a shipment of a **security sensitive biological agent (SSBA)** has not been received within 24 hours of when it was expected (HPTR 9[1][c][iii]). Established written provisions or procedures on how to handle such events will allow the licence holder to track and gain control of the shipment quickly and secure it in an appropriate licensed area without delay. In addition, where a licensed facility is authorized for activities involving SSBAs, all employees in the shipping and receiving areas are required to have a valid *Human Pathogens and Toxins Act* **Security Clearance (HPTA Security Clearance)** issued by the PHAC if they pack or unpack shipments in the shipping and receiving area or if they plan to temporarily store SSBAs (HPTA 33, HPTR 28). Licensed facilities that conduct transfers of human pathogens and toxins under the same licence should include in their biosecurity plan a description of how these transfers will take place, including chain-of-custody documents and provisions for safeguarding the agents against theft, loss, or release. Further information on movement and transportation of infectious material and toxins is discussed in Chapter 20; information on regulatory oversight of pathogens, toxins, and other regulated infectious material, including transfers, is discussed in Chapter 23.

19.2 Inventories and Inventory Control Systems

An inventory is a list of biological assets in possession of a facility, and includes the identification of the pathogens and toxins in long-term storage both inside and outside of the containment zone. Inventories are key records in areas where activities with human and animal pathogens and toxins are conducted that allow authorized personnel who are accountable for the control of this material to prevent it from being misused, misplaced, stolen, or released. Additionally, inventories allow individuals working with human and animal pathogens and toxins to be aware of the nature and scope of the material present so that appropriate precautions are used to prevent personnel **exposure** or release. An inventory control system is a process to manage and locate materials in the facility or containment zone. They are common to any quality assurance program and are required in quality management systems, including the International Organization for Standardization (ISO) standard ISO 9001, ISO 15189, National Standard of Canada (CAN)/ Canadian Standards Association (CSA) standard CAN/CSA Z15190, the Organisation for Economic Co-operation and Development (OECD) *Principles of Good Laboratory Practice*, and the European Committee for Standardization (CEN) *Laboratory Biorisk Management Standard*.[9,10,11,12,13]

19.2.1 Inventory Elements

The minimum inventory requirements for pathogens and toxins in long-term storage in regulated containment zones are specified in Matrix 4.10 of the CBS. A description of the inventory control system is also required as part of the biosecurity plan (CBS Matrix 4.1). The level of detail of the information to capture in the inventory records and the complexity of the inventory control system will vary depending on the risks associated with the pathogens or toxins and the needs of the facility or containment zone. At minimum, the inventory of pathogens, toxins, and other regulated infectious material in long term storage must include (CBS Matrix 4.10):

- a description of the pathogen or toxin, in sufficient detail to identify the level of risk of the material, including:
  - the pathogen or toxin name (e.g., genus, species, name of toxin); and
  - risk group(s);
- storage location (e.g., room, freezer); and
- any pathogens, toxins, or other regulated infectious material stored outside of the containment zone (for CL2 and **containment level** 3 [CL3] only).
- For RG3 and RG4 pathogens, and SSBA toxins only, the inventory must also include:
  - the specific identification of the pathogens, toxins, and other regulated material (e.g., genus, species, strain, subtype, batch number); and
  - a means to allow for the detection of a missing or stolen sample in a timely manner (e.g., include quantity information, such as number of vials or, for SSBA toxins, amount by weight [in mg or µg] for stocks in storage).

It may be advantageous for any facility to establish an inventory system in order to document and manage this information in a consistent manner, or the required information may be integrated into an existing inventory or inventory system. Additional information can be captured to create a more useful and robust inventory system. What additional information can be included depends on the facility's activities and needs. For example, compliance with quality management standards such as ISO 9001 or ISO 15189 may require documentation of additional or different information. The following is a list of additional elements that may be considered to create a comprehensive and robust inventory system of pathogens and toxins in any containment zone:

- additional information of the pathogen or toxin, such as:
  - strain or subtype; and
  - parental organism of toxin;
- an indication of the pathogenicity of the strain (e.g., attenuated vaccine strain, highly pathogenic strain, drug resistant variant), where appropriate;
- vial or sample identification (e.g., tube number, batch number, barcode number), where appropriate;
- form of the materials (e.g., lyophilized, suspension, concentrate, pellet, stab culture);
- number of vials, quantity (e.g., concentration, volume) contained in the vials, where appropriate or relevant;
- precise storage location (e.g., freezer, shelf, rack, box);
- location(s) of use;
- name and contact information of the responsible person;
- special characteristics (e.g., restrictions of use noted on a licence or animal pathogen import permit);
- manufacturer's name or source;
- dates of receipt, acquisition, or generation of the material;
- dates of removal, use, transfer, inactivation, or disposal;
- expiry date (e.g., lyophilized cultures);
- reference to associated documentation, such as:
  - import authorizations (i.e., licence and animal pathogen import permits) and transfer letters;
  - approved method of **decontamination** or inactivation;
  - **Pathogen Safety Data Sheets (PSDSs)**; and
- where the inventory includes SSBAs:
  - list of authorized individuals who have access;
  - list of individuals with a valid HPTA Security Clearance.

19.2.2 Inventory Review and Updates

In order to be useful, inventories need to be updated regularly; it is difficult to determine if a sample is missing if the inventory does not accurately reflect the material in possession at the time. Inventories should be updated annually and whenever a sample is used, transferred, inactivated, or disposed of, and whenever new material is identified as the result of diagnostic testing, receipt, generation, or production. Institutions are encouraged to develop internal policies outlining timeframes for regular and scheduled inventory reviews to compare material in storage with the inventory list and to update accordingly. For routine inventory checks, verifying a representative subset of the material may be a suitable approach.

19.2.3 Inventory Control Systems and Reporting

Inventories of pathogens, toxins, and other regulated infectious material in long-term storage should be readily available and easily searchable, whether an electronic or a paper system is used. Inventory control systems, such as record books, inventory software, or database systems, can be used to manage inventories of pathogens and toxins.[14] A notification process (i.e., internal and external reporting) should be in place for identifying, reporting, and remediating any problems, including inventory discrepancy, storage equipment failure, security breaches, or disposal or release of materials. For security and storage considerations, it is encouraged that facilities minimize the quantities of pathogens and toxins that comprise the inventory, whenever possible.

Licensed facilities are not required to submit regular reports of their inventories to the PHAC; however, it is expected that they are able to describe their record-keeping system(s) upon request (e.g., How would you find sample "X" in your facility? Do you have pathogen "Y" in your inventory?). In addition, licence holders are required to notify the PHAC without delay if they inadvertently produce or come into possession of a human pathogen or toxin that they are not authorized to possess (HPTA 12[2]) or have reason to believe that a human pathogen that was in their possession has been stolen or is otherwise missing (HPTA 14).

19.3 Storage and Labelling

Controls should be implemented to limit access to pathogens and toxins stored within a facility to authorized personnel for their specified purpose. Security barriers may include locks, security storage equipment, tamper-evident storage containers, locked storage compartments inside cabinets or refrigerators, or storage areas located inside **restricted access** areas. Whenever possible, pathogens and toxins should be stored inside the containment zone where they are handled, or in a zone at the same containment level. RG2 and RG3 pathogens and toxins may be stored outside of the containment zone, provided that additional security measures are implemented (CBS Matrix 4.6). Samples being banked or stored for long periods of time should be appropriately labelled (i.e., clearly and permanently) and meet the current requirements of the Workplace Hazardous Materials Information System (WHMIS).[15]

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CHAPTER 20

MOVEMENT AND TRANSPORTATION OF INFECTIOUS MATERIAL OR TOXINS

CHAPTER 20 – MOVEMENT AND TRANSPORTATION OF INFECTIOUS MATERIAL OR TOXINS

The **movement** and **transportation** of **infectious material** and **toxins** (or **biological material** suspected of containing them) is an essential part of routine **laboratory** procedures in both research and diagnostic settings. For the purposes of this chapter, a distinction is made between movement and transportation, with movement denoting the action of moving material within a **containment zone** or building, and transportation denoting the action of transporting material to another building or location, within Canada or abroad. This distinction is required because the transportation of infectious substances falls under the *Transportation of Dangerous Goods Act* (TDGA), *Transportation of Dangerous Goods Regulations* (TDGR), and *Dangerous Goods Regulations* (DGR) issued by the International Air Transport Association (IATA), and is discussed separately.[1,2,3] This chapter also provides information pertaining to the regulatory requirements for the **transfer**, importation, and exportation of **pathogens** and toxins.

20.1 Movement of Infectious Material or Toxins

Whenever infectious material or toxins are moved within a containment zone or building, the implementation of **good microbiological laboratory practices** will help prevent **contamination** and inadvertent spills. Procedures to prevent leaks, drops, spills, or similar events during the movement of infectious material or toxins within the containment zone, or between containment zones within the same building will also serve to prevent the **release** of pathogens and toxins. The minimum requirements regarding the movement of infectious materials or toxins are specified in Matrix 4.6 of the *Canadian Biosafety Standard* (CBS), 2nd Edition.[4]

20.1.1 Movement of Infectious Material or Toxins within a Containment Zone

When moving infectious material or toxins within a containment zone (e.g., from a freezer to a **biological safety cabinet [BSC]**, from an incubator to a BSC, from a BSC to a microscope, or **waste** to an autoclave), the infectious material or toxins should be adequately protected from being dropped, tipped, or spilled. The precautions taken by personnel to prevent mishaps should correlate with the inherent **risk** associated with the infectious material or toxins (i.e., the greater the risk associated with the material, the greater the care that should be taken when moving it).

Closed containers provide **primary containment** for the movement of infectious material and toxins. Moving infectious material or toxins within a containment zone using closed containers, in conjunction with a cart when necessary (e.g., for large number of specimens, **large volumes**, or heavier items), will help reduce the likelihood and extent of a drop, spill, or leak. Labelled containers will promote timely and appropriate spill response and post-exposure follow-up in the event of a spill or leak. Leak-proof, impact resistant containers

are recommended, and specially designed containers equipped with lid clamps are commercially available. Externally threaded tubes with screw caps should be used instead of snap-cap tubes or internally threaded tubes with screw caps to prevent leaking and minimize contamination of the lid surface. With higher risk agents and multiple samples, carts with rails or raised edges should be used and absorbent material placed on each cart shelf; cart pans may also be used. Samples should be loaded in a manner that will prevent them from being tipped or spilled if a collision occurs. Individuals should move slowly and with caution whenever carrying infectious material or toxins. Following established directional traffic and workflow patterns within the containment zone, based on a **local risk assessment (LRA)**, will help facilitate the movement of personnel and materials from "clean" areas (i.e., areas of lower contamination) to "dirty" areas (i.e., areas of higher contamination) in a manner that minimizes the spread of contamination. A biological spill kit available inside the containment zone allows for a prompt, appropriate cleanup in the event of a spill.

20.1.2 Movement of Infectious Material or Toxins between Containment Zones within the Same Building

Using leak-proof and impact resistant containers to move infectious material and toxins between containment zones in the same building will help prevent a spill or leak if a container is dropped. In the event that an **incident** occurs, such as the container is dropped, breaks, or its contents are spilled, the use of appropriate labels on the container to identify the contents and the hazards will assist with the appropriate response. Surface **decontamination** of containers performed prior to removal from the containment zone helps prevent the spread of infectious materials and toxins. This includes the movement of waste to a centralized decontamination area within the building, but outside the containment zone. Large or heavy items should be transported on carts and loaded in a manner that will prevent them from tipping. A cart designed with guard rails or raised edges can be considered to protect the items from falling off the cart during relocation. An **emergency response plan (ERP)** for infectious material or toxins stored outside the containment zone, and spill kits available outside the containment zone, will allow for a prompt, appropriate response in the event of a spill. Wet or dry ice used to keep specimens or samples cold during transit should always be used in accordance with the current requirements of the Workplace Hazardous Materials Information System (WHMIS)[5]. In order to prevent gas buildup, dry ice should never be placed inside an airtight secondary container.

20.2 Transportation of Infectious Material or Toxins

Materials known to contain or suspected of containing pathogens or toxins that are being transported or being offered to a commercial carrier for transport are considered dangerous goods and are subject to the requirements outlined in national (i.e., TDGR) and international regulations. These regulations provide details on the classification, packaging, labelling, documentation, and certification requirements that are designed to safeguard such materials during transport in order to protect the safety of shipping and receiving personnel, transportation workers, commercial carriers, emergency responders, the **community**, and to prevent inadvertent release into the environment.

20.2.1 Domestic and International Transportation Regulations

Biological material, including waste, containing pathogens or toxins that is transported in Canada is governed by the TDGA and TDGR, administered by Transport Canada. Each province and territory has adopted the TDGR as its own legislation. The TDGR define the classification, labelling, packaging, and documentation requirements necessary for shipping biological material and infectious substances within Canada. These regulations also require that a person who handles the package in the course of packaging for shipment, offers for transport, transports, or receives biological material or infectious substances be trained in the TDGR and hold a valid certificate of training. Finally, shippers of high risk infectious materials (i.e., most Risk Group 4 [RG4] and certain Risk Group 3 [RG3] pathogens) may be required to have an Emergency Response Assistance Plan (ERAP) approved by Transport Canada to respond to any shipping emergency within Canada. For more information on the TDGR, including exemptions that may exist based on the distance between properties please contact Transport Canada or visit its website.

The international transportation of biological material and infectious substances is governed by international regulations developed from the *Recommendations on the Transport of Dangerous Goods* (Model Regulations) by the United Nations (UN) Committee of Experts on the Transport of Dangerous Goods. Based on the UN Model Regulations, the International Civil Aviation Organization (ICAO) outlines the standards and requirements for the safe air transport of dangerous goods, including infectious substances, in the *Technical Instructions for the Safe Transport of Dangerous Goods by Air*.[6] The ICAO Technical Instructions have been adopted by and apply in most countries worldwide, including Canada. The International Air Transport Association (IATA), an international association representing 230 commercial airlines, issues the DGR annually. These regulations set forth the ICAO requirements for the safe packaging and transport of dangerous goods, including infectious substances, as they apply to the airline industry. As the majority of carriers (both passenger and courier/cargo) around the world are members of this organization, anyone shipping infectious substances internationally is subject to the IATA DGR and the ICAO requirements. Additionally, any shipment of biological material or infectious substances travelling within another country/territory may be subject to transportation regulations specific to the local jurisdiction. For more information on the Technical Instructions or the DGR, please contact ICAO or IATA, respectively, or visit their websites.

20.2.2 Considerations for Shipping and Receiving

The TDGA and TDGR apply to shipments containing dangerous goods from the time a shipper (i.e., consignor) prepares or packages the dangerous goods for transportation by a carrier, including the carrier taking possession of the package (e.g., pickup by courier) and operation of the carrier, until receipt by the intended recipient (i.e., consignee). **Facilities** should establish internal procedures for the movement and transportation of packages containing pathogens and toxins between the shipping and receiving area and the rest of the **facility**. Following these procedures will promote the safe transfer of materials, and continued compliance with the TDGA, TDGR, *Human Pathogens and Toxins Act* (HPTA), *Human Pathogens and Toxins Regulations* (HPTR), *Health of Animals Act* (HAA), and *Health*

of Animals Regulations (HAR).[7,8,9,10] The person who packages material known to contain or suspected of containing pathogens or toxins for shipment must be trained and hold a valid certification in safe transport of dangerous goods, in accordance with the TDGR, or be directly supervised by an individual with a valid certificate of training. Packaging of infectious material and toxins for transportation is best performed by containment zone personnel in a containment zone of the appropriate **containment level**. Final packaging (i.e., placement inside secondary shipping container, labelling) of sealed shipping containers of infectious material may be acceptable to be performed by personnel outside of the containment zone (e.g., in shipping and receiving areas), provided that the primary container is sealed and thoroughly surface decontaminated.

Personnel, including shipping and receiving personnel, responsible for shipments (e.g., packaging, opening packages) involving **security sensitive biological agents (SSBAs)** require a valid ***Human Pathogens and Toxins Act*** **Security Clearance (HPTA Security Clearance)** issued by the Public Health Agency of Canada (PHAC), in addition to the appropriate training in accordance with the TDGR. Where containers of SSBAs are being packaged for transportation in shipping and receiving areas in a facility, these areas are then required to be identified as a part of the facility where SSBAs are present and accessible on the **licence** for **controlled activities** with human pathogens and toxins. Only after the SSBA has been packaged in accordance with the TDGR, and the outer package does not identify that it contains an SSBA, is it acceptable for it to be offered for transportation to a carrier without an HPTA Security Clearance.

A package is not considered received until the intended recipient (i.e., consignee) has taken possession of it, and the TDGR (including training certification requirements) apply to the recipient of the package. The outer layer(s) of packaging that surrounds sealed primary containers of infectious material or toxins may be unpacked in the shipping and receiving area, as determined by an LRA; a BSC is recommended for this activity in case there has been a break or breach of the primary container inside the package. Vials containing infectious material or toxins are only to be opened at their required containment level (CBS Matrix 4.6). Recipients of shipments of SSBAs must have an HPTA Security Clearance issued by the PHAC. This means that personnel working in shipping and receiving areas must have a valid HPTA Security Clearance to pack or unpack shipments of SSBAs in this area or if the facility temporarily stores SSBAs in this area before delivery to the recipient. If SSBA packages are not identified or accessed at any time by shipping and receiving personnel, then the shipping and receiving area personnel may not need HPTA Security Clearances. HPTA Security Clearances are discussed in more detail in Chapter 6; investigation and reporting of incidents involving shipments of SSBAs are discussed in Chapter 18.

20.2.3 Transportation of Infectious Material or Toxins between Buildings

Organizations such as universities and colleges that have several buildings containing a multitude of containment zones may transport infectious material or toxins between buildings. In this case, infectious material and toxins are to be documented and packaged in an appropriate manner (i.e., at a minimum in labelled containers that are sealed, leak-

proof, and impact-resistant) to protect against their release during movement or transport, and in accordance with the TDGR when applicable. Additionally, pathogen and toxin accountability measures (e.g., **inventory**) and regulatory requirements relating to the movement or transfer of regulated materials, described in Chapters 19 and 23, respectively, need to be considered when material is transported between buildings within the same organization/entity.

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CHAPTER 21

WORKING WITH RISK GROUP 1 BIOLOGICAL MATERIAL

CHAPTER 21 – WORKING WITH RISK GROUP 1 BIOLOGICAL MATERIAL

Risk Group 1 (RG1) **biological material** consists of **microorganisms**, nucleic acids, or proteins that are either unable or unlikely to cause human or animal **disease**, and therefore are not generally considered **pathogens**. Nonetheless, this material may still pose a low risk to the health of individuals and animals. For example, *Bacillus subtilis*, an RG1 bacterium widely used as a probiotic (i.e., a live bacteria added to food or consumed as a supplement to confer a health benefit to the host), has also been associated with numerous cases of food poisoning and other negative health effects.[1,2,3,4,5] RG1 organisms may also be **opportunistic pathogens** that pose a particular threat to immunocompromised or immunosuppressed individuals (e.g., through medical therapy, pregnancy, diabetes, or other conditions). Due to the low risk to public health and the animal population associated with RG1 biological material, the *Canadian Biosafety Standard* (CBS), 2nd Edition does not specify requirements applicable to **laboratories** or other **facilities** where activities with RG1 biological material are conducted.[6] Nevertheless, it is recommended that RG1 material be handled safely using safe work practices and conducted in a laboratory or animal area that incorporates basic laboratory design. Where there is an increased risk (e.g., immunocompromised individual working with an opportunistic RG1 pathogen), consideration should be given to using **containment level** 2 (CL2) operational procedures or moving the work into a CL2 zone. The PHAC and the CFIA have developed additional guidelines and recommendations on best practices for **facility** design and the safe handling of RG1 biological materials; please visit the PHAC or CFIA website for further information. This chapter describes some general recommendations only and should not be interpreted as requirements.

21.1 Physical Design Considerations

Work with RG1 material is often performed in **laboratory work areas**, **large scale production areas**, or **animal work areas** that incorporate only basic facility design and engineering controls to limit the spread of biological material. These work areas are frequently described as containment level 1 (CL1) zones and provide the foundation of **biosafety** upon which the requirements for all higher levels of **containment** are built. Biosafety at CL1 is achieved through a basic level of operational practices in conjunction with minimal physical elements for safe laboratory design that serve to protect personnel and the environment from the biological material being handled.

Laboratory work areas for activities with RG1 material have no specific physical design features beyond those suitable for a well-designed and functional laboratory space for handling biological material (e.g., handwashing sinks, signage). Floors and surfaces (e.g., benchtops, chairs) should be easy to clean and able to withstand regular cleaning; floors should be slip-resistant to prevent incidents, especially where floors may be wet. Allowing sufficient space to manoeuver between benches and chairs will reduce the risk of workers being jarred. Laboratory materials should be stored safely and away from high traffic areas and doors. Laboratory work areas should be separated from public and **administrative areas** by a door. In addition, due to the **large volumes** of liquids containing biological material, large scale production areas should be designed to prevent the **release** of viable organisms to the sanitary sewer (e.g., by capping or raising floor drains).

CL1 animal work areas should be designed and operated in accordance with the Canadian Council on Animal Care (CCAC) *Guidelines on Laboratory Animal Facilities*.[7] Floors and walls should be able to withstand impacts (e.g., from animal cages) and repeated washing and cleaning (e.g., with a pressure washer). Defined traffic flow patterns through the work area minimize the spread of **contamination**. **Support areas** such as cold storage (e.g., fridge, freezer, walk-in cold room) and dedicated cage washing areas are encouraged.

21.2 Operational Practice Considerations

21.2.1 Risk Assessments, Personal Protective Equipment, and Training

Risk assessments are recommended, even with RG1 material, in order to identify hazards and develop strategies to mitigate the risks. **Local risk assessments (LRAs)** carried out for all activities will help identify risks and to develop safe work practices. The risk assessment for RG1 material can be quite simple and, in some scenarios, there may be no significant risk. For example, if there is a risk of contaminating the hands, gloves may be recommended; however, if the material being handled is considered completely safe to handle (e.g., bread mould, yogurt **culture**), gloves are not required from a biosafety perspective. In contrast, the LRA could identify a risk specific for a particular worker (e.g., an immunocompromised or immunosuppressed individual). Risk assessments are further discussed in Chapter 4.

Personal protective equipment (PPE) protects individuals from **exposure** to the material they are handling, and prevents the material from contaminating other areas. A wide range of PPE can be used to protect against risks, including lab coats, gloves, safety goggles, and respirators. The need for PPE and selection of appropriate type(s) of PPE are determined by completing an LRA. It is critical that PPE be used correctly in order for it to be effective. PPE is further discussed in Chapter 9.

Training aims to provide a rudimentary understanding of biosafety as well as safety in general. It is encouraged that all personnel be educated and trained (preferably before work with RG1 material is started) on the potential hazards present in their work environment, basic biosafety, the correct use of PPE and laboratory equipment, and proper, safe work practices and techniques. Training is further discussed in Chapter 8.

21.2.2 Good Microbiological Laboratory Practices

The term "**good microbiological laboratory practices**" describes a basic code of practice and techniques established in microbiology laboratories that can be employed in any work area where similar laboratory-related activities involving *any* microorganisms are performed.[8,9] These practices aim to minimize the spread of contamination generated by the material being manipulated as well as to safeguard the material against contamination from the environment to protect its quality or purity. At the same time, good microbiological laboratory practices provide a basic level of protection to the individual laboratory worker and the environment from the microorganisms being manipulated. Good microbiological laboratory practices provide the foundation upon which all operational practices for handling **infectious material** at higher levels of containment are based.

Good microbiological laboratory practices include the following:

- oral pipetting is strictly prohibited;
- eating, drinking, smoking, applying cosmetics, handling contact lenses, storing food or utensils in the work area is strictly prohibited;
- hair that may become contaminated while working should be restrained (e.g., tied back or fastened with a clip) or covered;
- jewellery (e.g., rings or long necklaces) that may come in contact with biological material or that may puncture protective gloves should not be worn while working;
- open wounds, cuts, scratches, and grazes should be covered with waterproof dressings;
- work stations (e.g., benchtops) should be kept free of clutter to avoid cross-contamination and to facilitate cleaning and **disinfection**;
- all personnel, including visitors and trainees, should wear suitable footwear (e.g., shoes that cover the entire foot with no or low heels) and PPE (e.g., lab coats, aprons, gloves, protective eyewear) appropriate to the procedure;
- personal belongings (e.g., purses, bags) and street clothing (e.g., coats, boots) should be stored separately from PPE and from work stations where biological material is handled;
- aseptic techniques should be used when manipulating open samples of RG1 biological material to provide basic containment and quality control;
- work surfaces should be cleaned and decontaminated using a suitable disinfectant and an appropriate contact time after work with RG1 biological material is complete;
- all items that come into contact with biological material, including liquid or solid **wastes**, should be decontaminated before disposal or reuse; hands should be washed with soap and water or otherwise disinfected (e.g., sanitized) after handling specimens that contain microorganisms (if gloves are not worn), after handling infected animals, immediately after removing gloves, and before leaving the work area;
    - disposable gloves used when handling RG1 biological material should be discarded after use and never reused;
    - all contaminated clothing and PPE should be decontaminated before laundering when a known or suspected exposure has occurred;
- PPE should be removed in a manner that minimizes the spread of contamination to the skin and hair;
- procedures for the safe use of sharp objects should always be followed (e.g., avoid use whenever possible, use safe alternatives or safety-engineered sharps devices, avoid bending, shearing, breaking, or re-capping needles, and discard used sharps in a puncture-resistant sharps container).

21.3 Routine Practices and Universal Precautions

Routine practices and universal precautions are infection control guidelines developed for health care and veterinary environments to protect individuals from exposure to potential sources of pathogens.[10,11] They aim to prevent the transmission of pathogens through the occupational contact with primary subjects (e.g., patients, animals), specimens and blood (e.g., tissue specimen, whole blood, serum, plasma), or other body fluids (e.g., urine, feces, saliva, milk).[12] Routine practices are based on five major elements: risk assessments, hand hygiene, PPE, environmental controls (e.g., suitable facilities for the disposal of waste, dirty linen, and sharps), and administrative controls (e.g., education, sharps safety program, aseptic technique).[13] Many of the elements of good microbiological laboratory practices are also common to both universal precautions and routine practices. It is considered prudent to treat any specimen of blood, body fluid, or tissue as though it contained a human pathogen and to follow routine practices to protect personnel and other individuals from exposure. These precautions will prevent the potential spread or release of pathogens, as microorganisms and other pathogens may be transmitted from symptomatic and asymptomatic humans or animals.[13] Consequently, it is recommended that routine practices be followed in work areas where such specimens are handled.

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CHAPTER 22

DESIGN CONSIDERATIONS FOR NEW CONTAINMENT ZONES

CHAPTER 22 – DESIGN CONSIDERATIONS FOR NEW CONTAINMENT ZONES

The principal objective of **containment zone** design is to provide an environment where **pathogens** and **toxins** can be safely handled and stored. Given the wide range of pathogens, toxins, and sample types that may be handled in a containment zone, and the variety of work that may take place (e.g., **laboratory** manipulation, work with **small-sized animals** or **large-sized animals**, **large scale** production), there is no single ideal design for **containment facilities**. Much of the information presented in this chapter will apply primarily to **large animal containment zones (LA zones)** and **high containment zones**, and may be considered when planning a **containment level** 2 (CL2) containment zone. Chapter 3 of the *Canadian Biosafety Standard* (CBS), 2nd Edition, should be consulted for the minimum **physical containment requirements** specific to each containment level (CL2-CL4).[1]

Physical containment can include **primary containment devices** such as **biological safety cabinets (BSCs)**, **primary containment caging**, and sealed centrifuge cups. In many containment zones, particularly in high containment zones, this will also include a laboratory's physical structure and engineering controls.

A comprehensive review of laboratory design is beyond the scope of this document and there are numerous resources on the subject that may be consulted.[2,3,4] Instead, this chapter will present a brief overview of design factors related to **biosafety**, containment, and **biosecurity**. References to the topic-related requirements specified in the CBS are indicated by matrix number where appropriate.

22.1 Planning

The physical containment requirements that apply to a containment zone are determined by a number of factors, including the pathogens, toxins, and other **infectious material** to be handled or stored (e.g., **risk group**, type of pathogen or material) and the type of activities to be performed (e.g., ***in vitro*** versus ***in vivo*** activities, laboratory scale versus large scale, animal species and size) (Chapter 3 of the CBS). The unique features and equipment in most specialized laboratories generally call for additions to the basic design of individual laboratories, rather than substitutions, and are identified at the planning stage. The overall design will be selected according to laboratory function as well as other factors such as cost, specialized equipment, workspace flexibility, and occupancy.

A planning team should be assembled to consider and discuss all aspects of the new laboratory. Regular communications, using plain language and terminology that can be reasonably understood by all team members, will help keep team members informed throughout the planning process. Formal documentation of key decisions made during the planning process, with signatures of approval from team members, will promote traceability. The planning team should be composed of individuals with a range of knowledge and experience. The team members can include **senior management**, financial officer(s), architect(s)/ engineering firm, the **biological safety officer (BSO)** or biosafety representative, a health and safety representative, scientists, laboratory workers, maintenance staff, and representatives from existing and newly constructed laboratories with similar functions. The Public Health Agency of Canada (PHAC) and the Canadian Food Inspection Agency (CFIA) can be consulted for guidance and assistance throughout the planning process.

22.1.1 Commissioning

Commissioning is a key component of the overall plan for the containment zone. During the commissioning process, a new or newly renovated containment zone undergoes an intensive quality assurance process that begins during the design phase and continues through construction and occupancy. The process is carried out by containment zone personnel and technical or qualified personnel (e.g., architects; engineering technologists; heating, ventilation, and air conditioning [HVAC] system specialists; engineers) to confirm that the finished containment zone, equipment, and **containment systems** will operate in accordance with the design intent as well as the specifications in the CBS.

A commissioning plan developed early in the design planning stages will facilitate both the construction and commissioning processes. The plan may define the scope, standards, roles and responsibilities, testing sequence, and deliverables of the commissioning process. More specifically, the plan will outline all the steps in the commissioning process, including system documentation, equipment start-up, control system calibration, testing and balancing, and performance testing.

Commissioning during the construction phase typically confirms that containment zone systems are designed, installed, functionally tested, and operate in accordance with the design intent. In addition, the applicable performance and **verification** testing specified in Matrices 5.1 to 5.3 of the CBS will be performed. For high containment zones, reports of these tests will be requested by the PHAC and/or the CFIA to facilitate compliance verification of facilities in relation to **licence** applications and **animal pathogen import permit** applications.

22.2 General Building Layout

The handling and storage of infectious material and toxins not only presents a **risk** to laboratory workers, but poses a biosecurity risk as well. Containment zones, office space and common areas are physically separated to prevent unintentional spread of pathogens or toxins beyond the containment zone. This section provides guidance on the relative location of the different functional areas, including **support areas**.

22.2.1 The Containment Zone

The segregation of laboratory and non-laboratory activities within the containment zone minimizes the potential for cross-**contamination** between work areas (e.g., between **laboratory work areas** and **administrative areas**). This may be achieved by locating offices, public and administrative areas, lunch rooms, washrooms and common areas outside the containment zone. Where that is not the case (e.g., washrooms within the confines of CL2 laboratory work area), procedures can be implemented and followed to maintain defined "clean" and "dirty" areas within the containment zone. Containment zones, and the concept of defining the **containment zone perimeter**, are discussed in Chapter 3.

The building layout can help control access to the containment zone (e.g., access to the containment zone via a secondary corridor rather than directly from public areas, locating offices outside of containment zones). Limiting or restricting containment zone access to **authorized personnel** contributes to biosafety and biosecurity by providing access only to those who have the appropriate training, knowledge, and, where applicable, a valid *Human Pathogens and Toxins Act* **Security Clearance (HPTA Security Clearance)** (CBS Matrix 4.5). The need for unauthorized persons to access these areas (e.g., students meeting with a professor) without entering the containment zone, can be addressed by considering the location of offices.

Within the containment zone, dedicated paper and computer work stations located away from benches and other areas where infectious materials and toxins are handled minimizes the risk of contaminating office materials that may be difficult to decontaminate (e.g., paper, notebooks), or electronic devices that may become damaged by **decontamination**. In animal containment zones, it is preferable for the **facility** design to include preparation rooms for laboratory activities that do not directly involve the animals (e.g., preparing/mixing feed, samples or inoculants), located in a space that is physically separated from the rooms where the animals are housed (i.e., **animal rooms** and **animal cubicles**). This configuration prevents the contamination of work materials, enhances personnel safety, and minimizes the **exposure** of experimental animals to noise and other activities that can lead to distress and unpredictable behaviour (CBS Matrix 3.1).

Locating high containment zones away from external envelope walls provides increased control of **biocontainment** systems (e.g., HVAC system), in addition to protecting the **containment barrier** from being breached during an environmental disaster (e.g., tornado, earthquake). Locating high containment zones away from exterior envelope walls can also help enhance security protection from **outsider threats** (e.g., break-in, theft).

Where clothing change areas are provided, they can be located in rooms separated from work areas by a door, or an area at the containment barrier with designated **"clean" change areas** and **"dirty" change areas**, which may be separated using a line demarcation on the floor (CBS Matrix 3.3). High containment zones, CL2 **large scale production areas**, and CL2 LA zones (i.e., CL2-Ag zones) have designated change areas located in **anterooms** where **inward directional airflow (IDA)** is maintained (Matrices 3.3 and 3.5 of the CBS). In these anterooms, the change rooms are separated into a "clean" area located outside the containment barrier and a "dirty" area directly inside the barrier, often separated by a walk-through body shower (CBS Matrix 3.3).

22.2.2 Laboratory Support Areas

The location, size, and number of rooms needed to support laboratory activities are important considerations in the design phase. Examples of support areas include rooms containing refrigeration equipment (e.g., freezers, cryostorage), integral cold rooms, or walk-in freezers for storage of perishable laboratory specimens, samples, reagents, or animal carcasses; areas for the storage of laboratory consumables (e.g., disposable plasticware, clean cages, animal feed and bedding), cleaning supplies (e.g., mop and bucket), or non-perishable samples

and reagents; and, in **small animal containment zones (SA zones)**, cage washing areas or barrier cage washers. There are special design considerations for biocontainment in systems such as the electrical, communication, HVAC, and plumbing systems in some areas.

Storing clean cages, feed, bedding, and other supplies outside the containment zone and bringing them into the zone as needed will avoid clutter; however, support areas within the containment zone may be considered for some storage needs, as well as for the preparation of surgical procedures, disposal of carcasses, or other activities. Biological **waste** is handled as hazardous waste until it is decontaminated for disposal. Rooms designed for, and dedicated to, decontamination and temporary storage of biological waste can reduce the risk of contamination by preventing the accumulation of waste in work areas.

High containment zones will include technologies that cross the containment barrier (e.g., fumigation ports, **pass-through chambers**, double-door autoclaves, and feed chutes). Locating these in a secure area will contribute to biosecurity and help maintain containment.

22.2.3 Electrical Systems

Containment zone equipment including refrigeration equipment, centrifuges, BSCs and specialized devices may have specific electrical needs, including the location of outlets. Anticipating the location of larger equipment and the anticipated maximum electrical demand at the planning stage will alleviate the need for subsequent modifications.

The continued operation of equipment critical for infectious material and toxin containment (e.g., BSCs, ventilated cage racks) during emergency situations is crucial. Uninterruptible power supply (UPS) systems and emergency generators maintain power to critical equipment so it can continue to function when the power supply fails. Battery-powered emergency lighting is recommended for all containment zones, especially where an interruption of power may be experienced before emergency power is supplied. In high containment zones, critical equipment may include HVAC and security systems, as well as equipment essential for personnel safety (e.g., lighting and positive-pressure suits).

22.3 Building Mechanical Systems

It is an important design consideration for containment zone systems and critical controls to be accessible outside the containment barrier (Matrices 3.5 and 3.6 of the CBS). These include air supply and exhaust systems (e.g., ductwork, fans and **high efficiency particulate air [HEPA] filters)**, circuit breakers, light ballasts and starters (for high containment zones), backflow preventers for the water supply, and shut-off valves. Locating these components outside the containment barrier will facilitate repairs, maintenance, testing and certification, cleaning, inspections, and emergency shut-off by personnel. Similarly, consideration should be given to the design of drainage piping for **effluent decontamination systems** such that it is readily accessible for repairs, maintenance, and inspection. **Limited access** or **restricted access** to containment zone systems and controls contributes to both biosafety and biosecurity. Minimizing and shielding protruding obstructions (e.g., lighting, electrical

fixtures, exposed plumbing) in animal containment zones will prevent animals from chewing or pulling on them, will help avoid injury to animals or personnel, and prevent rips or tears to **personal protective equipment (PPE)**.

Centralizing services including conduits, electrical wiring, and plumbing lines that are run through the containment barrier will reduce the number of penetrations that need to be sealed and tested for integrity.

22.3.1 Communication System

A communication system (e.g., telephone, intercom system, or two-way radios) can be used to minimize the **movement** of notebooks or paper and personnel into and out of the containment zone, and increases personnel safety in the event of an emergency.

Communication systems allow the timely transfer of information gathered in the containment zone and are essential for communications between personnel in the event of an emergency. Consequently, communication should be maintained with minimum interruptions during a power outage. Verification of communication systems confirms that they are operating as designed. Examples of communication devices include telephones, fax machines, intercom systems, two-way radios, panic buttons, computers, and notes or hand signals observed through a window.

22.3.2 Air Handling

HVAC systems enable temperature and humidity to be maintained at comfortable levels, and operate so that sufficient air changes per hour are provided under normal operation to maintain air quality, based on facility function. The HVAC system maintains inward directional airflow (IDA), an essential component of the containment barrier in zones where it is required. Inward directional airflow (IDA) and HEPA filters are described in Chapter 10, and the applicable physical requirements are specified in Matrix 3.5 of the CBS.

Where inward directional airflow (IDA) is provided, monitoring devices such as differential pressure gauges allow personnel to verify that it is being maintained as intended before they enter the containment zone. In cases of HVAC system failure, personnel need to be made aware immediately. It is important that alarms signalling HVAC system failure inform personnel both inside and outside the containment zone so that personnel are adequately informed to evacuate, to avoid entering the containment zone, to quickly initiate emergency procedures (e.g., terminate work, shut down equipment, exit), and to initiate repairs. Ideally, building automation systems can be programmed to provide maintenance warnings or pre-alarms to help prevent HVAC system failure.

HEPA filters in the air exhaust (high containment zones) and air supply ductwork (containment level 4 [CL4] only) protect the ductwork from contamination and prevent the **release** of **airborne pathogens** or aerosolized infectious material or toxin from the facility. Locating the HEPA filter(s) as close as possible to the outside of the containment barrier minimizes the length of contaminated ductwork. Airtight ductwork, tested ***in situ*** by **pressure decay testing** in accordance with American Society of Mechanical Engineers (ASME) N511 (test

pressure determined in accordance with ASME AG-1), between the containment barrier and HEPA filters or **isolation dampers**, allows for gaseous decontamination and prevents the release of infectious material or toxins[5,6]. Exhaust ductwork designed to withstand the maximum pressure achievable by the HVAC system when under extreme negative pressure, will prevent a biocontainment breach if the air supply or exhaust system malfunctions.

When designing HVAC systems for containment zones, consideration should be given to the appropriate placement of large equipment that can generate heat and disrupt airflow (e.g., refrigerators, freezers, incubators, autoclaves). Additional factors such as the presence of cylinders of hazardous gases (e.g., chlorine gas), liquid nitrogen, or toxic chemicals may be additional considerations when determining HVAC system specifications (e.g., increased air changes to maintain air quality).

22.3.2.1 Class II B2 Biological Safety Cabinets

Class II B2 BSCs are hard-ducted to the exhaust ductwork, and will therefore be linked to room airflow (i.e., they exhaust significant air quantities). A change in room ventilation will affect the functioning of the BSC, and failure of the BSC can affect room ventilation. Class II B2 BSCs can produce a reversal of airflow from the face of the BSC (i.e., **puff-back**) when the exhaust fan serving the cabinet fails, posing a risk to personnel. Designing the laboratory ventilation system accordingly can help prevent puff-back (e.g., supply blower brake, isolation damper for BSC air intake). BSCs are further discussed in Chapter 11.

22.3.2.2 Chemical Fume Hoods

Chemical fume hoods are hard-ducted to the exhaust ductwork, and will therefore be linked to room airflow (i.e., they exhaust significant air quantities). A change in room ventilation will affect the functioning of the fume hood, and failure of the fume hood can affect room ventilation. Chemical fume hoods are not designed for the manipulation of infectious material or toxins, and consideration should be given to minimizing the placement of chemical fume hoods in high containment zones; instead, Class II B2 BSCs, which are designed to handle infectious material or toxins as well as volatile chemicals and radionuclides, should be considered. Chemical fume hoods are further discussed in Chapter 12.

22.3.3 Plumbing

Appropriate design of supply and drain plumbing can contribute to containment by preventing the release of contaminated liquids into either the drinking water distribution system or the sanitary sewer. This is achieved by installing backflow preventers and isolation valves, which are required in CL2 LA zones (i.e., CL2-Ag) where **prions** are handled, and in CL3 (which includes CL3 LA zones [i.e., CL3-Ag]), and CL4 (CBS Matrix 3.6). Manuals for selection, installation, maintenance and testing of **backflow prevention** devices are available from the Canadian Standards Association (CSA).[7] Capped or raised floor drains incorporated into large scale production areas will prevent the release of infectious material or toxins into sanitary sewers, and allow the material to be decontaminated before being released.

Drainage traps create a water seal that prevents contaminated air within the containment zone from entering the piping, sewer or effluent decontamination systems. Where negative differential air pressures maintain inward directional airflow (IDA), a **deep seal trap** will prevent the water seal from being siphoned out of the trap, protecting against a breach of containment.

Separating drain lines and associated piping prevents the contamination of drain lines and piping that service areas outside the containment zone. Plumbing vent lines that are HEPA filtered or independent from those of lower containment levels and non-containment zone areas also protect against a breach of containment. The requirements pertaining to plumbing are specified in Matrix 3.6 of the CBS.

22.3.3.1 Sinks

The availability of handwashing sinks within the containment zone facilitates handwashing by personnel upon exit. It is preferable to have dedicated handwashing sinks, and to have them located near the points of exit from the containment zone. Locating handwashing sinks outside CL2 containment zones may be acceptable if appropriate measures (e.g., automatic doors or appropriate **standard operating procedures [SOPs]**) are taken to enable personnel to access handwashing sinks without contaminating other surfaces (e.g., door handles).

Handwashing sinks with "hands-free" capability, such as electronic eyes/infrared sensors, foot pedals, or elbow-controlled taps, prevent contamination of the sink area and the potential for recontaminating washed hands. The cost of hands-free faucets has become more reasonable in recent years, and they are rapidly becoming the norm in public washrooms. All levels of containment should be encouraged to upgrade to hands-free faucets on handwashing sinks as facilities are renovated.

22.3.3.2 Emergency Shower and Eyewash

Emergency eyewash and shower equipment, where required, provide on-the-spot treatment to flush out, dilute, and remove any infectious material or toxins that have contaminated the eyes, face, or body. The need for and placement of emergency eyewash stations and showers within a containment zone is based on an assessment of the activities performed and provincial/territorial occupational health and safety legislation. These devices are installed in accordance with American National Standards Institute (ANSI)/ International Safety Equipment Association (ISEA) standard Z358.1.[8] Further guidance on emergency shower and eyewash equipment is available from the Canadian Centre for Occupational Health and Safety (CCOHS).

22.4 Decontamination Technologies

Integration of dedicated **decontamination technologies**, such as autoclaves, effluent decontamination systems, and services to allow for full room gaseous decontamination, greatly impact the physical design of containment zones and present particular considerations during the design phase. Decontamination, including autoclaves, effluent decontamination systems,

and gaseous decontamination, is discussed in detail in Chapter 15. This section provides guidance on the special physical considerations for these technologies when designing and building a containment zone.

22.4.1 Autoclaves

Autoclaves are used to sterilize materials and reagents, and to decontaminate waste prior to disposal. Locating them outside the containment barrier will lead to additional procedures being put in place for the safe movement of contaminated waste. In high containment zones, autoclaves within the confines of the containment barrier allow for the decontamination of materials, including waste, prior to their removal from the containment zone. Locating double-door barrier autoclaves on the containment barrier facilitates the process. Pre-vacuum autoclaves function by first pumping air out of the decontamination chamber, prior to decontamination. This air is contaminated and must be filtered (e.g., with HEPA or 0.2μ filter) to prevent the release of pathogens and toxins. Consideration should be given to locating pre-vacuum autoclaves such that the air filter is accessible for routine inspection (e.g., annual) and replacement when necessary.

Autoclaves function with steam (self-generated or from a central supply) that condenses upon cooling, leading to potentially contaminated liquid waste. Autoclave condensate can either be channelled to containment zone drains for decontamination along with other containment zone liquid waste, or go through an autoclave auto-decontamination cycle that decontaminates the condensate prior to its release. It is important to note that it is the end user's responsibility to confirm that the auto-decontamination cycle provided with the autoclave has been programmed and verified for correct operation during the autoclave installation and that this cycle is effective against the pathogens or toxins being handled. Discharges from the autoclave chamber safety relief valves are similarly directed to containment zone drains, even when the body of the autoclave is located outside the containment barrier.

22.4.2 Effluent Decontamination Systems

An effluent decontamination system captures all liquid waste materials leaving the containment zone and decontaminates the waste prior to its release into sanitary sewers. In high containment zones, the effluent decontamination system should be designed to collect and treat all liquid waste generated within the containment zone (e.g., showers, toilets, floor drains in animal rooms, laboratory sinks). In high containment facilities, rooms that house an effluent decontamination system serving as the **primary decontamination technology** should be designed to contain the full volume of the largest holding tank in order to facilitate cleaning and decontamination in the event of a system failure. It may be possible to address this by the use of a sump with a feedback loop to secondary tanks. Effluent decontamination systems are discussed in Chapter 15, and the applicable physical containment requirements are specified in Matrix 3.8 of the CBS.

Accurate labelling of all drainage piping leading to an effluent decontamination system allows the correct identification of these components to facilitate a faster response in the event of a failure or leak. Utilizing gravity flow by sloping drain piping towards the effluent decontamination system reduces the risk of blockage in the piping, as does a mechanism to break down (e.g., grinder), collect (e.g., trap, strainer), and remove sludge and sediment. Where the drainage system is directly connected to the effluent decontamination vessels (i.e., without holding tanks), the inclusion of a mechanism to prevent full vessel pressure is a consideration in the event of failure of an inlet valve.

22.4.3 Whole Room Decontamination

Should the need arise, rooms can be decontaminated in their entirety by chemical decontamination of all surfaces or by using gaseous decontamination methods (CBS Matrix 4.8). Surfaces and materials (e.g., paint, caulking, adhesives) resistant to repeated exposures to the chemicals used will preserve the integrity of the containment barrier (CBS Matrix 3.4). The location of fumigation ports needed for certain gaseous methods should be carefully selected to facilitate effective decontamination.

22.5 Building Physical Components

The containment zone contains physical structures and materials that contribute to the containment of pathogens and toxins as well as providing security measures (biosecurity). These include windows, doors, containment zone surfaces (e.g., floors, walls), laboratory equipment, furniture, as well as the materials associated with them. This section provides guidance on containment zone physical structures and materials in order to facilitate compliance with the requirements specified in the CBS.

22.5.1 Windows on the Barrier

Windows located on the containment zone perimeter form part of the physical containment barrier. In CL2 laboratory work areas, non-fixed (i.e., openable) windows are acceptable, depending on the pathogen(s) in use (i.e., where prions and security sensitive biological agents [SSBAs] are not handled), provided that controls such as screens are in place to prevent the entry of insects and animals that are potential pathogen hosts or vectors (CBS Matrix 3.2). Consideration should be given to the location of non-fixed windows in relation to primary containment devices (e.g., BSC), whose airflow may be affected by drafts.

For CL2 SA zones, CL2 large scale production zones, large animal containment zones (LA zones) at all containment levels, and all high containment zones, fixed windows that are sealed maintain containment (biosafety) and biosecurity. Safety and security films and window glazing can protect against forced entry, breaking of window glass, and other environmental concerns, as well as reduce visibility into containment zones from the outside.

Windows that allow visual monitoring of activities in laboratory work areas, large scale production areas, and **animal work areas** inside high containment zones from an office or other area outside the containment barrier improves personnel safety and allows for quick emergency response and assistance. Other devices such as closed-circuit television (CCTV) may be an effective alternative where windows are not appropriate. Viewing windows into animal rooms and animal cubicles allow personnel within the containment zone to monitor animals without entering the rooms or cubicles; however, windows on the containment barrier are to prevent the public from viewing animal rooms, animal cubicles, and **post mortem rooms [PM rooms]**) as this could pose a biosecurity risk and compromise animal well-being (CBS Matrix 3.2).

22.5.2 Doors and Access

A door is an integral part of the containment barrier that separates the containment zone from public and administrative areas, while also providing a **security barrier** to limit access to the zone. The door will be the location where entry requirements (e.g., PPE, authorized persons) are posted to notify those who enter of the risks within the zone. Security barriers (i.e., a physical structure designed to prevent entry by unauthorized personnel), such as locked doors and **controlled access systems** can be incorporated to increase the security of a containment zone and to restrict access to authorized personnel only. Biosecurity and physical security considerations are further discussed in Chapter 6.

Doorways should be constructed large enough to allow for the passage of any large pieces of equipment (e.g., BSC, mass spectrophotometer) and any large-sized animals (e.g., cows, horses, moose) that may need to be moved into or out of the containment zone.

22.5.3 Door Interlocks and Anterooms

An anteroom is a room or a series of rooms at the entrance of CL2 large scale production areas, CL2 LA zones, and all high containment zones. Anteroom doors are used to separate the "clean" areas from the "dirty" areas, and allow for the movement of personnel or animals into or out of the containment zone. Preventing the anteroom doors from being opened simultaneously using an interlocking system will reduce the possibility of potentially contaminated air migrating outside the containment zone. Similarly, door **interlocks** and visual and audible alarms prevent personnel from opening both sides of a barrier autoclave or pass-through chamber simultaneously. In certain cases, it may be acceptable to use operational procedures to achieve the same intent as mechanical or electronic door interlocks.

Manual overrides on mechanically or electronically interlocked doors (e.g., button placed adjacent to each interlocked door) allow the manual release of interlocks, permitting personnel to open multiple doors simultaneously so multiple individuals can exit at the same time. This is critical in life-threatening emergency situations when personnel safety is the priority.

Sealable doors allow air leakage under normal operating conditions yet are capable of being sealed (e.g., gasket, weather stripping, door jambs, and door inserts or covers) to withstand gaseous decontamination. This can also be achieved with a tight fitting door and using tape and plastic film to create a seal around the door. **Airtight doors** found in some CL4 work areas and containment level 3 (CL3) LA zones (i.e., CL3-Ag zones) are always sealed when closed to prevent loss of containment in the event of HVAC system failure.

22.5.4 Materials and Surface Coverings

Containment facilities need to be designed so that they can be easily cleaned and decontaminated. The design and choice of materials used for walls, ceilings, floors, and barrier devices are critical for the containment zone to have the structural stability to withstand internal and external stresses.

Cleanable and resistant surface materials and finishes (e.g., paint, epoxy, and other protective finishes) provide protection against the stresses associated with activities performed inside the containment zone, which may include repeated decontamination (e.g., chemical, gaseous), frequent high pressure washing in animal containment zones, and activities causing impacts and scratches (e.g., movement of large-sized animals across floors, equipment resting on surfaces, animal cages). Other examples of surfaces in work areas that may become contaminated as a result of the procedures in use or spills of infectious material and require decontamination include animal holding units, interiors of drawers, cabinets, and shelves. Non-absorbent materials may include stainless steel, epoxy resin surfaces, or chemical resistant plastic laminate for benchtops, and urethane or vinyl for stools and chairs (CBS Matrix 3.4).

Containment barrier surfaces that are resistant to scratches, stains, moisture, chemicals, heat, impact, repeated decontamination, and high pressure washing, in accordance with function, will prevent the release of pathogens and toxins, and protect against the contamination of inaccessible spaces (e.g., under cracked paint). Similarly, benchtops that are a seamless one-piece design or sealed at seams will prevent contamination. Coved benchtops or installation of a backsplash sealed against the wall will provide a continuous barrier to prevent contaminated liquids from reaching inaccessible surfaces where the bench abuts a wall.

Continuous, non-pervious floors that are coved up the walls and cabinets help prevent spills from penetrating underneath. Slip-resistant floors (e.g., textured surfaces) can help avoid slips and falls by personnel and animals when the surface is wet. In animal containment zones, impact resistant floors can withstand the weight of animals and associated equipment without becoming gouged or cracked. In addition, floors can be designed to withstand prolonged contact with animal urine. Due to the **large volumes** of liquid required to clean animal rooms, animal cubicles, and PM rooms, floors can be sloped directly toward the floor drains in order to prevent pooling of contaminated liquids.

Using washable, hard non-porous paints on floors, walls, and ceilings will protect these surfaces and make them easier to clean and decontaminate. Solid-core materials (e.g., stainless steel, solid resin) should be used whenever possible; wood is not recommended when it can be avoided. Unfinished wooden and wood-finish walls or floors are not appropriate because they can absorb potentially infectious material, particularly liquids, making decontamination virtually impossible. Wood should only be used when it is sealed properly to prevent the absorption of liquid contamination. Where used, it is important to frequently inspect it to identify early any scratches or other damage from routine wear and tear that may result in reduced resistance to liquids, thereby increasing risk of absorbing infectious material. Surface materials that prevent the penetration of gases and liquids provide room integrity, facilitate surface and room decontamination, and help to contain any large volumes of contaminated liquids that may be present (e.g., animal wastes, large scale process fluids). Requirements relating to materials and surfaces are specified in Matrix 3.4 of the CBS.

22.5.5 Equipment and Furniture

Certain laboratory equipment will have specific operational needs and the location of large equipment should be considered when planning the floor space. For example, the operation of BSCs can be easily affected by air disturbances in their proximity, including as a result of nearby refrigeration equipment that generates a large amount of heat. As such, BSCs should be located away from high traffic areas, doors, non-fixed windows, and air supply/exhaust diffusers and other sources that may disrupt the protective air curtain of the BSC (CBS Matrix 3.7). BSCs are further discussed in Chapter 11.

It is always important to position large and integral equipment (e.g., large reusable equipment for large scale activities, fermenters) in the containment zone to allow for safe workflow and traffic flow patterns. Further safety considerations for equipment commonly used for biological work in laboratories are discussed in Chapter 12; large scale activities are further discussed in Chapter 14.

Furniture constructed from wood or with exposed wood surfaces is not practical in containment zones. Wood should only be used when it is sealed properly to prevent the absorption of liquid contamination. Instead, non-absorbent materials, such as urethane or vinyl, can be used for stools and chairs in containment zones so that they are liquid-resistant and can be easily cleaned and decontaminated when necessary. It is often preferable to have easily reconfigurable furniture (e.g., lab benches, shelving, and work stations) that allows for modification in order to accommodate large equipment and changing work priorities. Smooth rims and corners on furniture, drawers, benches, doors, handles, and shelving should always be considered to protect against compromising PPE (e.g., rip or tear). In addition, spaces between benches, cabinets, and equipment should be accessible for cleaning and decontamination when necessary, as well as to allow for the servicing of equipment. For example, it is unadvisable to position a Class II BSC in a limited concave space, as this might not allow the BSC certifier adequate space to remove panels from the cabinet when performing on-site field testing and certification of the unit.

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CHAPTER **23**

REGULATORY OVERSIGHT OF HUMAN AND ANIMAL PATHOGENS AND TOXINS IN CANADA

CHAPTER 23 – REGULATORY OVERSIGHT OF HUMAN AND ANIMAL PATHOGENS AND TOXINS IN CANADA

Controlled activities with human **pathogens** or **toxins** conducted in Canadian **facilities**, such as public health **laboratories**, teaching and research laboratories, diagnostic laboratories in hospitals, and vaccine production plants, are regulated under the *Human Pathogens and Toxins Act* (HPTA) and the *Human Pathogens and Toxins Regulations* (HPTR).[1,2] Importation of animal pathogens, or animals, animal products or by-products, or other organisms that carry an animal pathogen or part of one that retains its **pathogenicity** (e.g., toxins) into Canada are regulated under the *Health of Animals Act* (HAA) and the *Health of Animals Regulations* (HAR).[3,4] **Zoonotic pathogens**, capable of causing **disease** in human and animal hosts, that are imported into Canada are regulated under the HPTA, HPTR, HAA, and HAR.

23.1 Regulatory Authorities

The Public Health Agency of Canada (PHAC) is the national authority on **biosafety** and **biosecurity** for human pathogens and toxins. The PHAC is responsible for the regulation of human pathogens and toxins under the authority of the HPTA and the HPTR and the importation or **transfer** of **terrestrial animal pathogens** and toxins, with the exception of **non-indigenous animal pathogens, emerging animal disease** pathogens, and animal pathogens in animals, animal products, animal by-products, or other organisms, under the authority of the HAA and the HAR.

The Canadian Food Inspection Agency (CFIA) is the national expert on biosafety and biosecurity for foreign animal diseases and emerging animal diseases. The CFIA is responsible for the regulation of the importation or transfer of non-indigenous animal pathogens and emerging animal disease pathogens, as well as animals, animal products, and animal by-products that contain a terrestrial animal pathogen, under the authority of the HAA and HAR. The CFIA is also responsible for the regulation of the importation or transfer of aquatic animal pathogens and bee pathogens under the HAA and HAR.

The *Canadian Biosafety Standard* (CBS), 2nd Edition, 2015 is a harmonized national standard for the handling and storing of human and terrestrial animal pathogens and toxins in Canada.[5] The CBS specifies the **physical containment requirements**, **operational practice requirements**, and **performance and verification testing requirements** for **containment zones** where human and terrestrial animal pathogens and toxins are handled or stored. By condition of **licence** or **animal pathogen import permit**, the CBS establishes the criteria for any containment zone where human or terrestrial animal pathogens or toxins are to be safely handled or stored. The CBS is used by the PHAC and the CFIA to verify the ongoing compliance of facilities regulated under the HPTA and HPTR, and **importing** animal pathogens under the HAA and HAR in support of applications and renewals for licences for controlled activities with human pathogens or toxins, animal pathogen import permits, and, where applicable, the **facility certification** (and recertification) of containment zones.

23.2 Controlled Activities with Human Pathogens and Toxins

As specified in Section 7(1) of the HPTA, unless otherwise exempted (discussed in Section 23.2.1, below), a licence must be obtained from the PHAC to authorize any of the following controlled activities with human pathogens and toxins:

- possessing, handling or using a human pathogen or toxin;
- producing a human pathogen or toxin;
- storing a human pathogen or toxin;
- permitting any person access to a human pathogen or toxin;
- transferring a human pathogen or toxin to another **facility**;
- importing or **exporting** a human pathogen or toxin;
- releasing or otherwise abandoning a human pathogen or toxin; or
- disposing of a human pathogen or toxin.

The PHAC can issue a licence under the HPTA and HPTR to authorize one or more controlled activities with human pathogens and toxins. The licence specifies which of the controlled activities identified in Section 7(1) of the HPTA are authorized by the PHAC. A controlled activity that is not listed on the licence is not authorized under that licence. The licence also specifies the facility or facilities in which the controlled activities are authorized. An application to amend a licence can be submitted to add a new controlled activity or new facility not originally authorized on the licence.

The person to whom the licence has been issued is identified as the "licence holder". The HPTA and HPTR detail specific requirements and obligations of a licence holder. A summary of these is described in Chapter 1 of the CBS, although regulated parties are encouraged to refer to the specific sections of the HPTA and HPTR for a complete understanding of their obligations under the HPTA and HPTR. The licence holder can be an individual person or an organization (e.g., a corporation, society, company, firm, partnership, or association of persons); the licence holder may be an organization that represents multiple facilities (e.g., a university or district health authority), although an individual will have to be identified to sign the licence application on behalf of the organization. Selection of the licence holder is determined by the organization and can be supported by existing internal administrative oversight arrangements.

23.2.1 Exclusions and Exemptions

23.2.1.1 Exclusions from the Human Pathogens and Toxins Act

As specified in Section 4 of the HPTA, the HPTA does not apply to a human pathogen or toxin that is in an environment in which it naturally occurs if it has not been cultivated or intentionally collected or extracted. This includes a human pathogen or toxin that:

- is in or on a human suffering from a disease caused by that pathogen or toxin;
- has been expelled by a human suffering from a disease caused by that pathogen or toxin;
- is in or on a cadaver, body part, or other human remains; or
- is a drug in dosage form whose sale is permitted or otherwise authorized under the *Food and Drugs Act* or a human pathogen or toxin contained in such a drug.[6]

On the condition that procedures are not performed to intentionally increase the concentration (e.g., centrifugation, chromatography) or the amount (e.g., **propagation** or culture) of the pathogen or toxin, the natural environment of a pathogen or toxin can include blood, plasma, other bodily fluids, and tissue samples. For example, primary specimens collected from humans (e.g., blood specimens) known or suspected to contain a human pathogen or toxin are excluded from the HPTA and, by consequence, the HPTR, provided the human pathogen or toxin itself is not the object of cultivation or intentional collection or extraction. As a result, such specimens do not require a licence and are not subject to any requirements in the HPTA. The natural environment of some human pathogens can also include a living animal host, or primary specimens, such as blood, plasma, excretion, other bodily fluids, tissue samples, body parts (or carcass), collected from an animal host; however, a human pathogen in an animal that has been experimentally or intentionally infected is not in its natural environment and is therefore not excluded from the HPTA and HPTR (and is subject to all requirements, including a licence). Human pathogens that are also animal pathogens (i.e., zoonotic pathogens) would still require an animal pathogen import permit issued by the PHAC or the CFIA for importation into Canada (further discussed in Section 23.3).

23.2.1.2 Exemptions

There are several exemptions from the specific licence requirements under the HPTA. As specified in Section 7(2) of the HPTA, a licence is not required for:

- any activity to which the *Transportation of Dangerous Goods Act, 1992* applies (discussed in Chapter 20);[7] or
- the export of human pathogens or toxins authorized under the *Export and Import Permits Act* (discussed in Section 23.5.3).[8]

As specified in Section 37 of the HPTA, a licence is also not required for:

a. an inspector or analyst carrying out their functions under the HPTA;

b. a peace officer carrying out their functions under any federal or provincial Act or a person providing assistance to that peace officer;

c. any person who, in the course of their employment, outside a facility in which controlled activities are authorized, collects a sample for the purpose of laboratory analysis or diagnostic testing; or

d. in exigent circumstances, any person carrying out their functions under any federal or provincial Act.

As specified in Section 27 of the HPTR, the following controlled activities with human pathogens and toxins are exempted from the licence requirements under the HPTA and HPTR:

- *Laboratory analyses or diagnostic testing* (HPTR 27[1]): A person who carries out laboratory analyses or diagnostic testing with a human pathogen (other than a **prion** or **security sensitive biological agent [SSBA]**) does *not* require a licence on condition that:
  - they are not cultivating or otherwise producing a human pathogen (e.g., tests such as blood counts, blood chemistry tests, centrifugation of blood specimens to separate serum or plasma); or
  - if there is any production (e.g., propagation, culture, or concentration) of a human pathogen, it is exclusively performed in a sealed container that prevents the **release** of the pathogen, and the container remains sealed and unopened until it is decontaminated (including all contents) before its disposal or reuse.
- *Veterinary practices* (HPTR 27[2]): A veterinarian (or anyone under their supervision) who is registered under a provincial or territorial law is exempt from the licence requirements under the HPTA and HPTR when conducting diagnostic testing or laboratory analyses with an RG2 human pathogen on the condition that any controlled activities are conducted in the course of providing care to animals in a clinical practice in that province or territory.

Even when a facility is exempt from the licence requirements of the HPTA and HPTR, persons knowingly conducting any controlled activity involving a human pathogen or toxin continue to have a responsibility to take all reasonable precautions to protect the health and safety of the public against the **risks** posed by that activity (HPTA 6). Persons that are exempt from the licence requirements are still subject to other sections of the HPTA. They remain prohibited from the possession of smallpox (HPTA 8), and their facilities may be subject to inspection by the PHAC. For persons exempt from the licence requirements under the HPTA and HPTR, it is generally considered best practice to demonstrate, by following the physical and operational practice requirements specified in the CBS, that all reasonable precautions have been taken to protect the health and safety of the public against the risks associated

with the materials in their possession. Additional guidelines are available from the PHAC to further support those who are exempt from the licence requirements where following the CBS requirements may not be achievable; please contact the PHAC directly for further information, or visit the PHAC website (www.publichealth.gc.ca/pathogens).

23.2.2 Types of Licences

The PHAC issues four different types of licences, based on the inherent risks associated with the human pathogen(s) or toxin(s) (i.e., **risk group**, prion, SSBA, quantity) and the factors outlined above. The PHAC may issue to an applicant, up to the maximum period as specified in HPTR 2(2), a licence as follows:

1. a licence, up to a maximum of 5 years, that authorizes controlled activities with:
   i. RG2 human pathogens, and/or
   ii. RG3 prions, and/or
   iii. toxins that are not prescribed under HPTR section 10 (i.e., excludes SSBA toxins in quantities above their assigned **trigger quantity**);
2. a licence, up to a maximum of 3 years, that authorizes controlled activities with toxins that are prescribed under HPTR 10 (i.e., SSBA toxins in quantities above their assigned trigger quantity)
3. a licence, up to a maximum of 3 years, that authorizes controlled activities with RG3 human pathogens (this may include or exclude RG3 human pathogens that are also SSBAs); and
4. a licence, up to a maximum of 1 year, that authorizes controlled activities with RG4 human pathogens (this may include or exclude RG4 human pathogens that are also SSBAs).

The general conditions that apply to every licence are specified in Section 4 of the HPTR. Additional conditions of licence may also be imposed (HPTA 18[4]). The licence holder is responsible to inform all persons conducting controlled activities authorized by the licence of its conditions (HPTA 18[6]). The licence holder and all persons conducting controlled activities under a licence must comply with those conditions (HPTA 18[7]).

The "Pathogen and Toxin Licence" is the regulatory authorization document issued by the PHAC to a licence holder and is both a licence for controlled activities with human pathogens and toxins under the HPTA, and, when also identified on the document itself, an animal pathogen import permit for the importation or movement of terrestrial animal pathogens under the authority of the PHAC, in accordance with the HAR. The importation of animal pathogens is further discussed in Section 23.3. Applications for new licences or for amendments or renewal of licences can be submitted electronically through the Biosecurity Portal, accessible through the PHAC website (www.publichealth.gc.ca/pathogens).

Before issuing a licence, the PHAC must be of the opinion that the conduct of the controlled activity (or activities) with human pathogens and toxins in any facility under that licence would not pose undue risk to the health or safety of the public. Therefore, the amount and detail of information submitted in a licence application is proportional to the risk and types of human pathogens and toxins. For example, there are heightened security risks associated with SSBAs; therefore, the PHAC will require documentation that demonstrates how these risks will be controlled and managed for any licence application for controlled activities with an SSBA. As such, applicants applying for a licence involving SSBAs are required to submit their biosecurity plan, in addition to the requirement to obtain or apply for HPTA Security Clearances for any individuals working under that licence. Biosecurity Plans and HPTA Security Clearances are discussed further in Chapter 6.

Furthermore, as specified in Section 3 of the HPTR, an applicant who intends to carry out **scientific research**, before issuing the licence, the PHAC must determine that the person has developed a plan that sets out administrative measures for managing and controlling biosafety and biosecurity risks during the period in which the licence is in effect. A detailed **risk management plan**, or *Plan for Administrative Oversight for Pathogens and Toxins in a Research Setting* (the "Plan"), is required to be submitted in support of a licence application where scientific research is intended. These Plans are intended to be very high level (i.e., at the institutional/organizational level) and are not intended to include or repeat any regulatory elements already captured through other means, such as the CBS. Further details on the Plan, including a summary of the elements to be included, can be found in Appendix A. Administrative controls are further described in Chapter 5.

23.3 Importation of Animal Pathogens into Canada

The importation into Canada of an animal pathogen or part of one that retains its pathogenicity (e.g., toxins) or animals, animal products (e.g., cream, milk, eggs non-fertilized ova, semen), animal by-products (e.g., blood, serum, tissues, cells, bones, flesh, skins, hides, hair, feathers, wool), or other organisms that carry an animal pathogen or part of one that retains its pathogenicity is regulated by the PHAC or the CFIA under the authority of the HAA and HAR. Importation of animal pathogens under the HAA and HAR is authorized by the issuance of an animal pathogen import permit by the PHAC or the CFIA. A person who wishes to import an animal pathogen, toxin, or other regulated material must obtain an animal pathogen import permit prior to the time of importation of the material (HAR 51). The HAA and HAR detail specific requirements and obligations of a person handling material imported under an animal pathogen import permit. A summary of these is described in Chapter 1 of the CBS, although regulated parties are encouraged to refer to the specific sections of the HAA and HAR for a complete understanding of their obligations under the HAA and HAR.

The PHAC issues animal pathogen import permits under the authority of the HAR for:

- cultures of indigenous terrestrial animal pathogens;
- purified or synthesized samples of toxins derived from indigenous terrestrial animal pathogens; and
- indigenous terrestrial animal pathogens or part of one carried in or on a substance other than an animal, animal product, animal by-product, or other organism (e.g., human specimens, plant tissues).

The CFIA issues animal pathogen import permits under the authority of the HAR for:

- cultures of non-indigenous terrestrial animal pathogens;
- cultures of emerging animal disease pathogens;
- purified or synthesized samples of toxins derived from non-indigenous terrestrial animal pathogens or emerging animal disease pathogens;
- animals, animal products, animal by-products, and other organisms carrying an animal pathogen or part of one;
- non-indigenous terrestrial animal pathogens, emerging animal disease pathogens, or parts of one carried in or on a substance other than an animal, animal product, animal by-product, or other organism (e.g., human specimens, plant tissues);
- aquatic animal pathogens (in any form); and
- bee pathogens (in any form).

The PHAC issues a regulatory authorization document titled a "Pathogen and Toxin Licence", which is an animal pathogen import permit for the importation or movement of terrestrial animal pathogens under the authority of the PHAC, in accordance with the HAR, when identified on the document itself. The movement to another location (e.g., a containment zone not identified on the original animal pathogen import permit) of material that has been imported into Canada under an animal pathogen import permit is prohibited, unless it has been pre-authorized on the animal pathogen import permit under which it was imported or authorized under another permit (e.g., written authorization or Transfer Permit) issued for that purpose by the appropriate agency (the PHAC or the CFIA) for the material (HAR 51.1[a]). Likewise, introduction into an animal (e.g., inoculation) of material that has been imported under an animal pathogen import permit is also prohibited, unless it has been pre-authorized on the animal pathogen import permit under which it was imported or authorized under another permit for that purpose issued by the appropriate agency (the PHAC or the CFIA) for the material (HAR 51.1[b]). Please refer to the conditions of permit for further information on prohibitions and restrictions that may also apply. For further information on transfers, please contact the appropriate issuing agency (the PHAC or the CFIA).

23.3.1 Facility Certification for the Importation of Animal Pathogens

Facility certification is the formal acknowledgement from the CFIA that a containment zone or facility where imported animal pathogens will be handled or stored complies with the physical **containment**, operational practice, and performance and verification testing requirements specified in the CBS. Before issuing an animal pathogen import permit, the CFIA must be satisfied that the activities for which the permit is issued would not result in the introduction into Canada, into another country from Canada, or the spread within Canada of the pathogen (HAR 160[1.1]). Applicants of an animal pathogen import permit from the CFIA may be subject to facility certification or compliance verification to demonstrate satisfactory evidence that the containment zone meets the necessary requirements.

For higher containment zones, the facility certification process may include an onsite inspection, a review of as-built drawings and specifications, **commissioning** and performance and verification testing reports of critical physical containment systems, the **Biosafety Manual**, the containment zone SOPs, and, for work with RG4 pathogens, a review of training records. The performance and verification testing of containment systems required for facility certification is specified in Chapter 5 of the CBS. For lower containment zones, compliance verification could include the completion of a compliance checklist.

Facilities certified by the CFIA may require annual recertification. Recertification may comprise a review of documentation such as program intent and the performance and verification testing reports of critical containment systems to verify that the zones continue to comply with the requirements specified in the CBS. A request to change the **program intent** is to be submitted to the CFIA along with updated SOPs and the Biosafety Manual before implementation of any changes to the program intent. Changes to program intent may include, but are not limited to, the introduction of new pathogens, new animal species, or changes to procedures which could alter the risk of personnel exposure or the risk of pathogen release from the containment zone. In some cases, an on-site inspection may be required by the CFIA to verify continued compliance before facility recertification is granted. Further information, instructions, checklists, and forms on the specific documents required and the facility certification and recertification process can be obtained from the CFIA website or by contacting the CFIA directly.

23.4 Activities with Zoonotic Pathogens

Zoonotic pathogens are capable of causing disease in both human and animal hosts. As a result, they are subject to regulation under the HPTA and HPTR as human pathogens (described in Section 23.2) and the HAA and the HAR as animal pathogens (described in Section 23.3). The importation of cultures of zoonotic pathogens may require authorizations from both the PHAC (as human pathogens) and the CFIA (as animal pathogens). For further information on the regulation of zoonotic pathogens, please contact either the PHAC or the CFIA, or visit their websites.

23.5 Additional Regulatory Considerations for Pathogens and Toxins

This section describes additional federal legislation that may also impact the importation, production, and the exportation of pathogens and toxins beyond the regulatory authorities of the PHAC and the CFIA.

23.5.1 Importation of Human and Animal Pathogens and Toxins

The Canada Border Services Agency (CBSA) provides integrated border services that support national security and public safety priorities and, at the same time, facilitate the free flow of persons and goods. Under the *Customs Act*, CBSA Customs Officers have the authority to detain and examine any goods at the Canadian border.[9] The CBSA provides administrative support at Canadian points of entry for imported pathogens and toxins under the authorities of the PHAC or the CFIA. The activity of importing pathogens and toxins by an individual or an entity for sale or for any industrial, occupational, institutional, or other similar use in Canada is considered by the CBSA to be importing for "commercial use".[10] The Government of Canada has established an integrated Single Window Initiative (SWI), administered by the CBSA, to reduce the paper burden associated with goods imported for commercial use by providing an electronic interface for all required importation documentation.[11] Through the SWI, importers (or licence holders) and their customs brokers can electronically submit import or export data, accounting documents, and any other information to the CBSA that is required to comply with and any relevant importation legislation. In turn, the CBSA transmits this information to the appropriate government department(s) or agency (or agencies) responsible for regulating the goods to assess the information and provide any necessary border-related decisions. This electronic interface streamlines and simplifies the importation process by permitting pre-border clearance of imported goods, thereby reducing delays at the border. This also facilitates the processing of low-risk goods through customs and allows resources to focus on higher-risk goods that could pose potential threats to Canada's safety and security. For more information regarding the SWI, please contact the CBSA or visit its website.

Through the SWI, the PHAC receives from the CBSA an electronic record of each importation that contains human or animal pathogens or toxins under a licence or animal pathogen import permit issued by the PHAC. The CBSA may detain and refer imports of human and terrestrial animal pathogens and toxins at the request of the PHAC to determine that the import requirements under the HPTA, HPTR, HAA, and HAR have been met. CBSA officers follow the PHAC's direction to release or refuse entry of these goods based on the PHAC's decision. In order to complete importation documentation, it is necessary for licence holders or importers to provide their appropriate Pathogen and Toxin Licence number or animal pathogen import permit number, the Canadian Product Category (CPC), and the Intended Use Code (IUC) of the imported material in advance to their purchasing department or customs broker.[12] At the time of publication, SWI is not a mandated process. Interested brokers have commenced the certification process with CBSA to move to SWI, and will continue to do so during the implementation phase that is scheduled to be complete in 2017. For more detailed information on importing goods regulated by the PHAC, including the CPC and IUC codes, please visit the PHAC website (www.publichealth.gc.ca/pathogens).

When importing an animal pathogen under an animal pathogen import permit issued by the CFIA, the import permit holder (or delegate) is responsible to make certain that a copy of the animal pathogen import permit and any other necessary importation documentation accompanies the shipment across the border. The CFIA has established an electronic interface with the CBSA, similar to the SWI, that allows importers and brokers to transmit release information on CFIA-regulated goods to the CBSA.[13] In turn, the CBSA transmits this information to the CFIA to make a decision regarding the admissibility of the goods, and, through the interface, can transmit its decision directly to the CBSA inspector. The CBSA then makes a final decision. Through this system, importers and brokers have easy access to current information on import requirements, and additional data (codes) that must be included in the release message. In addition, under the HAA, the CBSA may designate inspectors under the *Canada Border Services Agency Act* to enforce the HAA.[14] Further information on CFIA-regulated importations, such as Harmonized System Codes for importations of animal pathogens, toxins, and other material regulated by the CFIA under the HAA and HAR, can be obtained through the Automated Import Reference System (AIRS) on the CFIA website (http://www.inspection.gc.ca/airs).

23.5.2 Regulation of New Substances (New Organisms) in Canada

Certain new living organisms, including **microorganisms**, proposed for importation into or production within Canada are subject to the *Canadian Environmental Protection Act* 1999 (CEPA 1999) and the *New Substances Notification Regulations (Organisms)* (NSNR[O]).[15,16] A new substance (e.g., a microorganism developed through **biotechnology**) requires notification under CEPA 1999 prior to importation into or manufacture in Canada, if it is not already found on the *Domestic Substances List* (DSL). This legislation aims to protect both the environment and human health from potentially harmful new substances that are animate products of biotechnology (i.e., living organisms, including both naturally occurring and genetically modified forms). The NSNR(O) applies to both microorganisms used in microbial products or to produce various biomolecules, as well as a variety of "higher" organisms, such as fish, livestock, and insects (depending on the use). The NSNR(O) includes exemptions for microorganisms that are research and development organisms not intended for introduction outside of a containment facility. Persons conducting research and development activities with new microorganisms resulting from biotechnology should refer to the NSNR(O) for a complete understanding of the exemptions and when they can be applied. To avoid regulatory duplication, those organisms regulated under the *Seeds Act*, *Feeds Act*, *Fertilizers Act* (all administered by the CFIA), and HAA (with respect to veterinary biologics, administered by the CFIA), and the *Pest Control Products Act* (administered by the Pest Management Regulatory Agency) are exempt from the NSNR(O) for products or activities already covered by the legislation.[17, 18,19, 20] The NSNR(O) does not apply to a microorganism that is imported for use that is regulated under other acts or regulations (e.g., HPTA, HAA).

Environment and Climate Change Canada, in conjunction with Health Canada, is responsible for conducting environmental and indirect human health risk assessments, respectively, for new organisms in products regulated under the *Food and Drugs Act* (e.g., novel foods, human biologics, and food additives) and recommending any needed risk management measures. Enforcement of the NSNR(O) is the responsibility of Environment and Climate Change Canada's Enforcement Branch. For more information regarding the NSNR(O), please contact Environment and Climate Change Canada or visit its website (http://www.ec.gc.ca/subsnouvelles-newsubs/default.asp?lang=En&n=E621534F-1).

23.5.3 Exportation of Pathogens from Canada

When transporting regulated materials to another country, it is the responsibility of the person sending the material (i.e., the shipper) to make certain that all necessary documentation accompanies the shipment, including any importation documents required by the recipient country. Prior to the export of human pathogens or toxins, the person exporting the material must take reasonable care to be satisfied that the recipient has the appropriate containment zone in which to handle the material and that the recipient will conduct activities in accordance with any applicable **biosafety** and **biosecurity** standards and policies in the foreign jurisdiction (HPTR 4[1]). For example, before shipping a sample of an RG2 human

pathogen or toxin to an address in the United States, the person intending to export the material should verify and document that the intended recipient has access to and will work with the material in a suitable containment facility that meets biosafety level 2 as described in the current edition of *Biosafety in Microbiological and Biomedical Laboratories*, published by the United States Centers for Disease Control and Prevention (CDC) and the United States National Institutes of Health (NIH).[21] As a further example, before shipping a sample of an RG2 human pathogen or toxin to an address in France, the person intending to export the material should verify and document that the intended recipient has access to and will work with the material in a suitable containment facility that meets **containment level** 2 (CL2) as described in the current edition of *Manuel de Sécurité et de Sûreté Biologiques*, published by the Société Française de Microbiologie.[22]

Canada is a State Party to the 1972 *Convention on the Prohibition of the Development, Production and Stockpiling of Bacteriological (Biological) and Toxin Weapons and on their Destruction*, commonly known as the Biological and Toxin Weapons Convention (BTWC). The BTWC aims to prevent the proliferation of biological and toxin weapons through the prohibition of the development, production, stockpiling, acquisition, or retention of microbial or other biological agents or toxins, whatever their origin or method of production, of types and in quantities that have no justification for prophylactic, protective, or other peaceful purposes, and of weapons, equipment, or means of delivery designed to use such agents or toxins for hostile purposes or in armed conflict.

To help fulfil their obligations under the BTWC and the Chemical Weapons Convention (CWC), many national governments around the world, including Canada, participate in the informal forum known as the Australia Group and have developed harmonized export controls for chemical weapons and chemical weapon precursors, human, animal, and plant pathogens and toxins with **dual-use potential**, and dual-use manufacturing facilities, equipment, technology, and software, as well as other items that could be used to test or disseminate controlled chemical or agents or for protection against them. The Australia Group maintains common lists for export control, including the *List of Human and Animal Pathogens for Export Control*, which can be viewed on its website (www.australiagroup.net).[23]

In Canada, such controls have been implemented through Groups 2 and 7 of the schedule to the *Export Control List* (ECL). The Export Controls Division of Global Affairs Canada (GAC) is responsible for the administration of export controls for strategic goods and technology under the authority of the *Export and Import Permits Act*.[8] Residents of Canada wishing to export any goods or technology listed on the ECL must first receive a Permit to Export from GAC. For more information, please contact GAC or visit its website (www.exportcontrols.gc.ca).

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CHAPTER 24

GLOSSARY

CHAPTER 24 – GLOSSARY

It is important to note that while some of the definitions provided in the glossary are universally accepted, many of them were developed specifically for the *Canadian Biosafety Standard* (CBS), 2nd Edition or the *Canadian Biosafety Handbook* (CBH), 2nd Edition; therefore, some definitions may not be applicable to facilities that fall outside of the scope of the CBS and the CBH. The words and phrases defined in this glossary appear in **bold type** upon first usage in each chapter throughout the CBH.

Accident	An unplanned event that results in injury, harm, or damage.
Administrative area	Dedicated room or adjoining rooms that are used for activities that do not involve infectious material and toxins. Administrative areas do not require any containment equipment, systems, or operational practices. Examples of administrative areas include offices, photocopy areas, and meeting/conference rooms.
Aerosol	A suspension of fine solid particles or liquid droplets in a gaseous medium (e.g., air) that can be created by any activity that imparts energy into a liquid/semi-liquid material.
Airborne pathogen	A pathogen that is capable of moving through or being carried by the air.
Airtight doors	Doors that are designed to allow no leakage of air (0%) under normal operating conditions and to withstand pressure decay testing and gaseous decontamination. Airtight doors can be achieved with inflatable or compression seals.
Animal cubicle	A room or space designed to house an animal (or animals) where the room itself serves as primary containment. These spaces are used to house large-sized animals (e.g., livestock, deer), or small-sized animals that are housed in open caging (i.e., not primary containment caging).
Animal health surveillance program	A program that monitors the health of animals brought into and housed in a containment facility in order to identify, treat, and/or prevent infections or diseases that may either affect research results or that may cause laboratory acquired infections/intoxications in facility personnel.

Animal pathogen	Any pathogen that causes disease in animals; including those derived from biotechnology. In the context of the *Canadian Biosafety Standard* and the *Canadian Biosafety Handbook*, "animal pathogen" refers only to pathogens that cause disease in terrestrial animals; including those that infect avian and amphibian animals, but excluding those that cause disease in aquatic animals and invertebrates.
Animal pathogen import permit	A permit issued by the Public Health Agency of Canada or the Canadian Food Inspection Agency for the importation into Canada of: animal pathogens or toxins; animals, animal products, animal by-products, or other organisms carrying an animal pathogen or part of one; under Section 51(a) and (b) of the *Health of Animals Regulations*.
Animal room	A room designed to house animals in primary containment caging. These spaces are used to house only small-sized animals (e.g., mice, rats, rabbits).
Animal work area	A room or space dedicated to housing or conducting activities with animals.
Anteroom	A room, or series of rooms, inside the containment zone, used to separate "clean" areas from "dirty" areas (i.e., area with a lower risk of contamination from those with a higher risk of contamination), for personnel and animal entry/exit across the containment barrier, and for entry to/exit from animal rooms, animal cubicles, and post mortem rooms. The negative differential air pressures required in containment zones where inward directional airflow is provided can be more effectively maintained through the presence of an anteroom. An anteroom may also provide appropriate space at the entry/exit point(s) to don, doff, and store dedicated containment zone clothing and additional personal protective equipment, as required.
(Biological) assets	All of the pathogens, infectious material, and toxins in the possession of a facility. Other assets include materials, equipment, non-infectious material, animals, knowledge and information (e.g., protocols, research findings), and personnel in a facility.

Authorized personnel	Individuals who have been granted unsupervised access to the containment zone by the containment zone director, biological safety officer, or another individual to whom this responsibility has been assigned. This is dependent on completing training requirements and demonstrating proficiency in the standard operating procedures, as determined to be necessary by the facility.
Autologous cells	Cells derived from an individual's own body.
Backdraft protection	A system that protects the air supply to the containment zone from contamination in the event of a reversal of airflow. High efficiency particulate air (HEPA) filters or isolation dampers are commonly used to prevent contamination from reaching areas of lower containment.
Backflow prevention	A system that protects the water supply to the containment zone from contamination. Many types of backflow devices also have test ports so that they can be checked to ensure that they are functioning properly.
Biocontainment	See "containment".
Biological material	Pathogenic and non-pathogenic microorganisms, proteins, and nucleic acids, as well as any biological matter that may contain microorganisms, proteins, nucleic acids, or parts thereof. Examples include, but are not limited to, bacteria, viruses, fungi, prions, toxins, genetically modified organisms, nucleic acids, tissue samples, diagnostic specimens, live vaccines, and isolates of a pathogen (e.g., pure culture, suspension, purified spores).
Biological safety cabinet (BSC)	A primary containment device that provides protection for personnel, the environment, and the product (depending on BSC class), when working with biological material.
Biological safety officer (BSO)	An individual designated for overseeing the facility's biosafety and biosecurity practices.
Biosafety	Containment principles, technologies, and practices that are implemented to prevent unintentional exposure to infectious material and toxins, or their accidental release.

Biosafety Manual	A facility-specific manual that describes the core elements of a biosafety program (e.g., biosecurity plan, training, personal protective equipment).
Biosecurity	Security measures designed to prevent the loss, theft, misuse, diversion, or intentional release of pathogens, toxins, and other related assets (e.g., personnel, equipment, non-infectious material, and animals).
Biosecurity risk assessment	A risk assessment in which the pathogens, toxins, infectious material, and other related assets (e.g., equipment, animals, information) in possession are identified and prioritized, the threats and risks associated with these materials are defined, and appropriate mitigation strategies are determined to protect these materials against potential theft, misuse, diversion, or intentional release.
Biotechnology	The application of science and engineering to the direct or indirect use of living organisms or parts or products of living organisms in their natural or modified forms.
Cell line	A cell population with uniform genetic characteristics derived from a single cell or homogenous tissue provided by a source of human or animal (including avian, amphibian, or insect) origin. Primary cell lines are grown from a primary specimen derived from a single clinical or research subject. Immortalized cell lines can proliferate indefinitely due to mutation, either through spontaneous mutations resulting from infection by a virus, or through genetic modification using recombinant DNA technology.
Chemical fume hood	An enclosed workspace that is ventilated by an induced flow of air through the front opening and is intended to protect personnel from hazardous gases, vapours, mists, aerosols, and particulates generated during the manipulation of chemical substances.
"Clean" change area	The designated space where dedicated personal protective equipment is donned when entering the containment zone, animal cubicle, or post mortem room. The "clean" change area is considered to be free from contamination when entry and exit procedures are routinely followed. In high containment zones, the "clean" change area is located outside the containment barrier.

Closed system	An apparatus or process system designed to contain biological material and prevent its release into the surrounding environment.
Commissioning	A process whereby a newly constructed containment zone, or a newly modified or renovated containment zone, is subjected to a series of performance and verification tests to ensure that the finished containment zone, including equipment and containment systems, will operate in accordance with the physical design intent and specifications and is ready to be put into operation, or resume activities involving pathogens and toxins, respectively.
Community	Encompasses both human (i.e., the public) and animal populations.
Confinement	A situation where only certain containment components are implemented. During specific periods of time subsequent to inoculation with certain pathogens, natural excretions and casual contact with infected animals would not pose a significant risk for pathogen transmission. Thus, while the infected animals should always remain adequately confined, they are not housed and maintained within a containment facility.
Containment	The combination of physical design parameters and operational practices that protect personnel, the immediate work environment, and the community from exposure to biological material. The term "biocontainment" is also used in this context.
Containment barrier	The boundary between "clean" and "dirty" areas (i.e., between the laboratory work areas, animal rooms, animal cubicles, or post mortem rooms, and outside of that containment area). Where inward directional airflow is provided, a physical containment barrier of air is established to protect against airborne or aerosolized infectious material or toxins from reaching the "clean" areas.
Containment level (CL)	Minimum physical containment and operational practice requirements for handling infectious material or toxins safely in laboratory, large scale production, and animal work environments. There are four containment levels ranging from a basic laboratory (containment level 1 [CL1]) to the highest level of containment (containment level 4 [CL4]).

Containment system	Dedicated equipment that functions to provide and maintain containment. This includes, but is not limited to, primary containment devices (e.g., biological safety cabinets), heating, ventilation, and air conditioning (HVAC) and control systems, and decontamination systems (e.g., autoclaves).
Containment zone	A physical area that meets the requirements for a specified containment level. A containment zone can be a single room (e.g., containment level 2 [CL2] laboratory), a series of co-located rooms (e.g., several non-adjoining but lockable CL2 laboratory work areas), or it can be comprised of several adjoining rooms (e.g., containment level 3 [CL3] suite with dedicated laboratory areas and separate animal rooms, or animal cubicles). Dedicated support areas, including anterooms (with showers and "clean" and "dirty" change areas, where required), are considered to be part of the containment zone.
Containment zone perimeter	The outermost physical boundary of a containment zone (i.e., the walls, doors, windows, floors, and ceilings that enclose a single containment zone).
Contamination	The undesired presence of infectious material or toxins on a surface (e.g., benchtop, hands, gloves) or within other materials (e.g., laboratory samples, cell cultures).
Controlled access system	A physical or electronic system designed to restrict access to authorized personnel only.
Controlled activities	Any of the following activities referred to in Section 7(1) of the *Human Pathogens and Toxins Act*: possessing, handling or using a human pathogen or toxin; producing a human pathogen or toxin; storing a human pathogen or toxin; permitting any person access to a human pathogen or toxin; transferring a human pathogen or toxin; importing or exporting a human pathogen or toxin; releasing or otherwise abandoning a human pathogen or toxin; or disposing of a human pathogen or toxin.
Critical door	Any door directly located on the containment barrier of a containment zone, animal cubicle, or post mortem room where inward directional airflow is required.

Culture	The *in vitro* propagation of microorganisms, tissue cells, or other living matter under controlled conditions (e.g., temperature, humidity, nutrients) to generate greater numbers or a higher concentration of the organisms/cells. In the context of the *Canadian Biosafety Standard* and the *Canadian Biosafety Handbook*, "cell culture" refers to cells derived from a human or animal source.
Decontamination	The process by which materials and surfaces are rendered safe to handle and reasonably free of microorganisms, toxins, or prions; this may be accomplished through disinfection, inactivation, or sterilization.
Decontamination technology (plural: decontamination technologies)	Equipment proven by validation to render materials safe to handle and reasonably free of microorganisms, toxins, or prions. Examples include autoclaves, incinerators, tissue digesters, and effluent decontamination systems.
Deep seal trap	A plumbing drain trap that has an effective head or depth that is sufficient to maintain a water seal, in accordance with air pressure differentials (i.e., water is neither siphoned into the room nor pushed through the trap). These traps have a water seal greater than 102 mm (4 inches) in depth, and a trap seal of 127 mm to 152 mm (5 to 6 inches).
Diagnostic activities	Activities (e.g., antibody assay, nucleic acid testing, histology, clinical chemistry) involving primary specimens for the purpose of identifying an infection, intoxication, or disease. These activities are regularly carried out in hospitals and clinical laboratories.
"Dirty" change area	The designated space inside the containment barrier where contaminated personal protective equipment is doffed when exiting the containment zone, animal cubicle, or post mortem room. The "dirty" change area is considered to be contaminated or potentially contaminated during normal operations.
Disease	A disorder of structure or function in a living human or animal, or one of its parts, resulting from infection or intoxication. It is typically manifested by distinguishing signs and symptoms.

Disinfection	Process that eliminates most forms of living microorganisms; disinfection is much less lethal to infectious material than sterilization.
Dual-use potential	Qualities of a pathogen or toxin that allow it to be either used for legitimate scientific applications (e.g., commercial, medical, or research purposes), or intentionally misused as a biological weapon to cause disease (e.g., bioterrorism).
Dunk tank	A disinfectant-filled vessel located at or on the containment barrier that allows for the safe removal of material and samples from containment zones via surface decontamination achieved through immersion.
Effluent decontamination system	Equipment connected to the drain plumbing used to decontaminate, through heat or chemical means, the liquid waste (i.e., effluent) produced in a containment zone prior to release into sanitary sewers.
Emergency response plan (ERP)	A document outlining the actions to be taken and the parties responsible in emergency situations such as a spill, exposure, release of infectious material or toxins, animal escape, personnel injury or illness, power failure, fire, explosion, or other emergency situations (e.g., flood, earthquake, hurricane).
Emerging animal disease	A new infectious disease resulting from the evolution or change of an existing pathogenic agent, a known infectious disease spreading to a new geographic area or population, or a previously unrecognized pathogenic agent or disease diagnosed for the first time and which has a significant impact on animal health. Emerging animal disease pathogens are handled as non-indigenous animal pathogens due to the high risk of serious negative effects associated with these pathogens.
Enzootic	A term that describes a disease (or pathogen) that is regularly present in an animal population.
Exporting	The activity of shipping (e.g., transferring or transporting) pathogens, toxins, or other regulated infectious material from Canada to another country.

Exposure	Contact with, or close proximity to, infectious material or toxins that may result in infection or intoxication, respectively. Routes of exposure include inhalation, ingestion, inoculation, and absorption.
Exposure follow-up report	A tool used to report and document incident occurrence and investigation information for an exposure incident previously notified to the Public Health Agency of Canada.
Exposure notification report	A tool used to notify and document preliminary information to the Public Health Agency of Canada of an exposure incident.
Facility (plural: facilities)	Structures or buildings, or defined areas within structures or buildings, where infectious material or toxins are handled or stored. This could include individual research and diagnostic laboratories, large scale production areas, or animal housing zones. A facility could also be a suite or building containing more than one of these areas.
Facility certification	The formal acknowledgement from the Canadian Food Inspection Agency (CFIA) that a containment zone or facility where imported animal pathogens will be handled or stored complies with the physical containment, operational practice, and performance and verification testing requirements described in the *Canadian Biosafety Standard*. Recertification refers to the renewal of the facility certification issued by the CFIA following a streamlined review process.
Good microbiological laboratory practices	A basic laboratory code of practice applicable to all types of activities with biological material. These practices serve to protect workers and prevent contamination of the environment and the samples in use.
Gross contamination	The accumulation of organic material (e.g., bedding, feed, excrement, blood, and tissues) on a surface that can be removed by physical methods, such as scraping, brushing, and wiping.

Handling or storing	"Handling or storing" pathogens, toxins, or infectious material includes possessing, handling, using, producing, storing, permitting access to, transferring, importing, exporting, releasing, disposing of, or abandoning such material. This includes all controlled activities involving human pathogens and toxins specified in Section 7(1) of the *Human Pathogens and Toxins Act*.
High concentration	Infectious material or toxins that are concentrated to a degree that increases the risks associated with manipulating the material (i.e., increases the likelihood or consequences of exposure).
High containment zones	Containment zones (i.e., laboratory work areas, animal rooms and cubicles, post mortem rooms, areas for large scale production), including all dedicated support areas, in containment level 3 (CL3), containment level 3-Agriculture (CL3-Ag), and containment level 4(CL4).
High efficiency particulate air (HEPA) filter (also: HEPA filtration)	A device capable of filtering 99.97% of airborne particles 0.3 μm in diameter, the most penetrating particle size. Due to the effects of impaction, diffusion, and interception, HEPA filters are even more efficient at trapping and retaining particles that are either smaller or larger than 0.3 μm in diameter.
Human *Pathogens and Toxins Act* Security Clearance (HPTA Security Clearance)	An authorization following verification of an individual's background and reliability status issued by the Public Health Agency of Canada under Section 34 of the *Human Pathogens and Toxins Act*.
Importing	The activity of bringing (e.g., transferring or transporting) pathogens, toxins, or other regulated infectious material into Canada from another country.
Incident	An event or occurrence with the potential of causing injury, harm, infection, intoxication, disease, or damage. Incidents can involve infectious material, infected animals, or toxins, including a spill, exposure, release of infectious material or toxins, animal escape, personnel injury or illness, missing infectious material or toxins, unauthorized entry into the containment zone, power failure, fire, explosion, flood, or other crisis situations (e.g., earthquake, hurricane). Incidents include accidents and near misses.

Infectious dose	The amount of pathogen required to cause an infection in the host, measured in number of organisms.
Infectious material	Any isolate of a pathogen or any biological material that contains human or animal pathogens and, therefore, poses a risk to human or animal health.
Insider threat	An authorized individual with access to secured assets, containment zones, or facilities as part of his/ her job that may pose a biosecurity risk.
In situ	Latin for "on site" or "in place"; describes a fixed location at which a procedure or experiment is conducted.
Interlock	A device or mechanism for coordinating the function of components (e.g., to prevent two doors being open simultaneously, or to ensure a supply fan shuts down in the event of an exhaust fan failure).
Internal accountability system	The responsibilities established for all personnel in a facility to safeguard pathogens, infectious material, and toxins.
Intoxication	A substance-induced disorder or disease resulting in a symptomatic or asymptomatic condition, or other physiological change resulting from an exposure (i.e., ingestion, inhalation, inoculation, or absorption) to a toxin produced by or isolated from a microorganism. This includes a similar response from exposure to a synthetically produced microbial toxin.
Inventory (plural: inventories)	A list of (biological) assets associated with a containment zone identifying pathogens, toxins, and other infectious material in storage both inside and outside of the containment zone.
In vitro	Latin for "within glass"; describes experimentation involving components of a living organism within an artificial environment (e.g., manipulation of cells in petri dish), including activities involving cell lines or eggs.

In vivo	Latin for "within the living"; describes experimentation conducted within the whole living organism (e.g., studying the effect of antibiotic treatment in animal models).
Inward directional airflow (IDA)	Air that always flows from areas of lower containment or lower contamination risk to areas of higher containment or higher contamination risk, as the result of a negative air pressure differential within the containment zone created by a ventilation system.
Isolation damper	A shut-off valve used to seal off air supply and exhaust air ductwork to/from a containment zone, as well as plumbing vent lines to allow the decontamination of high efficiency particulate air (HEPA) filters. Isolation dampers also provide backdraft protection in the event of heating, ventilation, and air conditioning (HVAC) system failure or a reversal of airflow, and prevent puff-back in certain types of biological safety cabinets.
Laboratory (plural: laboratories)	An area within a facility or the facility itself where biological material is handled for scientific or medical purposes.
Laboratory acquired infection/ intoxication (LAI)	Infection or intoxication resulting from exposure to infectious material, infected animals, or toxins being handled or stored in the containment zone.
Laboratory work area	Area inside a containment zone designed and equipped for *in vitro* research, diagnostics, and teaching purposes.
Large animal containment zone (LA zone)	Animal containment zone comprised of two or more co-located or adjoining rooms of equal containment level where animals are housed in animal cubicles (i.e., the room itself provides the primary containment). An LA zone may include, for example, large-sized animals, such as livestock or deer, housed in cubicles or, cubicles where small-sized animals, such as mice or raccoons, are housed in open caging (i.e., not primary containment caging). Post mortem rooms, where present, are considered to be part of an LA zone.

Large scale	Activities generally involving volumes of toxins or the *in vitro* culture of infectious material on a scale of 10 litres or greater. This could be a single vessel with a volume of 10 litres or greater, or based on the processes and pathogen used, could be multiple vessels with a total volume of 10 litres or greater. It is determined in consultation with the Public Health Agency of Canada and/or the Canadian Food Inspection Agency on a case-by-case basis, whether or not particular activities conducted in a containment zone are required to follow the increased or unique requirements for large scale production areas.
Large scale production area	A room or space where activities involving the production of toxins or the *in vitro* culture of biological material on a scale of 10 litres or greater are conducted.
Large-sized animal	Refers to the physical size of the animal; large-sized animals are generally too large to be housed in primary containment caging, and are therefore housed in an animal cubicle. Examples include cows, horses, moose, deer, and sheep.
Large volume	A volume of infectious material or toxins that is sufficiently large to increase the risk associated with the manipulation of the material (i.e., increases the likelihood or consequences of exposure or release).
Licence	An authorization to conduct one or more controlled activities with human pathogens or toxins issued by the Public Health Agency of Canada under Section 18 of the *Human Pathogens and Toxins Act*.
Limited access	Access that is only permitted to authorized personnel and other authorized visitors through either operational means (e.g., having authorized personnel actively monitor and check all individuals entering a designated area) or through the use of a physical barrier (e.g., a controlled access system, such as key-locks or electronic access card).
Local risk assessment (LRA)	Site-specific risk assessment used to identify hazards based on the infectious material or toxins in use and the activities being performed. This analysis provides risk mitigation and risk management strategies to be incorporated into the physical containment design and operational practices of the facility.

Long-term storage	In the context of the *Canadian Biosafety Standard* and the *Canadian Biosafety Handbook*, the possession of material (i.e., pathogens, toxins, and other regulated infectious material) beyond 30 days of receipt or creation.
Median effective dose (ED_{50})	The amount of a toxin that will cause a particular effect in 50% of the test population.
Median lethal dose (LD_{50})	The amount of a toxin that is lethal to 50% of the test population.
Medical surveillance program	A program designed to prevent and detect personnel illness related to exposure to infectious material or toxins. The focus of the program is primarily preventive, but provides a response mechanism through which a potential infection or intoxication can be identified and treated before serious injury or disease occurs.
Microorganism	A cellular or non-cellular microbiological entity, capable of replication or transferring genetic material and that cannot be reasonably detected by the naked human eye. Microorganisms include bacteria, fungi, viruses, and parasites, and may be pathogenic or non-pathogenic in nature.
Movement	The action of moving (e.g., bringing, carrying, leading, relocating) people, material (including infectious material or toxins), or animals from one physical location to another physical location in the same building. This can include movement within the same containment zone, to a different containment zone, or to another location within the same building.
Non-indigenous animal pathogen	A pathogen that causes an animal disease listed in the World Organisation for Animal Health's *OIE-Listed diseases, infections and infestations* (as amended from time to time) and that is exotic to Canada (i.e., foreign animal disease agents that are not present in Canada). These pathogens may have serious negative health effects to the Canadian animal population.
Open caging	Caging intended to restrict animals to an area (e.g., animal pens). This type of caging does not prevent the release of pathogens and toxins and, therefore, does not meet the requirements for primary containment caging.

Operational practice requirements	Administrative controls and procedures followed in a containment zone to protect personnel, the environment, and ultimately the community, from infectious material or toxins, as outlined in Chapter 4 of the *Canadian Biosafety Standard*.
Opportunistic pathogen	A pathogen that does not usually cause disease in a healthy host but can cause disease when the host's resistance is low (e.g., compromised immune system).
Organic load	The amount of organic material (e.g., bedding, litter, feed, manure) present on a surface or in a solution.
Outsider threat	An individual without authorization or access to secured assets, containment zones, or facilities, who may not have a formal relationship with the facility and may pose a biosecurity risk.
Overarching risk assessment	A broad risk assessment that supports the biosafety program as a whole and may encompass multiple containment zones within an institution or organization. Mitigation and management strategies reflect the type of biosafety program needed to protect personnel from exposure and to prevent the release of pathogens and toxins.
Pass-through chamber	Interlocked double-door compartment situated on a containment barrier that allows the safe movement of materials into and out of containment zones.
Pass-through technology	Equipment with double-door compartments situated on a containment barrier that allows the safe movement of materials into and out of the containment zone. Examples include double-door barrier autoclaves, pass-through chambers, dunk tanks, barrier cage washers, and feed chutes.
Pathogen	A microorganism, nucleic acid, or protein capable of causing disease or infection in humans or animals. Examples of human pathogens are listed in Schedules 2 to 4 and in Part 2 of Schedule 5 of the *Human Pathogens and Toxins Act*, but these are not exhaustive lists. Examples of animal pathogens can be found through the Automated Import Reference System on the Canadian Food Inspection Agency website.

Pathogenicity	The ability of a pathogen to cause disease in a human or animal host.
Pathogen risk assessment	The determination of the risk group and appropriate physical containment and operational practice requirements needed to safely handle the infectious material or toxins in question.
Pathogen Safety Data Sheet (PSDS)	Technical document describing the hazardous properties of pathogens and recommendations for their safe handling. A PSDS may include information such as pathogenicity, drug susceptibility, first aid treatment, personal protective equipment, and risk group classification. PSDSs were formerly called material safety data sheets for infectious material.
Performance and verification testing requirements	Performance and verification tests that are necessary to demonstrate compliance with the physical containment requirements, as outlined in Chapter 3 of the *Canadian Biosafety Standard* and, in some cases, the operational practice requirements, as outlined in Chapter 4 of the *Canadian Biosafety Standard*. The performance and verification testing requirements are listed in Chapter 5 of the *Canadian Biosafety Standard*.
Personal protective equipment (PPE)	Equipment and/or clothing worn by personnel to provide a barrier against infectious material or toxins, thereby minimizing the risk of exposure. PPE may include, but is not limited to, lab coats, gowns, full-body suits, gloves, protective footwear, safety glasses, safety goggles, masks, and respirators.
Physical containment requirements	Physical barriers in the form of engineering controls and facility design used to protect personnel, the environment, and ultimately the community, from pathogens or toxins, as outlined in Chapter 3 of the *Canadian Biosafety Standard*.
Post mortem room (PM room)	A room within the containment zone where animal necropsies and dissections are conducted.
Pressure decay testing	A method of quantifying the leak rates of a sealed environment.

Primary containment	The first level of physical barriers designed to contain pathogens and toxins and prevent their release. This is accomplished by the provision of a device, equipment, or other physical structure situated between the infectious material or toxins and the individual, the work environment, or other areas within the containment zone. Examples include biological safety cabinets, glove boxes, and animal microisolators. In animal cubicles, the room itself provides primary containment, and personal protective equipment serves as primary protection against exposure.
Primary containment caging	Animal caging serving as a primary containment device to prevent the release of infectious material and toxins. Examples include ventilated filter-top cages and ventilated micro-isolator cage rack system, with or without high efficiency particulate air (HEPA) filters.
Primary containment device	Apparatus or equipment that is designed to prevent the release of infectious material or toxins and to provide primary containment (i.e., provide a physical barrier between the individual and/or the work environment and the biological material). Examples of primary containment devices include biological safety cabinets, isolators, centrifuges with sealable cups, process equipment, fermenters, microisolator cages, and ventilated cage racks.
Primary decontamination technology	The initial validated equipment or process used to decontaminate waste from the containment zone before disposal, incineration, or release to sanitary sewers. This serves to remove or inactivate infectious material or toxins through a process of disinfection, sterilization, or inactivation. This may be followed by a secondary decontamination process.
Prion	Small proteinaceous infectious particle generally considered to be responsible for causing a group of neurodegenerative diseases in humans and animals known as transmissible spongiform encephalopathies.
Process equipment	Specific equipment used to carry out a manufacturing procedure involving biological material. This term is generally used to describe equipment used in large scale processes (e.g., industrial fermentation equipment).

Program intent	A description of the planned work to be performed in a containment zone. This includes, but is not limited to, the scope of work (e.g., diagnostic, teaching, research, large scale production, *in vitro* work, *in vivo* work), a list of pathogens, toxins, and other regulated infectious material to be handled or stored, a list of animal species to be involved in *in vivo* work with pathogens and toxins in the zone, and a list of procedures that may create aerosols.
Propagation	The act of multiplying pathogens under controlled laboratory conditions.
Puff-back	The reversal of airflow from the face of a Class II type B2 biological safety cabinet due to failure of the exhaust fan.
Release	The discharge of infectious material or toxins from a containment system.
Representative load	A simulation batch of materials of a similar type (e.g., gloves, plastics, liquids) and quantity used to validate a decontamination method for routine loads.
Restricted access	Access that is strictly controlled to authorized personnel only by means of a physical barrier (i.e., a controlled access device or system, such as an electronic access card, access code, etc.).
Risk	The probability of an undesirable event (e.g., accident, incident, breach of containment) occurring and the consequences of that event.
Risk group (RG)	The classification of biological material based on its inherent characteristics, including pathogenicity, virulence, risk of spread, and availability of effective prophylactic or therapeutic treatments, that describes the risk to the health of individuals and the public as well as the health of animals and the animal population.
Risk management plan	A plan that provides the foundation and organizational arrangements for designing, implementing, monitoring, reviewing, and continually improving risk management throughout the organization.

Scientific research	As defined in Section 1 of the *Human Pathogens and Toxins Regulations*: the following types of systematic investigation or research that are carried out in a field of science or technology by means of controlled activities: a) basic research, when the controlled activities are conducted for the advancement of scientific knowledge without a specific practical application; b) applied research, when the controlled activities are conducted for the advancement of scientific knowledge with a specific practical application; c) experimental development, when the controlled activities are conducted to achieve scientific or technological advancement for the purpose of creating new – or improving existing – materials, products, processes, or devices.
Sealable doors	Doors that are designed to allow leakage of air under normal operating conditions yet are capable of being sealed to withstand pressure decay testing and gaseous decontamination (e.g., three-sided or four-sided gasket, four-sided door jamb).
Security barrier	A physical obstruction designed to prevent access to pathogens, infectious material, toxins, or other related assets by unauthorized personnel (e.g., locked doors, controlled access systems, or padlocked storage equipment) that increases the security of a containment zone by restricting access to authorized personnel only.

Security sensitive biological agents (SSBAs)	The subset of human pathogens and toxins that have been determined to pose an increased biosecurity risk due to their potential for use as a biological weapon. SSBAs are identified as prescribed human pathogens and toxins by Section 10 of the *Human Pathogens and Toxins Regulations*. This means all Risk Group 3 and Risk Group 4 human pathogens that are in the List of *Human and Animal Pathogens for Export Control*, published by the Australia Group, as amended from time to time, with the exception of Duvenhage virus, Rabies virus and all other members of the Lyssavirus genus, Vesicular stomatitis virus, and Lymphocytic choriomeningitis virus; as well as all toxins listed in Schedule 1 of the *Human Pathogens and Toxins Act* that are listed on the *List of Human and Animal Pathogens for Export Control* when in a quantity greater than that specified in Section 10(2) of the *Human Pathogens and Toxins Regulations*.
Senior management	The ultimate authority responsible for delegating appropriate biosafety authority. Senior management is responsible for ensuring that adequate resources are available to support the biosafety program, to meet legal requirements, and that biosafety concerns are appropriately prioritized and addressed.
Seroconversion	A change in the antibody titre of an individual's serum from a seronegative to a seropositive state, indicating the development of antibodies in response to an infection or immunization.
Small-sized animal	Refers to the physical size of the animal; small-sized animals are small enough to be housed in primary containment caging. Examples include rodents, rabbits, ferrets, chickens, and non-human primates. Small-sized animals may also be housed in an animal cubicle (e.g., when open caging is used).
Small animal containment zone (SA zone)	Animal containment zone comprised of one or several co-located or adjoining rooms of equal containment level where animals are housed in animal rooms inside primary containment caging (e.g., microisolators). An SA zone may contain, for example, mice, rats, rabbits, ferrets, or non-human primates, provided that they are housed in primary containment caging.

Standard operating procedure (SOP)	A document that standardizes safe work practices and procedures for activities with infectious material and toxins in a containment zone, as determined by a local risk assessment.
Sterilization	Process that completely eliminates all living microorganisms, including bacterial spores.
Strict animal pathogen	A pathogen that causes disease exclusively in animals (i.e., not capable of causing disease in humans).
Support area	Area containing the necessary material and functionality to support the containment zone. This could include, but is not limited to, storage and preparation areas as well as the change rooms in high containment zones.
Synthetic biology	Synthetic biology is a rapidly evolving interdisciplinary field of research that combines biology and engineering for the design, redesign, or fabrication of novel or existing natural biological components and systems.
Terrestrial animal pathogen	A pathogen that causes diseases in terrestrial animals, including avian and amphibian animals, but excluding aquatic animals and invertebrates.
(Microbial) Toxin	A poisonous substance that is produced or derived from a microorganism and can lead to adverse health effects in humans or animals. Human toxins are listed in Schedule 1 and Part 1 of Schedule 5 in the *Human Pathogens and Toxins Act*.
Training needs assessment	An evaluation performed to identify the current and future training needs of the facility (organization, containment zone) and to identify gaps in the current training program.
Transfer	A change in possession of pathogens, toxins, or other regulated infectious material between individuals from the same or different facilities (i.e., the movement from the place or places specified in the licence or animal pathogen import permit to any other place).

Transmissible spongiform encephalopathy (TSE)	A fatal progressive neurodegenerative disease affecting humans and/or animals that is generally accepted to be caused by prions.
Transportation	The act of transporting (e.g., shipping or conveyance) infectious material or toxins to another building or location (i.e., different address), within Canada or abroad, in accordance with the *Transportation of Dangerous Goods Act and Regulations*.
Trigger quantity	The minimum quantity above which a toxin regulated by the *Human Pathogens and Toxins Act* is considered a "prescribed toxin" and, therefore, a security sensitive biological agent, as described by Section 10(2) of the *Human Pathogens and Toxins Regulations*.
Validation	The act of confirming that a method achieves its objective by observing that specific parameters have been met (e.g., using biological indicators to confirm that a given autoclave cycle can decontaminate a representative load of waste). Validation infers that a method is suitable for its intended purpose.
Ventilated cage changing station	Equipment specifically designed to change bedding and other contents from animal cages that: a) directs the air away from the user into the interior of the unit at a sufficient velocity to protect the user from potential exposure to any infectious material or toxins; and b) filters the exhaust air prior to release from the unit, thereby preventing the potential release of infectious material or toxins into the environment.
Verification	The routine monitoring of equipment and processes to ensure continued efficacy between validations. This includes comparing the accuracy of a piece of equipment to an applicable standard or standard operating procedure (e.g., testing of a Class I biological safety cabinet in accordance with the manufacturer specifications).
Virulence	The degree or severity of a disease caused by a pathogen.

Vulnerability (plural: vulnerabilities)	A component of a biosecurity risk assessment that identifies weaknesses in a facility's physical security barriers, operational practices (e.g., biosecurity training), personnel security, transport security, information security, and program management.
Waste	Any solid or liquid material generated by a facility for disposal.
Zoonoses (singular: zoonosis)	Diseases that are transmissible between living animals and humans. Zoonoses include anthropozoonoses (i.e., diseases transmitted from animals to humans) and zooanthropoposes, also known as reverse zoonoses (i.e., diseases transmitted from humans to animals).
Zoonotic pathogen	A pathogen that causes disease in humans and animals, and that can be transmitted from animals to humans and vice versa (i.e., zoonoses). They are considered both human and animal pathogens.

CHAPTER 25

RESOURCES

25.1 General Resources

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Canadian Council on Animal Care: http://www.ccac.ca/

Canadian Food Inspection Agency: http://www.inspection.gc.ca/

Canadian Food Inspection Agency, Automated Import Reference System (AIRS):
http://www.inspection.gc.ca/airs

Canadian Food Inspection Agency, Biohazard Containment and Safety:
http://www.inspection.gc.ca/english/sci/bio/bioe.shtml

Canadian Food Inspection Agency, Veterinary Biologics:
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Environment and Climate Change Canada: www.ec.gc.ca/

Environment and Climate Change Canada, Biotechnology (Living Organisms):
http://www.ec.gc.ca/subsnouvelles-newsubs/default.asp?lang=En&n=E621534F-1

Global Affairs Canada: www.international.gc.ca/

Global Affairs Canada, Export and Import Controls:
http:// www.exportcontrols.gc.ca /

Government of Canada: http://www.canada.ca/

Health Canada: http://www.hc-sc.gc.ca/

Health Canada, Biologics and Genetic Therapies Division:
http://www.hc-sc.gc.ca/ahc-asc/branch-dirgen/hpfb-dgpsa/bgtd-dpbtg/index-eng.php

International Air Transport Association: www.iata.org/

International Civil Aviation Organization: www.icao.int/

OIE World Organisation of Animal Health: http://www.oie.int/

Public Health Agency of Canada: http://www.publichealth.gc.ca/pathogens

Public Health Agency of Canada, Biosecurity Portal:
http://www.publichealth.gc.ca/pathogens

Public Health Agency of Canada, e-Learning Portal:
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Public Health Agency of Canada, Laboratory Biosafety and Biosecurity:
http://www.publichealth.gc.ca/pathogens

Public Health Agency of Canada, Security Sensitive Biological Agents:
http://phac-aspc.gc.ca/lab-bio/regul/ssba-abcse-eng.php

Transport Canada: from www.tc.gc.ca/

Transport Canada, Transportation of Dangerous Goods:
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New Substances Notification Regulations (Chemicals and Polymers) (SOR/2005-247). (2015).

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APPENDIX A

PLAN FOR ADMINISTRATIVE OVERSIGHT FOR PATHOGENS AND TOXINS IN A RESEARCH SETTING

APPENDIX A – PLAN FOR ADMINISTRATIVE OVERSIGHT FOR PATHOGENS AND TOXINS IN A RESEARCH SETTING

There are characteristics of research environments where the need for innovation may be at odds with the regulatory framework intended to protect the health and safety of the public from the risks posed by human pathogens and toxins. The research sector also faces additional risk factors, such as autonomous research and researchers, perceived diffuse accountabilities, and complex reporting and governance structures, which are not always present in other sectors (e.g., diagnostic and private industry). As a risk mitigation approach to balance the public health and safety concerns with the importance of promoting Canadian research with human pathogens and toxins, the PHAC requires that a risk management plan (i.e., a *Plan for Administrative Oversight for Pathogens and toxins in a Research Setting* [the "Plan"]) be submitted in support of an application for a licence where scientific research is intended to be carried out (HPTR 3).

These Plans are intended to be very high level (i.e., at the institutional/organizational level) and are not intended to include or repeat any regulatory elements already captured through other means, such as the Canadian Biosafety Standard (CBS), 2nd Edition. A licence will not be issued to an applicant without the submission of a Plan; however, the quality and completeness of the Plan will not delay the issuance of a licence. The PHAC will work with the applicants to finalize their Plans as needed.

As defined in Section 1 of the HPTR, "scientific research" means the following types of systematic investigation or research that are carried out in a field of science or technology by means of controlled activities:

a) basic research, when the controlled activities are conducted for the advancement of scientific knowledge without a specific practical application;

b) applied research, when the controlled activities are conducted for the advancement of scientific knowledge with a specific practical application; and

c) experimental development, when the controlled activities are conducted to achieve scientific or technological advancement for the purpose of creating new — or improving existing — materials, products, processes or devices.

Administrative controls in relation to a biosafety program management in any facility are described in Chapter 5. The Plan is intended to provide an overview of the administrative controls already in place and will need to capture the following ten elements:

Element 1:	Commitment from **senior management** to manage and control biosafety and biosecurity risks at the institution/organization. • Example: biosafety policy, code, strategy, and could encompass other safety areas or be incorporated into other risk management documents.
Element 2:	Delineation of the roles and responsibilities for committees, individuals, departments, etc., that have a role in the control/management of biosafety and biosecurity risks. • Example: could be demonstrated by diagrams, flow-charts, terms of reference for committees.
Element 3:	Establishment of a single point of contact to provide guidance on the Plan and a senior level "champion" who can represent biosafety issues at a senior level on his/her behalf. • Example: utilization of an already well-established system, such as BSO linkage to an Occupational Health and Safety Director who represents safety at senior level meetings.
Element 4:	Overview of how biosafety and biosecurity risks, including those from research with **dual-use potential**, are *identified* at the institution/organization. • Example: reference to CBS requirements or explanation of the approach taken if an all hazards approach is used.
Element 5:	Overview of how biosafety and biosecurity risks, including those from research with dual-use potential, are *assessed* once they have been identified at an institutional/organizational level. • Example: the processes used to determine the overall biological risk at an institutional level or how multiple levels are involved.

Element 6:	Overview of how the biosafety and biosecurity risks, including those from research with dual-use potential, are *managed and controlled* at an institutional/organizational level • Example: mechanisms in place, such as internal permit systems, off-site control mechanisms, internal inspections, release of research grants and funds based on compliance, institutional biosafety committee (IBC) role.
Element 7:	Description of all work areas covered by the Plan (research areas, teaching, off-site, etc.). • Example: explain how all of the work areas are linked into the internal permitting system, how different areas such as teaching areas or off-site areas have special permits or are captured; include how areas are assessed for containment requirements and how appropriate space is assigned for the work being done.
Element 8:	Description of all individuals covered by the Plan (researchers, faculty, students, etc.). • Example: indicate linkages to the human resources system within all departments to capture all individuals covered by the Plan and how they are made aware of their need for compliance.
Element 9:	Summary of how the Plan is communicated. • Example: how regular communication at the interfaces between those responsible for the oversight of biosafety and biosecurity risks, such as individuals and committees, and between functions (e.g., research and administration) takes place.
Element 10:	Overview of the procedures to review and monitor the Plan. • Example: a chart to indicate the timeline for continual review or modifications, a summary of the indicators or factors that are used as triggers to update and communicate the Plan.

APPENDIX B

PROPER HANDWASHING TECHNIQUE

APPENDIX B – PROPER HANDWASHING TECHNIQUE

Handwashing is the most common method for decontamination of hands and the most effective means for preventing the transmission of infection. Handwashing, using soap and clean running water is an effective way to remove visible soil and/or organic material and eliminate all types of pathogens from the surface of the hands.

Proper Handwashing (soap and water)[1]

1. Running water should be used to wet hands.
2. Enough soap should be used to lather all surfaces of the hands, including fingers, fingertips, between fingers, palms, backs of hands and thumbs, base of thumb, and if a ring is worn, on and under the ring.
3. The palms and backs of each hand should be rubbed vigorously, interlocking and interfacing fingers to ensure finger and thumbs are rubbed to remove visible soil and/or organic material (this task should take 15 to 30 seconds).
4. Hands should be rinsed thoroughly in a downward position under running water.
5. Hands should be dried thoroughly by patting with a single-use towel.
6. Manual faucets should be turned off with paper towels, ensuring that hands are not recontaminated in the process.
7. Skin products should be applied regularly to maintain healthy skin.
8. The complete handwashing procedure (going to sink, wetting hands, applying soap, lathering, rinsing and drying) should take 40 to 80 seconds.

Considerations On the Use of Alcohol-based Hand Sanitizers

- Use of alcohol-based hand sanitizers should be limited, as they are not as effective as handwashing with soap and water and cannot eliminate all types of pathogens.[2]
- Alcohol-based hand sanitizers may not be as effective as handwashing when hands are visibly dirty or greasy.
- A hand sanitizer that has been demonstrated to be effective against the pathogen(s) or toxin(s) in use in the containment zone may be an alternative where handwashing sinks are not easily accessible to avoid the spread of contamination. In this instance, handwashing should follow as soon as a suitable handwashing sink is available.
- Alcohol-based hand sanitizers should not be applied to wet hands, as this will dilute the alcohol.
- The manufacturer's instructions should be followed; all hand surfaces should be rubbed until product has dried to allow for the appropriate contact time.

- Alcohol-based hand sanitizers should be allowed to dry prior to contact with an oxygen-rich environment and prior to putting gloves on.
- Paper towel should not be used to dry hands or wipe product off hands before it can dry.
- Hand wipes (impregnated with plain soap, antimicrobials, or alcohol) should not be used as an alternative to antimicrobial soaps or alcohol-based hand sanitizers for hand antisepsis.

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INDEX

INDEX

access
- anteroom 32, 167
- containment zone 22-24, 32, 167, 256, 263
- security sensitive biological agents 78, 80

accident 214, 222
accountability 67, 76-77, 235
- internal accountability system 59, 235-236
- transportation 236

administrative area 18, 248, 255, 263
aerosol 39, 126, 146, 156, 161
- aerosol-generating equipment and processes . . . 39, 141, 146-149, ... 152, 168, 179
- animal work 156, 158, 161, 168, 170
- protection from 108
- release 28, 118, 258

airborne pathogens 28, 31, 107
airflow 259
. . . . . *See also* biological safety cabinet
- inward directional airflow. . . 28, 32, 121, ... 136, 167, 256, 258
- reversal of airflow 32, 133, 259

airtight doors 120, 264
alarm 96, 118, 258, 263
- equipment . . . . . 139- 140, 151, 198, ... 200, 263

animal containment zone 22, 158-168
- design 29-30, 165-168, 256

animal cubicle 22, 28, 160
- containment zone design . 158, 166-167
- decontamination 170-171, 185
- personal protective equipment 105- 106, 109-110

animal health surveillance program 156
animal pathogen
- accountability 60, 234
- non-indigenous . . . 43-44, 59, 268, 274
- risk group classification 38
- risk management 50

animal pathogen import permit . . 39, 43, 60-61, ... 68, 229, 268, 273, 277
animal room 22, 31, 160, 263
- containment zone design 158, 166
- personal protective equipment 105- 106, 109

animal work area . . . . . 21,249, ... *See also* animal containment zone
anteroom 21, 31-32
- animal work area 166-167
- door interlocks 263
- inward directional airflow. . . 32, 118, 167

asset 72, 234
- inventory 237

autoclave 193-196, 261
- prion 203
- validation 183
- validation and verification. 183-185

backdraft protection 119, 139
backflow prevention 259
biocontainment 16
. . . . . *See also* containment
biological safety cabinet 126-143
- class I 126
- class II. 129-136
- class II type B2 133,259
- class III 136
- installation 139, 265
- proper use 140-143
- spill response 218
- testing and certification 139-140
- ultraviolet light 143

biological safety officer 60-61
- incident reporting 224

biological spill kit 216
biosafety manual 64
- emergency response plan 215
- equipment maintenance 146
- facility certification. 275
- medical surveillance 84
- training 94
- waste management 208

biosecurity 72-80
- biosecurity plan 64, 75-77
- Human Pathogens and Toxins Act Security Clearance . . . 78-80
- information security 77
- physical security 75
- program management 58
- response plans 77
- security sensitive biological agents 43

biotechnology 10-11, 45-47, 278
body protection 106-107
caging 158
- open 160
- primary containment 22,160
- washing 171

cell line 11-12, 48-49
change area 110, 256
CL3 19
CL4 19
configuration 31
cold storage 167
commissioning 255
confinement. 171-172
container
movement 242
sharps. 210-211
waste 211
containment
assessment. 39-40
barrier. 28, 118
containment zone perimeter . . . 23-24, 28
containment zone work areas 21
support areas 249, 256
systems 118, 275
containment levels 16–19
containment zone 21-32
animal 158-168
design 254-264
facility certification. 275
controlled access system 75, 167, 263
controlled activities 269
critical door. 32, 118
culture 11, 210,
. . . . . *See also* cell line,
. . . . . *See also* large scale
decontamination 182-204, 208
animal work. 170-171
chemical decontamination shower. 32
chemical disinfectants 189-193
effluent 198-199
high efficiency particulate air filter
. . . . . .122, 197, 203
in situ 122, 139, 179
prion 203
surface 143, 185
toxins 201-202
decontamination technology
autoclave 195, 261
gaseous decontamination 196-198
incineration 200
irradiation 199
validation and verification. 184
deep seal trap 260
diagnostic activities. 49
disinfectant 182
chemical disinfectant 185-193
dunk tanks 200
footbath 105, 112
dual-use potential 41, 43, 47, 73, 279
dunk tank 200
effective dose 42
effluent decontamination system
. . . . . 198-199, 261-262
animal facilities. 170
emergency response plan . . . 65, 77, 214-216
emerging animal disease 43, 59, 268
enzootic 9, 174
exhaust. 119, 258
animal facilities. 160
biological safety cabinets 126, 135
chemical fume hoods. 151
fermenters 179
exporting 278
exposure
exposure assessment 42
exposure risks. 52
follow-up report. 227
incidents 222
laboratory exposures 84-86
notification report 86, 226
reporting 66, 228
response planning. 65, 88
eye protection 105-106,113
doffing 111
donning 110
eyewash. 260
face protection. 105-106, 113
mask. 107-108
facility
certification 275
design 158-168, 248, 254
shared facilities. 62, 80
fermenters 179
foot protection 104-105, 250
freezer 23, 167, 256
gloves
. . . . . 102, 106, 108, 110,
. . . . . *See also* hand protection

good microbiological laboratory practice 65, 249-250
gross contamination 161, 188
hand protection 102-104
heating, ventilation, and air conditioning 51, 118, 258
high containment zone 88
airflow 119
cage washing areas 171
chemical fume hood 151, 259
compressed gas cylinders. 152
containment zone barrier 28
containment zone perimeter. 22
design 256-257
effluent decontamination. 261
gaseous decontamination 196
performance and verification testing . .255
personal protective equipment 106, 110, 112
waste decontamination 193, 200, 261
high efficiency particulate air filter . . . 121-122
biological safety cabinet 126, 129, 133, 136
caging exhaust air. 160
decontamination 197
ductwork 258
fermenter exhaust air 179
plumbing vent lines 260
prion 203
respirators 108
Human Pathogens and Toxins Act
exclusions 270
exemptions 270-272
Human Pathogens and Toxins Act Security Clearance 76, 80
accompaniment and supervision 80
exemptions 79
process 78
shared workspace. 80
shipping and receiving. 236, 245
suspension and revocation 79
validity and portability 79
importation
animal pathogens 273
emerging animal disease pathogen 43, 274
non-indigenous animal pathogen . .43, 274
records 66
roles and responsibilities. 60
toxins derived from animal pathogens . . .40
importation for commerical use 276
incident
. . . . . *See also* spill response 214
follow-up 227
reporting and investigation . .66, 224, 229
response 77, 214, 230
incineration 200
prion 173, 203
infectious dose. 37
information security 77
insider threat 73,75-76
institutional biosafety committee. 62
interlock
air supply and exhaust system 119, 139-140
anteroom doors 32, 118, 263-264
internal accountability system . . . 59, 235-236
intoxication
. . . . . .9, 84
. . . . *See also* laboratory acquired infection/intoxication
inventory. 67, 77, 237-239
inward directional airflow 28, 32,118-121, 258
laboratory acquired infection/intoxication 9, 84-86
reporting and investigation 66, 226
laboratory work area 21, 248
large animal containment zone . . . 22, 88, 109-110, 158, 160-161, 254
large scale 21, 44, 180
containment assessment 39
large-sized animal 22, 156
equipment 168
handling and restraint 170
lethal dose 40, 42
licence
exclusions and exemptions 271
local risk assessment 3,52, 249

long-term storage 73, 76, 235, 237
medical surveillance program 64, 84-89
high containment zone 88
ongoing medical surveillance 87
post-exposure 88
pre-placement medical evaluation. 86
vaccinations 87
movement
accountability 236
materials across containment barrier. . . .151
within the containment zone . . . 242-243
within the same building 243
non-indigenous animal pathogen 43
importation and transfer 268
physical requirements 168
program intent 59
open caging 22, 160
opportunistic pathogen 6-7, 38, 248
organic load 188
overarching risk assessment 63
packages
shipping and receiving 245
transfer and transportation. 236
transfer or shipping 77
pass-through chamber 151
pathogen
accountability 234
pathogen risk assessment 36-37
pathogen safety data sheet 36
pathogenicity 37
modification 46
performance and verification testing
. . . . . .68, 255, 268, 275
personal protective equipment
. . . . . 51, 102-114, 249
animal containment zone 157, 161
body protection 106, 113
doffing 110-112
donning 109-110
eye and face protection 105,113
foot protection 104-105, 112, 250
hand protection 102, 112
respiratory protection 107-108, 114
personnel suitability 76
plan for administrative oversight
. . . . . 58, 273, 334-336
positive-pressure suit 19, 107
post mortem room. . . . 21-22, 161, 166, 168
pressure decay testing 120-121
Class III biological safety cabinet 140
ductwork 258
primary containment
. . . . . .*See also* biological safety cabinet,
. . . . . .*See also* animal cubicle
caging 22, 160
device 17, 51, 126,
. . . . . .160, 214, 254, 262
primary decontamination technology
. . . . . .171, 198, 261
prion 8, 42
animal work 170, 172-173
decontamination 203-204
equipment considerations 152
waste 210
program intent 59, 275
puff-back 133, 140, 259
records 66-67, 77, 237
criminal record check 78
training records 99
transfers 236
representative load 183
respiratory protection 107-108
restricted access 76-77, 167, 239
risk 36
assessment 3, 36
biosecurity 43, 72, 335
factors 37, 42
risk group 36, 38
risk group 1 . . . . . .16, 38, 248
risk group 2 38
risk group 3 38
risk group 4 38-39
risk management 50-52
risk management plan 58, 273, 278,
. . . *See also* plan for administrative oversight
sealable doors 120, 264
security barrier 75, 239, 263

security sensitive biological agents . . . . . , 43
inventory 237
notification and reporting 226, 228
shared facilities. 80
toxins 41,
senior management
roles and responsibilities. 60, 69, 74
seroconversion 47, 85, 88
shower
anteroom. 19, 28, 31, 166
chemical 182
emergency. 260
when to shower 167
signage
authorized personnel only signage . . .167
biohazard warning sign 23
small animal containment zone. . .22, 158, 160
decontamination 170
small-sized animal 22, 158, 160
spill response. 216-219
standard operating procedure. 51, 65
animal work. . . . . , 166
anteroom doors 118
decontamination 184
equipment 140, 194
personal protective equipment . . 109- 110
training 94, 169
waste disposal 208
sterilization 182
autoclave 193
indicator 184, 199
open flame 147
support area 21, 158, 249, 256
tailgating 96
toxins 9-10, 40-42
decontamination 201-202
intoxication 84
risk assessment 36
security sensitive biological agents . . . 43
training. 94-99
animal containment zones. 169
biosecurity training 95, 99
emergency response 215
evaluation 98
program content 94
program review 99
records 66, 99
trainees 97
training needs assessment. . 64, 94, 215
training program 64
transfer
records 66, 236
transfer air device. 119
transfer box 158, 169
transmissible spongiform encephalopathies
. . . . . .8, 172
transportation. 200
accountability measures 236
infectious material or toxins 243-246
regulations 244
trigger quantity. 41, 43, 79, 272
validation
decontamination processes and
technologies 183-184, 199
ventilated cage changing station
. . . . . .126, 156, 170
verification
decontamination processes and
technologies 184
virulence 37, 46
visitor 67
access 76
training 97
vulnerability. 73, 77
waste
animal waste
. . . . . .156, 170, 172, 201, 210
disposable personal protective equipment
. . . . . .105, 111
effluent 168, 170
human anatomical waste 209
human blood and body fluids 210
microbiological laboratory waste 210
movement 242-243
prion 172, 203, 210
sharps. 210-211
storage 210
transportation 244
waste management 208-211
windows. 75, 120, 262
observation windows. 167
zoonotic pathogens
. . . . .8, 40, 44, 86, 156, 268, 270, 276

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